ACID-BASE BALANCE AND METABOLISM IN SHORT-TERM, MAXIMAL EXERCISE

by

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ACID-BASE BALANCE AFTER MAXIMAL EXERCISE

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ABSTRACT

The acidosis accompanying short-term maximal exercise has been quantified and the mechanisms contributing to its control examined. Maximal exercise lasting 30 s was performed on a constant-velocity cycle ergometer. In 3 subjects, acid-base changes were examined across the working quadriceps femoris muscle after arterial and femoral venous catheterisation (Part A). The acid-base changes across the inactive forearm muscle were examined in 6 subjects following arterial and deep forearm venous catheterisation. Gas exchange was measured breath-by-breath during exercise and recovery (Part B). Muscle biopsies were taken from the quadriceps femoris muscle in 6 subjects and analysed for intracellular strong ion changes using neutron activation analysis (Part C).

The intracellular acid load was due to both increased CO_2 production and strong anion production; the muscle [lactate] increased to 30 mmol/kg w.w. after 30 s exercise. The CO_2 and strong ion concentration contributed 25% and 75%, respectively, to the increase in intracellular $[H^+]$. The weak acid concentration was assumed not to change during exercise and recovery. CO_2 and strong ions were removed from the intracellular fluid during recovery.

Initially CO_2 output from the muscle reduced the intracellular PCO_2 ; the femoral venous PCO_2 increased to 105 mm Hg. The increased CO_2 flux to the lungs increased the CO_2 elimination from the body; the CO_2 output increased to 3060 ml/min by the end of exercise. The lungs were effective in removing the excess CO_2 delivered to them as the arterial PCO_2 was less than resting levels throughout recovery. Elimination of excess CO_2 from muscle was complete by 3 min recovery.

Strong ion exchange occurred more slowly; lactate disappeared at a rate of 2 mmol/kg w.w./min. Immediately after exercise the intracellular-femoral venous [lactate] gradient was 40 mmol/l and favoured diffusion of lactate into the circulation. Approximately 55-60% of the lactate diffused from the muscle, the remaining lactate was oxidised or converted to glycogen. Lactate was taken up by the inactive forearm muscle; the v-a [lactate] difference was approximately 4.5 mmol/l. Only about 45% of the lactate taken up by the inactive tissue was oxidised, the remaining lactate was metabolised to other metabolic end points. Lactate uptake by inactive tissue reduced the anion concentration of the body and increased the strong ion difference across the inactive tissue. Recovery of acid-base balance is not complete until all the lactate has been removed from the body.

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1. THE ACIDOSIS OF EXERCISE: THE DEVELOPMENT OF PRESENT DAY CONCEPTS

The work described in this thesis examined the effects of heavy exercise of 30 seconds duration on whole body acid-base homeostasis. As will be seen, even exercise of this short a duration presents an extreme acid load that has to be dealt with adequately if serious impairment of metabolic and contractile processes is to be avoided. Thus the studies to be described examine the size of this load, the body's adaptive responses and the resultant changes in hydrogen ion concentration, all topics that have been researched extensively throughout the last century.

1.1 DISCOVERY AND ORIGIN OF LACTIC ACID

Lactic acid was known to be a constituent of muscle since the early 1800's when Berzelius first observed acid formation in the muscles of hunted stags. Berzelius (1848), referring to this work published in 1807, described the acid as being identical to that found in milk (Needham, 1971). Lehmann (1850) referred to the early work of Berzelius and stated "Berzelius recognized the existence of free lactic acid in muscular fluid...Berzelius thought that he had convinced himself that the amount of free lactic acid in a muscle is

proportional to the extent to which it has been previously exercised" (Needham, 1971). Du Bois Reymond (1859) reported that fresh resting muscle had a neutral or slightly alkaline reaction which changed to an acid reaction on activity or death (Needham, 1971). Ranke (1865) observed that excised muscle reached a constant maximal acid content. However, the maximal acid content of the muscle was reduced if the muscles were first tetanised in the living animal, demonstrating that the acid-forming substance was used during activity (Needham, 1971).

Ranke also observed that lactic acid injected into frogs brought on fatigue (Needham, 1971). The importance placed on lactic acid formation towards the development of fatigue by early researchers can be appreciated in the following statement: "It is the accumulation of products of change and not the exhaustion of supplies of oxidisable material which leads to fatigue and ultimately to death in rigor...the prime if not the sole cause of fatigue, and no less, of death in rigor, is the accumulation of lactic acid. Both phenomena are due to the effect of this upon the colloid machinery of the muscle" (Hopkins, 1921). Thus early workers believed that lactic acid formation from carbohydrate or carbohydrate-like substances was the immediate energy source for muscle contraction and that excess accumulation of lactic acid without its removal was responsible for the conditions of fatigue and rigor mortis.

The origin of lactic acid was thought by Hermann (1874) to

arise from an "inogen molecule", a complex oxygen-containing molecule, localised in the muscle. Hermann described the function of "inogen" in the following way: "The simplest expression for the chemical processes during onset of rigor and the active state is therefore probably the following: The muscle contains at any moment a store of a complicated N-containing substance, dissolved in the muscle contents and plasma (which one can designate for sake of brevity the energy-generating or 'inogen' substance) which is capable of splitting with development of energy; the products of the splitting are, amongst others: CO_2 , sarcolactic acid, perhaps glycerophosphate and a gelatinous protein body separating out and later contracting firmly" (Needham, 1971).

Bernard (1859) described the presence of glycogen in muscle and later Nasse (1869) discussed the possibility that glycogen was the source of lactic acid (Needham, 1971). Nasse (1877) observed a lower glycogen content in muscle after rigor and assumed that it would also be reduced by activity. When Nasse compared carbohydrate loss with the lactic acid content of the muscle he found the loss of carbohydrate to be greater and concluded that the lactic acid was likely derived from glycogen (Needham, 1971). However, Parnas and Wagner (1914) found that during fatigue and rigor, the disappearance of glycogen was equivalent to the formation of lactic acid (Needham, 1971). Meyerhof (1920b) confirmed and extended these findings and showed that during recovery the glycogen content of the muscle

increased in proportion to the difference between the total amount of lactic acid disappearing and the amount calculated as being oxidised (Needham, 1971).

1.2 LACTIC ACID, HYPOXIA AND CARBON DIOXIDE EVOLUTION

1.2.1 The effect of oxygen on lactic acid and carbon dioxide production

Pasteur (1861) demonstrated the importance of oxygen in regulating metabolism when he discovered that less sugar was used by yeast in the presence of oxygen than under anaerobic conditions (Needham, 1971). Fletcher (1902) demonstrated that the carbon dioxide output of stimulated, isolated muscle was greater in an atmosphere of oxygen compared to that of nitrogen or air. In addition, the time to fatigue was prolonged and the fatigue process, once initiated, could be reversed when the muscle was placed in an atmosphere of oxygen. Fletcher (1902) believed that the "chemical processes of muscular activity, like the survival processes of resting muscle, do not reach their natural end in the production of CO_2 without an adequate oxygen supply. With or without oxygen available at the moment, the excised muscle gives rise slowly if at rest, rapidly during activity, to bodies believed to be precursors of CO_2, and known to be poisonous, whose action is marked by the onset of fatigue and hastening of rigor mortis. It is reasonable to suppose that the beneficial action of

oxygen, in delaying both fatigue and rigor mortis, is a sign of the greater completeness with which in its presence the metabolic products of muscle are expressed in the form of liberated CO_2." Fletcher believed that in the presence of oxygen, lactic acid was oxidised to carbon dioxide, thereby preventing its accumulation and delaying the onset of fatigue.

Fletcher and Hopkins (1906-07) demonstrated that the lactic acid content of resting, excised muscle was less than 0.02 gm% (2 mmol/l) and increased to approximately 0.22 gm% (24 mmol/l) following stimulation to fatigue. They demonstrated that the lactic acid content of fatigued muscles was reduced during recovery and that the removal of lactic acid was faster in an atmosphere of oxygen than in nitrogen or air.

1.2.2 The effect of exercise intensity on lactic acid and carbon dioxide production

Fletcher (1913-14) showed that the carbon dioxide output from isolated muscle was related to the production of lactic acid, believing it to be "preformed CO_2 displaced from loose combination by the acid and escaping by diffusion from the muscle." Fletcher determined that the acid yield in the muscle was large enough to account for the total yield of carbon dioxide after excision if it was assumed that one molecule of acid displaced one molecule of carbon

dioxide "from combination within muscle substance."

Hill (1912) examined the heat production of isolated muscle and compared his results with those obtained by Fletcher and Hopkins (1906-07). Hill noted a similarity between the carbon dioxide output, lactic acid production and heat liberated by the muscle and was convinced that the three processes represented different aspects of the same reaction. According to Hill, muscle contraction was associated with the formation of lactic acid "from some precursor" with evolution of heat. As the acid was formed it combined with the sodium bicarbonate ($NaHCO_3$) of the tissues to form sodium lactate (NaLa) and carbon dioxide. Carbon dioxide was liberated at a rate proportional to the production of lactic acid. Hill's view was summarized according to the following reactions:

$$AHLa \longrightarrow A + HLa + Heat$$

$$HLa + NaHCO_3 \longrightarrow NaLa + water + carbon\ dioxide$$

where A was the unknown chemical precursor and HLa was lactic acid.

The oxygen utilised by the isolated muscle during recovery from stimulation is in excess of that utilised by resting, nonstimulated, nonfatigued muscle. The excess oxygen was believed to be used for the oxidation of a portion of the lactic acid produced during stimulation, thus providing the energy necessary to convert the

remaining lactic acid to glycogen (Meyerhof, 1920b). The efficiency of this process (ie. the total lactic acid removed/portion of lactic acid oxidised) in isolated muscle was between 4:1 and 6:1 (Meyerhof, 1920a; Meyerhof, 1920b; Hartree and Hill, 1923). Hill and Lupton (1923) measured the excess recovery oxygen (O_2 debt) in humans and were able to estimate the total amount of lactic acid produced during the exercise period by assuming an efficiency of 6:1 (ie. approximately 8.1 gm lactic acid/l O_2 debt). They observed that the O_2 debt and the estimated lactic acid production was related to the severity of the exercise. The fatigue associated with heavy exercise was attributed to an imbalance between lactic acid production and removal, more specifically to the increase in intramuscular hydrogen ion concentration associated with an excess lactic acid production.

Hill and Lupton (1923) also observed that following light to moderate exercise the respiratory exchange ratio (carbon dioxide output/oxygen intake) remained between 0.8 and 1.0. After heavy exercise the respiratory exchange ratio increased to values greater than 2.0 indicating that the carbon dioxide output was in excess of that predicted for the oxidation of lactic acid. The authors speculated that during moderate exercise the majority of the lactic acid liberated in the muscle did not combine with bicarbonate but was buffered by the protein and phosphates of muscle, a process which does not liberate carbon dioxide. However, in heavy exercise "only when the supply of suitable protein buffer has run out, and when the H^+

concentration inside the muscle has risen far enough, may we suppose the lactic acid to attack the bicarbonate, and so to drive off CO_2" (Hill and Lupton, 1923). Hill, Long and Lupton (1924) emphasized that "lactic acid in muscle does not, to any serious extent, directly turn out carbon dioxide from bicarbonate. It combines with sodium-protein and raises the hydrogen ion concentration: the elimination of carbon dioxide which results is the consequence of the induced activity of the respiratory system." According to this view it was not the combination of excess hydrogen ions with bicarbonate that elevated the carbon dioxide output but the stimulating effect of hydrogen ions on the respiratory centre that increased ventilation. The excess carbon dioxide output was presumably due to a mass action effect between carbon dioxide and bicarbonate.

Early studies by Hill, Long and Lupton (1924), Barr and Himwich (1923a; 1923b), Barr, Himwich and Green (1923), Owles (1930), Margaria, Edwards and Dill (1933) and Bang (1936) described the relationship between the blood lactic acid concentration and the intensity of the exercise. The work of Owles (1930) and Margaria et al. (1933) suggested that there was some critical level of energy output and exercising above this critical level led to increasing levels of lactic acid appearing in the blood. The stimulus for acid formation was thought to be an inadequate supply of oxygen in the working tissues. Bang (1936) demonstrated that for moderately severe exercise the appearance of lactic acid in the blood was confined to

those periods when the muscles were contracting anaerobically such as at the onset of exercise and during very severe exercise.

Much of this early work examined lactic acid levels in isolated animal muscle and in the blood of exercising humans. It was not until the introduction of the needle biopsy technique (Bergström, 1962) that acid-base balance and lactate metabolism could be studied in tissue of exercising humans. Karlsson and Saltin (1970) showed that constant-load exercise to exhaustion was associated with a steady increase in both blood and muscle lactate concentration to some peak value. The authors suggested that the high muscle lactate levels (16 mmol/kg w.w.) could be a limiting factor to performance. Karlsson, Diamant and Saltin (1971) found that during exercise of progressively increasing intensity, little lactate appeared in the muscles or blood until the intensity reached approximately 50-60% of the individual's maximal aerobic power, after which there was a rapid accumulation of both muscle and blood lactate until the individual was unable to continue.

Wasserman and colleagues (1973) attempted to account for the apparent simultaneous rise in blood lactate concentration and carbon dioxide output. They proposed the term "anaerobic threshold" to describe a critical oxygen intake above which further exercise was associated with increasing reliance on anaerobic energy metabolism. According to Wasserman, exercise at intensities below 50-60% of the

maximal aerobic capacity was accomplished with no lactic acid formation because the oxygen tension within the tissues was high enough to meet the oxygen requirements for aerobic metabolism. However, lactic acid was formed at exercise intensities above this critical level because the oxygen tension was reduced to levels too low to support aerobic metabolism. The blood lactate concentration increased as lactic acid was removed from the exercising muscle. The rise in plasma lactic acid concentration caused an equimolar fall in the plasma bicarbonate concentration. Carbon dioxide output at the lungs increased with increased formation of carbonic acid.

Jöbsis and Stainsby (1968) examined the NADH concentration to determine the mitochondrial redox state during exercise. They demonstrated that at exercise intensities associated with lactate production the NADH concentration was reduced indicating that the oxygen tension in the mitochondrion was high enough to support electron transport and NADH oxidation. Connett, Gayeski and Honig (1984) observed that lactate accumulated in fully aerobic, working dog hindlimb muscle. They concluded that lactic acid production was due to factors other than oxygen limitation of mitochondrial ATP production.

1.3 LACTIC ACID PRODUCTION AND PLASMA ACID-BASE BALANCE

1.3.1 Lactate and carbon dioxide-combining capacity

Early work in acid-base regulation during exercise in humans examined changes in carbon dioxide-combining capacity and "reaction" of the blood (ie. pH) with changes in the blood lactic acid concentration. The carbon dioxide-combining power is a measure of the metabolic, nonrespiratory component of the blood acid-base status and is measured as the bicarbonate concentration of separated plasma after equilibration to a PCO_2 of 40 mm Hg. Barr, Himwich and Green (1923) studied acid-base equilibrium in arterial and venous blood at rest and at 1 or 3 minutes recovery from exercise. They observed that mild exercise could be completed with minimal changes in arterial and venous carbon dioxide-combining capacity, PCO_2 and pH, but these variables all decreased with increasing severity of the exercise. The fall in carbon dioxide-combining capacity was always accompanied by an increase in the blood lactate concentration. Owles (1930) and Laug (1934) observed that the fall in carbon dioxide-combining capacity or bicarbonate concentration was inversely related to the increase in the blood lactate concentration and observed that these changes occurred in approximately equimolar amounts. This relationship would be expected if lactate and hydrogen ions were released from muscle in equimolar amounts and if the hydrogen ions were not buffered by other noncarbonic buffers. Laug (1934) demonstrated that the changes in the

concentrations of lactate and bicarbonate were not as closely related in situations where the venous PCO_2 increased, presumably because the bicarbonate concentration increased as the PCO_2 increased.

Barr and Himwich (1923b) examined the time course of acid-base changes during exercise and recovery and showed that the greatest changes in carbon dioxide-combining capacity and pH did not occur during exercise but occurred within the first few minutes of recovery. These changes were followed by a slow return to resting levels. The change in carbon dioxide-combining capacity and pH were related to lactate release from the previously active muscle. The authors were aware that the level of lactate in the blood was a balance between lactate output from the muscle and lactate removal from the blood; only when lactate output from working tissue was less than lactate removal from the blood did the carbon dioxide-combining capacity and pH begin to return slowly to control conditions.

Hill, Long and Lupton (1924) measured the lactic acid concentration of the blood at various times during recovery from moderate or heavy exercise. Lactate appeared in the blood soon after short bouts of heavy exercise indicating that the muscle and plasma capillary membranes were permeable to lactic acid. However, the peak blood lactate concentration was not observed until some minutes later. The authors believed that this delay was due to the fact that the lactate concentration in the muscle was higher than that found in the

blood immediately after exercise. They were aware that at the end of exercise the content of lactate within the muscle was reduced by diffusion into the blood and by "local oxidative recovery" (ie. restoration of lactic acid to glycogen) and believed that an equilibrium was eventually reached between the muscle and plasma. It was not known whether oxidative removal of lactate could occur in tissues other than those responsible for the initial breakdown but the authors attributed the slowness of recovery following severe exercise to diffusion of lactic acid from other tissues to the "localities where it can be dealt with by the recovery mechanism", suggesting that inactive tissue could not take part in the recovery process.

Barr and Himwich (1923a) observed that if heavy exercise was performed with the arms the lactate concentration was higher and the carbon dioxide-combining capacity was lower in the forearm vein than in the artery. However, after heavy leg exercise the lactate concentration in the forearm vein was lower and the carbon dioxide-combining capacity was higher than that found in the artery. The higher carbon dioxide-combining capacity of the inactive forearm vein was attributed to removal of lactic acid by the inactive tissue.

Eggleton and Evans (1930) examined the concentration of lactate in arterial and venous blood and in rested and electrically stimulated hindlimb muscles of dogs and cats. They demonstrated that after maximal stimulation lactate was not in equilibrium throughout

the body. In the first 12 minutes of recovery the lactate concentration was higher in the muscle than in arterial blood, but later in recovery this gradient was reversed. The lactate concentration gradients favoured the diffusion of lactate from the previously active muscle into the blood and from the blood into inactive muscle. Blood returning from the liver contained lower lactate levels than arterial blood indicating that the liver was also a source for lactate removal.

1.3.2 Lactate and hydrogen ion release from muscle

Much of the information regarding the acidosis of heavy exercise was gained by measuring the lactic acid content and the carbon dioxide-combining power of blood. The early workers found that the carbon dioxide-combining power decreased as the blood lactic acid concentration increased (Barr, Himwich and Green, 1923; Owles, 1930; Laug, 1934). More recently, interest has focussed on whether the lactate concentration accurately reflects the acid output by the muscle. Bouhuys and coworkers (1966) observed that during exercise the base deficit overestimated the acid output by the muscle as measured by the blood lactate concentration. The base deficit is a measure of the non-CO_2 acid production and is calculated from whole blood equilibrated to a PCO_2 of 40 mm Hg and a pH of 7.4 (Siggaard-Andersen, 1963). These authors attributed the discrepancy between the two measures to a difference between the in vitro and in vivo

equilibration curve for carbon dioxide (ie. the change in base deficit would be greater in vitro than in vivo).

Osnes and Hermansen (1971) demonstrated that following either continuous or intermittent exercise to exhaustion the increase in the blood lactate concentration was associated with an almost linear fall in plasma pH and plasma bicarbonate concentration. The increase in the plasma base deficit was greater than the increase in the plasma lactate concentration suggesting that more hydrogen ions appeared in the blood than lactate ions and/or that base was removed from the blood. Hermansen and Osnes (1971) observed that the pH of muscle homogenates decreased from 6.92 at rest to 6.41 immediately following maximal exercise to exhaustion and that the pH of arterialized capillary blood decreased from 7.42 at rest to 7.17. During the first few minutes of recovery the muscle pH began increasing whereas the plasma pH continued to decrease.

Sahlin and coworkers (1978a) demonstrated that during heavy exercise the arterial base deficit and lactate concentration increased in equimolar amounts indicating that hydrogen ions and lactate ions were simultaneously removed from the working muscle. Immediately after exercise the lactate concentration did not change but the base deficit continued to increase. The authors attributed this to a faster release of hydrogen ions compared to lactate ions in exhausted tissue. In support of this hypothesis, Sahlin and coworkers (1976)

demonstrated that by 20 minutes of recovery from maximal exercise the muscle pH had returned to resting levels but the muscle lactate concentration was still 20-30 mmol/kg d.w. higher than resting levels.

Benadé and Heisler (1978) measured the relative rate of efflux of hydrogen ions and lactate ions from stimulated muscle. They observed that hydrogen ion efflux was faster and exceeded lactate efflux by a factor of 14 and 50 in rat diaphragm and frog sartorius muscle, respectively.

Stainsby and coworkers (Barbee, Stainsby and Chirtel, 1983; Chirtel, Barbee and Stainsby, 1984) observed that the non-CO_2 acid output (determined by blood flow x arterial-venous base deficit difference) from an electrically stimulated canine hindlimb preparation exceeded the lactate output by 5 and 11 times during steady-state and non-steady-state, progressive exercise. They argued that in addition to lactic acid, other acids were released from muscle and that strong ions may have exchanged between the plasma and the muscle.

1.4 METABOLIC PRODUCTION OF HYDROGEN IONS IN MUSCLE DURING MAXIMAL EXERCISE

Maximal exercise is associated with an increase in the intramuscular hydrogen ion concentration. The increase in hydrogen

ion concentration occurs when hydrogen ions are produced in excess of their removal from the intracellular fluid. This section describes several reactions which are important to both energy and hydrogen ion production.

1.4.1 ATP hydrolysis

Adenosine triphosphate (ATP) is the immediate energy source for muscle contraction. Hydrolysis of ATP releases hydrogen ions according to the following reaction:

$$ATP \longrightarrow ADP + Pi + nH^{+} + energy$$

The stoichiometrical release of hydrogen ions depends upon the ionic state of ATP, ADP and Pi, and thus upon the concentration of magnesium, potassium and hydrogen ion complex-bound to the adenine nucleotides. The amount of hydrogen ions released per mole of ATP hydrolysed is 0.52 and 0.03 at pH 7.0 and 6.4, respectively (Hultman and Sahlin, 1980). The maximal observed increase in ADP is approximately 0.5 mmol/kg d.w., corresponding to a maximal release of 0.25 mmol H^{+}/kg d.w. (0.05 mmol/l muscle water) at pH 7.0 and 0.02 mmol H^{+}/kg d.w. (0.005 mmol/l muscle water) at pH 6.4. The release of hydrogen ions from ATP hydrolysis is small because ATP levels change very little during exercise.

1.4.2 Creatine phosphate hydrolysis

Creatine phosphate (CP) was described by Fiske and Subbarow (1929) as being an important component in muscle. Hydrolysis of CP was associated with the liberation of a relatively large amount of base that could function to neutralize any acid formed during muscular contraction. Lipmann and Meyerhof (1930) demonstrated that a greater amount of base was liberated when the pH of the reaction medium was reduced (Needham, 1971). They pointed out that intracellularly, a fall in pH would not only limit the formation of lactic acid but would also enhance the breakdown of CP. This pH effect would operate to maintain a neutral pH within the muscle.

Hydrolysis of CP is one of the mechanisms available for resynthesizing ATP and occurs according to the following reaction:

$$CP + ADP + nH^{+} \longrightarrow ATP + \text{creatine}$$

The concentration of ADP and ATP change very little during exercise, thus it is more appropriate to consider the net reaction:

$$CP + nH^{+} \longrightarrow \text{creatine} + Pi$$

$$pKa = 4.5 \qquad\qquad pKa = 6.8$$

The stoichiometric uptake of hydrogen ions is 0.38 mole per mole CP at

pH 7.0 and 0.70 mole per mole CP at pH 6.4. The maximal observed decrease in CP is 75 mmol/kg d.w., corresponding to an uptake of 29 mmol H^+/kg d.w. (6.7 mmol/l muscle water) and 52 mmol H^+/kg d.w. (12.0 mmol/l muscle water) at pH 7.0 and 6.4, respectively (Hultman and Sahlin, 1980). During heavy exercise, CP levels may decrease to 10-20% of resting levels (Karlsson and Saltin, 1970) and produce a brief alkaline pH shift within the muscle. CP is rapidly resynthesized in recovery (Harris et al., 1976), a process which liberates hydrogen ions (Hultman and Sahlin, 1980).

1.4.3 Lactic acid formation

During heavy exercise energy production is associated with the formation of lactic acid. Lactic acid (pKa 3.7) is fully dissociated at physiological pH values, thus the formation of lactate is associated with the production of hydrogen ions in equimolar amounts. Lactate values of 150 mmol/kg d.w. have been observed following heavy exercise and are associated with the production of 150 mmol H^+/kg d.w. (35 mmol/l muscle water) (Hultman and Sahlin, 1980). Lactic acid production is the major factor responsible for the increase in the hydrogen ion concentration during exercise. Sahlin et al. (1976) observed that the decrease in muscle pH following dynamic exercise was inversely related to the rise in muscle (lactate + pyruvate) concentration. Since the pyruvate concentration is normally very low, the change in pH is essentially related to the change in lactate

concentration.

1.4.4 Formation of hexose phosphates

The concentration of hexose phosphates in resting muscle is very low but during heavy exercise the concentration of glucose-6-P, fructose-6-P and fructose-1,6-bisP increases by as much as 35 fold (Jones et al., 1985). Associated with the increase in hexose phosphate concentration is the production of hydrogen ions according to the following reactions:

$$\underset{pKa = 6.8}{\text{glucose} + Pi} \longrightarrow \underset{pKa = 6.1}{\text{glucose-6-P}} + nH^+$$

$$\text{glucose-6-P} + nH^+ \longrightarrow \text{fructose-6-P} + nH^+$$

$$\underset{pKa = 6.11}{\text{fructose-6-P} + Pi} \longrightarrow \underset{pKa = 6.31}{\text{fructose-1,6-bisP}} + nH^+$$

The amount of hydrogen ions released by the hexose phosphates may be only 25% of that produced by lactic acid.

1.5 EFFECT OF AN INTRACELLULAR ACIDOSIS ON ENERGY METABOLISM AND MUSCLE FUNCTION

Failure to remove hydrogen ions from within the working muscle will eventually lead to an inability of the muscle to function efficiently. Hydrogen ions can affect performance by acting at a number of intracellular sites.

1.5.1 Enzyme activity

The immediate energy source for muscle contraction is ATP. The ATP content in resting muscle is very low, approximately 24 mmol/kg d.w., and during exercise, the ATP levels would be depleted in a matter of seconds if not for the fact that ATP is continually being resynthesized by CP breakdown, glycolysis and oxidative phosphorylation. Muscle tissue has a high glycolytic capacity and can change its glycolytic rate by about 100 times in a matter of seconds. With heavy exercise the ATP requirement is met by an increased rate of glycolysis, the associated accumulation of lactic acid will increase the intracellular hydrogen ion concentration. Hill (1955-56) reported that lactic acid production ceased at a pHi of approximately 6.3 indicating that excess hydrogen ion production has an inhibitory effect on energy production.

A change in hydrogen ion concentration can affect enzyme

activity in two ways. First, the enzyme molecule may be directly affected by a change in the charge of the ionizable groups of the enzyme, thereby altering the affinity of the enzyme for substrates and products or altering the native conformation of the protein molecule and influence catalytic activity. Second, a change in hydrogen ion concentration can change the ionic state of the substrate, product or inhibitors or activators of the enzyme, thereby indirectly influencing the catalytic activity.

Glycogen phosphorylase and phosphofructokinase (PFK), enzymes catalysing nonequilibrium reactions in the glycolytic pathway, are inhibited at low pH. Glycogen phosphorylase exists in two interconvertible forms; phosphorylase b, which is inhibited by ATP and glucose-6-phosphate, and phosphorylase a, which is less sensitive to these metabolites. Conversion of phosphorylase b to the more active "a" form is catalysed by phosphorylase b kinase, which is inhibited by low pH (Newsholme and Start, 1973).

Phosphofructokinase catalyses the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate. Trivedi and Danforth (1966) demonstrated that the activity of PFK in isolated frog and mouse muscle decreased rapidly as the pH of the incubation medium was lowered. The inhibiting effect of hydrogen ions could be due to accumulation of $HATP^{3-}$, a potent inhibitor of PFK (Lowry and Passonneau, 1966), or to a direct effect of hydrogen ions on the

protein molecule (Hultman and Sahlin, 1980).

Pyruvate undergoes oxidation to acetyl-CoA in the mitochondria. This reaction is catalysed by the enzyme complex pyruvate dehydrogenase (PDH). The PDH complex is subject to two types of regulation, product inhibition (acetyl-CoA and NADH) and by interconversion of an inactive to an active form. Ward and coworkers (1982) demonstrated that ratio of active/total PDH was greatest after aerobic exercise (88%), least after maximal isometric exercise (39%) and intermediate after intermittent supramaximal short-term exercise (60%). The authors speculated that the enzymes controlling the interconversion between the inactive and active forms of PDH may be pH sensitive.

1.5.2 Chemical equilibria

Besides having an effect on enzymatic reactions through a direct effect on the enzyme, hydrogen ions may also be consumed or produced in an equilibrium reaction. Acting in this way, an increase in intracellular hydrogen ion concentration can affect the position of the equilibrium and thus the concentration of substrates and products.

Related to this is the possibility that the increase in the hydrogen ion concentration could interfere with energy production by ATP hydrolysis. The energy liberated when one mole of ATP is

hydrolysed to ADP and Pi is dependent on the concentrations of ATP, ADP, Pi, free magnesium and hydrogen ions. During heavy exercise the concentrations of ATP, ADP, Pi and hydrogen ions change in such a way that the energy liberated by ATP hydrolysis is reduced; the energy liberated is approximately 54 kJ/mole ATP at rest and 50 kJ/mole ATP following heavy exercise (Sahlin, Palmskog and Hultman, 1978). This decrease in energy output may fall below the minimum energy requirement for cross-bridge formation between actin and myosin, thus preventing muscle contraction.

1.5.3 Effects on the contractile apparatus

Muscle contraction is initiated by the release of calcium from the sarcoplasmic reticulum into the cytosol. An increase in intracellular free calcium concentration to 10^{-7} - 10^{-6} M stimulates the myosin ATPase and causes splitting of ATP and cross-bridge formation. Portzehl, Zaoralek and Gaudin (1969) demonstrated that at a given free calcium concentration, a decrease in pH reduced the myosin ATPase activity of rabbit muscle fibres: At low pH values maximal ATPase activity could only be achieved by raising the free calcium concentration. Fuchs, Reddy and Briggs (1970) showed that a fall in pH was associated with a decrease in the apparent binding constant for calcium-troponin interaction suggesting that hydrogen ions interacted with the calcium-binding site on troponin. Nakamaru and Schwartz (1972) demonstrated that calcium-binding by the

sarcoplasmic reticulum was increased by a fall in pH. In skinned muscle fibres, a decrease in pH lowered the tension developed by the fibres and increased the calcium requirements to develop half-maximum tension (Donaldson and Hermansen, 1978).

1.6 DEFENSES AGAINST AN INTRACELLULAR ACIDOSIS

1.6.1 The steady-state distribution of hydrogen ions according to a Donnan equilibrium

It is now generally accepted that hydrogen ions are not in equilibrium across the cell membrane. However in the 1920's and early 1930's it was believed that small permeable ions, including hydrogen ions, were distributed across the muscle membrane according to a Donnan equilibrium (Donnan, 1924) and that regulation of intracellular pH (pHi) involved the redistribution of ions according to the relation: $[H^+]i/[H^+]o = [K^+]i/[K^+]o = \exp-(-Vm\ F/RT)$, where Vm is the membrane potential, F is the Faraday constant, R is the gas constant, and T is the absolute temperature. According to the Donnan theory of ionic equilibrium, the pHi should be approximately 5.9 if the membrane potential is -90 mV and the extracellular pH (pHo) is 7.4. Netter (1928) estimated the pH of the "pressed juice" of frog muscle and found it to be higher than the value predicted from the potassium ratio (Roos and Boron, 1981). Netter did not question the validity of applying Donnan's theory to the distribution of hydrogen ions but

attributed the discrepancy to either compartmentalization or to loss of carbon dioxide. Mond and Netter (1930) observed that potassium efflux from frog muscle was not reduced following repetitive stimulation even though pHi was assumed to fall (Roos and Boron, 1981). A fall in pHi would be expected to lower the extracellular potassium concentration and/or raise the intracellular potassium concentration thereby maintaining the equality of hydrogen ion and potassium distributions as required by the Donnan equilibrium. Since there was no evidence that the potassium concentration changed the authors postulated that the pHo fell in parallel with pHi.

1.6.2 The steady-state distribution of hydrogen ions according to an energy-requiring process

Fenn and Cobb (1934) were the first to demonstrate that hydrogen ions were not in equilibrium across the muscle membrane. They showed that frog skeletal muscle incubated at pHo 7.0 (equilibrated with 5% CO_2) had a pHi of 7.0, much higher than a pHi of 5.6 predicted from the potassium distribution. They concluded that potassium was distributed according to a Donnan equilibrium but that there was "some independent mechanism within the muscle which regulates the pH to approximate neutrality in spite of the demands of the membrane equilibrium." They also stated that "some continuous supply of energy would obviously be necessary for this purpose." Fenn and Maurer (1935) obtained an in vivo pHi of 6.9 after correcting for

the extracellular (chloride) space and assuming a pHo of 7.34. These authors believed that phosphocreatine and lactic acid mechanisms controlled the pHi more or less independently of the membrane equilibrium, at some continuous expenditure of energy, and kept the pHi on the alkaline side of the equilibrium value in spite of the physicochemical forces tending to make it more acid.

Hill (1955-56) observed that isolated frog muscle stimulated anaerobically to exhaustion was still able to produce lactic acid even when incubated at pH 4.5; the pHi would have to be 3.3 or less if governed by a Donnan equilibrium. Since lactic acid production ceases at approximately pH 6.3, Hill argued that the pHi of the muscle must still be above 6.3. Hill reviewed other evidence arguing against the passive distribution of hydrogen ions across the muscle membrane. He concluded that the muscle cell regulated its internal hydrogen ion concentration against diffusion and electrical potential gradients by actively removing hydrogen ions, analogous to the sodium pump.

Caldwell (1958) presented direct evidence that hydrogen ions were not passively distributed across the cell membrane. Using microelectrodes implanted inside muscle fibres of the crab and squid giant axons, Caldwell demonstrated that the pHi was normally near 7.0 and not related to the pHo in a manner predicted by a Donnan equilibrium. When fibres were incubated in solutions of varying pH, the internal pH approached but in most cases did not reach the

theoretical pH calculated from the Donnan equilibrium. In those cases where the pHi approached the theoretical values the fibres showed signs of serious deterioration. Caldwell concluded that "in vivo the pH of the fibres may be maintained at a value higher than that required by the Donnan theory by some process which leads to the extrusion of hydrogen ions from them at the expense of metabolic energy."

Compensatory processes appear to function within the resting, nonstimulated muscle to maintain the intracellular hydrogen ion concentration at levels lower than would exist if they were distributed according to a Donnan equilibrium. Diffusion of hydrogen ions into the cell would be expected based on its electrochemical gradient, the continuous removal of hydrogen ions from the intracellular space has to occur at the expense of some continual supply of metabolic energy. The maintenance of intracellular acid-base homeostasis is dependent on a system which not only neutralizes the passive transfer of hydrogen ions into or out of the cell, as would occur during resting conditions, but it must also regulate large changes in hydrogen ions occurring when hydrogen ions are produced or consumed through metabolism or through fluxes of weak acids and bases, as would occur during exercise. Siesjö and Messeter (1971) stated that the factors determining the hydrogen ion concentration in living tissue during acid-base changes may be divided into physicochemical buffering, consumption or production of non-CO_2

acids and transmembrane fluxes of hydrogen ions or bicarbonate ions.

1.7 PHYSICOCHEMICAL BUFFERING

1.7.1 Properties of weak acids and bases

When acid is added to the interior of a muscle fibre the immediate change in pH will be determined by the internal buffering power or physicochemical buffering of the cell. Physicochemical buffering is a property of weak acids and bases whereby these compounds minimize shifts in pH by reacting with exogenous hydrogen ions according to the reaction: $M^n + H^+ \rightleftharpoons HM^{n+1}$, where M^n is a weak base of valence n and HM^{n+1} is a weak acid of valence n+1. The resistance to a pH change depends on the concentrations of the buffers and their pK values. Henderson (1908) and Washburn (1908) were the first to observe that maximum buffering by a weak acid occurs when its dissociation constant equals the hydrogen ion concentration (ie. pKa = pH and $[M^n] = [HM^{n+1}]$); both authors limited their analysis to the neutral range of pH values around pH 7. Koppel and Spiro (1914) extended this observation to a wide range of pH values and in doing so they introduced the first general measure of "buffer action" (P):

$$P = d(S - So)/dpH$$

where dS is the amount of strong acid required to produce a small pH change (dpH) in the buffered solution, and dSo is the amount required to produce the same pH change in an unbuffered solution of the same pH. The authors demonstrated that for a monobasic weak acid ($HA \rightleftharpoons H^+ + A^-$) of apparent dissociation constant Ka:

$$P = (-2.3 \times Ka \times [H^+])/(Ka + [H^+])^2 \times [TA]$$

where the total concentration of acid $[TA] = [HA] + [A^-]$. Koppel and Spiro (1914) were the first to derive the conditions for maximal buffering by demonstrating that by setting $dP/dpH = 0$, P achieved its maximal value of -0.58 [TA] when $[H^+] = Ka$.

Michaelis (1922) modified the definition of buffering power (B') such that:

$$B' = dB/dpH$$

where dB is the amount of strong base added (Roos and Boron, 1981). A similar definition was developed by Van Slyke (1922). Within the pH range 3 to 11, Van Slyke's buffer value, B', is almost numerically identical to Koppel and Spiro's buffer value, P, except for being its negative value. This definition of buffering power is now generally accepted and the unit of buffer power (mmol/l/pH unit) is termed the "slyke" (Woodbury, 1974).

The reaction defining the buffer action "P" is valid only when the buffer concentration (ie. [TA]) is constant but not when one of the buffer partners can exchange with the surroundings. Van Slyke (1922) showed that in a closed system, where the carbon dioxide buffer concentration ($[CO_2] + [HCO_3^-]$) is constant, the buffer value of the CO_2 $-HCO_3^-$ pair, $B'CO_2$, is very small (less than 3 mmol/l/pH) and contributes only about 10% to the total buffering of the blood in a closed system (Roos and Boron, 1981). However in an open system when carbon dioxide rather than ($[CO_2] + [HCO_3^-]$) is constant the contribution of the CO_2-HCO_3^- pair to overall buffering is substantial. In blood, $B'CO_2$ is more than twice as great as the buffering provided by all other buffers combined. In an open system with PCO_2 constant, $B'CO_2$ is given by:

$$B'CO_2 = (d'[HCO_3^-]/d'pH)$$
$$= 2.3 \times s \times PCO_2 \times 10^{pH-pK}$$
$$= 2.3 \times [HCO_3^-]$$

where s is the solubility of carbon dioxide.

1.7.2 Physicochemical buffering in muscle

The muscle membrane is highly permeable to carbon dioxide so that the cell interior can be considered an open system with respect

to carbon dioxide. The total buffering power (B't) of the intracellular fluid is given by $B't = B'CO_2 + B'i$, where B'i is the intrinsic or non-CO_2 buffering power. Generally total buffering power is measured and if carbon dioxide is constant, the buffering power due to the CO_2 -HCO_3^- pair can be calculated. Hultman and Sahlin (1980) have calculated that the total amount of hydrogen ions taken up by physicochemical buffering during exhaustive cycle ergometer exercise (pHi change 7.0 to 6.6) is approximately 24-25 mmol/l muscle water, the main buffers being phosphates, bicarbonate and proteins.

Phosphate compounds include ATP, ADP, AMP, Pi, glyceraldehyde-P, dihydroxyacetone-P, glycerol-1-P, fructose-1,6-bisP, glucose-1-P, glucose-6-P, fructose-6-P and CP. All these phosphate compounds, except for CP, have pKa values in the range 6.1-7.0 but for most, their concentration in resting muscle is either too low or they are complex-bound to magnesium or proteins and have limited effectiveness as buffers. The concentration of inorganic phosphate in resting muscle is approximately 12 mmol/l (39 mmol/kg d.w.). The stoichiometrical uptake of hydrogen ions when the pH is reduced from 7.0 to 6.6 is 0.27 mole per mole of Pi, resulting in a total hydrogen ion uptake of 3.2 mmol/l muscle water (Hultman and Sahlin, 1980).

The concentration of hexose-P is very low in resting muscle but increases with exercise. Formation of these compounds during exercise will release hydrogen ions (Hultman and Sahlin, 1980). The

concentration of CP in resting muscle is high (76 mmol/kg d.w.) but its contribution to buffering is limited because of its low pKa value (4.5) (Hultman and Sahlin, 1980).

The concentration of bicarbonate in resting muscle is 10 mmol/l and falls to 3 mmol/l following heavy exercise (Sahlin et al., 1978). This decrease in bicarbonate is associated with an equivalent uptake of hydrogen ions.

The buffering power of free amino acids within the cell is low because of the pKa of the carboxyl and amino groups is less than 3 and greater than 9, respectively. In amino acids, peptides and proteins the buffering power is dependent on the acid-base behaviour of the R-group. Only histidine has a pKa value of the R-group (pKa 6.0) which falls within the physiological range. However, the concentration of histidine in muscle is low (0.38 mmol/l) and contributes little to intracellular buffering (Hultman and Sahlin, 1980). When amino acids are incorporated into peptides and proteins the pKa of the R-group changes. The pKa of the imidazole group of histidine increases from 6.0 to 6.8 when incorporated into the dipeptide carnosine. The carnosine content of human quadriceps femoris is 5.5 mmol/l; the stoichiometrical uptake of hydrogen ions when the pH falls from 7.0 to 6.6 is 0.27 mole per mole of carnosine, resulting in a total uptake of 1.5 mmol H^+/l muscle water (Hultman and Sahlin, 1980). The protein content in muscle is approximately 200

g/kg w.w.. With a histidine concentration of human muscle protein of 46 mmol/l (pKa 6.8), 12.5 mmol H^+/l muscle water will be taken up with a pH change from 7.0 to 6.6 (Hultman and Sahlin, 1980).

1.7.3 Buffer values of muscle

Heisler and Piiper (1972) determined that the total buffer value for rat diaphragm was 68 slykes. Larsen and Burnell (1978) reported a value of 66 slykes in dog skeletal muscle. Buffer values ranging from 40-70 slykes have been reported for leg muscle of various species (Roos and Boron, 1981). Sahlin, Harris and Hultman (1975) determined the buffer value in human quadriceps muscle to be 57 slykes during isometric leg exercise to fatigue. Similar values were obtained in trained (58 slykes) and untrained individuals (50 slykes) following isometric leg exercise to fatigue (Sahlin and Henriksson, 1984). During dynamic cycling exercise, the buffer value of the quadriceps muscle was 73 slykes (Sahlin et al., 1976). The difference was attributed to the different conditions existing in the muscle during isometric and dynamic exercise; in the former, the muscle is considered a closed system and the CO_2 -HCO_3^- buffer system has a minimal effect on intracellular buffering.

1.7.4 Buffer values of blood

The blood, like muscle, contains a number of weak acids and

bases which limit pH changes when acid or alkali are added to it; these include bicarbonate, hemoglobin and plasma proteins. Plasma proteins contribute only about one-sixth of the total buffer value of the blood. The buffer value of separated plasma, containing only plasma proteins, is approximately 3.9 slykes (Woodbury, 1974). The buffer value of hemoglobin in blood is 22.7 slykes (Woodbury, 1974). The buffer value of whole blood determined in vitro, separated and titrated with carbon dioxide, is approximately 26.6 slykes; that is, the buffer value of whole blood is equal to the sum of the buffer values of plasma proteins and hemoglobin. The buffer value of whole blood determined by titration with carbon dioxide without separation is approximately 31 slykes; the higher value is because of the negative transmembrane potential of red blood cells causing the bicarbonate concentration in the plasma to be greater than that of the red blood cells.

1.8 ACID EXTRUSION

Long-term, steady-state regulation of intracellular pH requires that the accumulation of hydrogen ions within the cell be balanced by the active removal of hydrogen ions (ie. acid extrusion). While active removal of hydrogen ions from the cell interior had been proposed for some time (Fenn and Cobb, 1934; Hill 1955-56), it was not until the early 1970's that hydrogen ion removal from the intracellular space was demonstrated. The mechanisms for acid

extrusion have been examined by acid loading the cell interior and monitoring the recovery of the intracellular pH. The methods of acid loading have been reviewed by Roos and Boron (1981) and Thomas (1984) and include exposure to weak acids, intracellular injection of acid, transient exposure to ammonium, intracellular dialysis or perfusion and reduction of external pH.

Messeter and Siesjö (1971) observed that when rat brain was exposed to carbon dioxide the pHi fell from pH 7.06 to approximately 6.93 after 15 min. After continued exposure to carbon dioxide the pHi gradually recovered, reaching 7.03 after 3 hr. The authors concluded that the recovery of pHi was due to active transport of hydrogen ions or bicarbonate across the cell membrane. Subsequent work examining the nature of the hydrogen ion extrusion have demonstrated at least three separate mechanisms for regulating pHi: a sodium-dependent, chloride/bicarbonate exchange; a sodium-independent, chloride/bicarbonate exchange; and a sodium/hydrogen exchange. These three mechanisms will be reviewed separately.

1.8.1 Sodium-dependent, chloride/bicarbonate exchange

Requirement for external bicarbonate : Thomas (1977) demonstrated in snail neurones that recovery of pHi during exposure to carbon dioxide was related to the bicarbonate concentration in the incubation medium; a fall in pHi of 0.25 units below control (at

external pH 7.5) was associated with acid extrusion rates of 0.2 mmol/l/min at 0 mmol HCO_3^-/l, 1.3 mmol/l/min at 4.5 mmol HCO_3^-/l and 2.3 mmol/l/min at 21 mmol HCO_3^- /l. A similar dependence on external bicarbonate was reported for squid giant axons (Boron and De Weer, 1976; Boron and Russell, 1983), crayfish neurones (Moody, 1981) and barnacle muscle (Boron, McCormick and Roos, 1981). Boron and coworkers (1981) demonstrated in barnacle muscle that the dependence on external bicarbonate followed simple Michaelis-Menton kinetics. In these studies, movement of strong ions were not carefully controlled.

Requirement for internal chloride : The dependence of acid extrusion on internal chloride was first examined in squid giant axons (Russell and Boron, 1976). Recovery of pHi was observed only in the presence of intracellular chloride and external bicarbonate. A unidirectional efflux of Cl-36 occurred from the axon following an acid load, but only when bicarbonate was present in the bathing medium. In squid axons, acid extrusion was related to the intracellular chloride concentration by Michaelis-Menton kinetics (Boron and Russell, 1983). Thomas (1977) used indwelling Cl-selective microelectrodes and demonstrated in snail neurones that recovery of pHi from an acid load was accompanied by a decrease in the intracellular chloride concentration; reducing the intracellular chloride concentration to low levels (2 mmol/l) almost eliminated the recovery of pHi. Moody (1981) also used Cl-sensitive microelectrodes in crayfish neurones and observed a "small but reproducable" fall in

the intracellular chloride concentration as the pHi recovered from an acid load. Boron and coworkers (Boron et al., 1978; Russell, Boron and Brodwick, 1983) also demonstrated a requirement for internal chloride in barnacle muscle.

Requirement for external sodium : Thomas (1977) observed that recovery of pHi in snail neurones was inhibited when external sodium was removed from the incubation medium. Recovery of pHi during a CO_2 -induced acid load was accompanied by a transient rise in the intracellular sodium concentration (as determined by indwelling Na-selective microelectrodes). Thomas proved that the rise in the intracellular sodium concentration was not related to inhibition of the sodium/potassium pump; inhibition of the sodium/potassium pump caused the intracellular sodium concentration to increase but an additional influx of sodium occurred as the pHi recovered from an acid load. In barnacle muscle, a unidirectional Na-22 influx occurred simultaneously with pHi recovery (Russell et al., 1983). The rate of acid extrusion was related to the external sodium concentration by simple Michaelis-Menton kinetics (Boron et al., 1981). Sodium-dependent acid extrusion has also been observed in squid giant axon (Boron and Russell, 1983) and crayfish neurones (Moody, 1981).

Inhibition by stilbene derivatives : The disulfonic stilbene derivatives 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS)

are known to block anion exchange in erythrocytes. In barnacle muscle, addition of SITS to the incubation medium blocked pHi recovery (Boron et al., 1978), Cl-36 efflux (Boron et al., 1978), and Na-22 influx (Russell et al., 1983). Similar effects were observed in squid giant axons (Russell and Boron, 1976; Boron and Russell, 1983). Thomas (1977) demonstrated that addition of SITS or removal of external sodium inhibited pHi recovery in snail neurones to a similar extent suggesting that both treatments were inhibiting the same process.

Dependence on internal and external pH : Thomas (1976) observed that the pHi of snail neurones recovered "roughly exponentially" after exposure to carbon dioxide. Boron and coworkers (Boron, McCormick and Roos, 1979; Boron et al., 1981) demonstrated that the acid extrusion was greatest at low values of pHi and declined to zero as pHi approached control levels (pHi 7.4). The rate of acid extrusion increased as the pHo was raised either by increasing the external bicarbonate concentration (at constant PCO_2) or by decreasing the PCO_2 (at a constant external bicarbonate concentration). The pH also has an effect in squid axons (Boron and De Weer, 1976) and snail neurones (Thomas, 1977).

Thus regulation of pHi by means of a sodium-dependent, chloride/bicarbonate exchange requires the presence of extracellular sodium and bicarbonate, and intracellular chloride. Acid extrusion is

activated by a fall in the pHi and widening of the pHi-pHo gradient increases the rate of acid extrusion. Blocking chloride/bicarbonate exchange prevents pHi recovery.

1.8.2 Sodium-independent, chloride/bicarbonate exchange

Aickin and Thomas (1977) demonstrated acid extrusion in mouse soleus muscle fibres. Reducing the external sodium concentration slowed but did not prevent the recovery of pHi. Addition of amiloride, a sodium/hydrogen exchange blocker, also reduced the rate of pHi recovery but did not completely inhibit the recovery process. Thus this acid extrusion mechanism does not have an absolute requirement for external sodium. Addition of SITS or removal of bicarbonate from the incubation medium also slowed pHi recovery (Aickin and Thomas,1977) indicating that external bicarbonate and possibly intracellular chloride were required in the recovery process. However the requirement for chloride was not rigorously investigated.

Thus mouse soleus muscle fibres possess a pHi regulating system which exchanges extracellular bicarbonate for intracellular chloride, but does not have an absolute requirement for external sodium. This mechanism contributes approximately 20% to the pHi regulating system in this particular system.

1.8.3 Sodium/hydrogen exchange

Aickin and Thomas (1977), demonstrated that although the acid extrusion mechanism in mouse soleus fibres does not have an absolute requirement for external sodium, reducing the external sodium concentration or adding amiloride to the incubation medium partially inhibits the recovery process. In mouse soleus muscle fibres sodium/hydrogen exchange accounts for approximately 80% of the pHi recovery. Addition of SITS and amiloride to the incubation medium completely abolished recovery of pHi following an acid load. Moody (1981) demonstrated that when crayfish neurones were acid loaded in a HCO_3^- -free incubation medium, pHi recovery was slowed. Addition of SITS to a HCO_3^- -containing medium slowed the rate of pHi recovery to that observed in the HCO_3^- -free medium. A sodium/hydrogen exchange process was also demonstrated in frog semitendinosis muscle (Abercrombie, Putman and Roos,1983; Abercrombie and Roos,1983).

Thus in some preparations a sodium/hydrogen exchange mechanism appears to regulate intracellular pHi following introduction of an acid load. The exchanger requires external sodium and is inhibited by amiloride.

1.9 STEWART'S RE-EXAMINATION OF THE FACTORS INFLUENCING ACID-BASE CONTROL

Regulation of intracellular acid-base balance requires that the hydrogen ions produced during metabolism be buffered within the cell or rapidly removed from the cell interior to prevent excessive increases in the hydrogen ion concentration. Evidence has been presented which suggests that following a rise in intracellular hydrogen ion concentration the hydrogen ions are removed from the cell interior by at least three separate mechanisms: a sodium-dependent, chloride/bicarbonate exchange; a sodium-independent, chloride/bicarbonate exchange; and a sodium/hydrogen exchange. All three mechanisms require that bicarbonate move into the cell and/or hydrogen ions move out of the cell to remove or neutralise the excess acid in the cell interior. However, as hydrogen and bicarbonate ions are influenced by several mechanisms these concepts require careful examination, including those of "ion exchanges" with hydrogen ions and bicarbonate. As will be developed below, actual exchange may not occur; the apparent exchange may be the result of independent changes occurring in or outside the cell.

Stewart (1981, 1983) argued that the concentration of hydrogen ions and bicarbonate ions within a system must be regarded as dependent variables rather than independent variables. The values for the dependent variables are determined by the independent variables

within the system and the equations which govern the system. Only changes in the independent variables can cause changes in the dependent ones. Dependent variable values cannot be set arbitrarily but must always adapt to the independent variable values. Considering intracellular acid-base regulation in terms of the exchange of hydrogen ions or bicarbonate ions between the intra- and extracellular space is meaningless since only changes in the independent variables will change the hydrogen ion concentration.

1.9.1 Factors determining the hydrogen ion and bicarbonate concentrations

The independent variables that regulate the concentrations of hydrogen ions and bicarbonate ions within a body fluid are the PCO_2, the strong ion difference ([SID]) and the concentration of weak acid ([ATOT]). The [SID] is equal to the difference between the sum of the strong base cation concentrations and the sum of the strong acid anion concentrations. The important strong cations are sodium, potassium, calcium and magnesium, and the strong anions are chloride, sulphate and lactate. Lactate is considered a strong anion because lactic acid (pKa 3.7) is completely dissociated at physiological pH values.

The relationship between the dependent and independent variables requires that certain physical and chemical principles be obeyed. They include the principles of conservation of mass and

electrical neutrality, and the requirement for dissociation equilibrium of water and weak acids. The components of most biological solutions are water, strong ions, bicarbonate, weak acids and their conjugate bases, dissolved CO_2, carbonate, carbonic acid, hydroxyl ions and hydrogen ions. In considering biological solutions containing strong ions, a weak acid (HA) and CO_2 at an externally regulated PCO_2, the following six equations must be satisfied:

Water dissociation equilibrium:

$$[H^+] \times [OH^-] = K'w$$

Weak acid dissociation equilibrium:

$$[H^+] \times [A^-] = Ka \times [HA]$$

Conservation of mass for "A":

$$[HA] + [A^-] = [ATOT]$$

Bicarbonate ion formation equilibrium:

$$[H^+] \times [HCO_3^-] = Kc \times PCO_2$$

Carbonate ion formation equilibrium:

$$[H^+] \times [CO_3^{2-}] = K3 \times [HCO_3^-]$$

Electrical neutrality:

$$[SID] + [H^+] - [HCO_3^-] - [A^-] - [CO_3^{2-}] - [OH^-] = 0$$

where K'w is the ion product of water, Ka is the weak acid dissociation constant, Kc is the dissociation constant for bicarbonate into dissolved CO_2 and hydroxide ions, and K3 is the dissociation constant for bicarbonate ions into hydrogen ions and carbonate. These

six simultaneous equations contain six unknown dependent variables, $[HA]$, $[A^-]$, $[HCO_3^-]$, $[CO_3^{2-}]$, $[OH^-]$ and $[H^+]$, and three known, independent variables, [SID], [ATOT] and PCO_2. The concentrations of dissolved carbon dioxide and carbonic acid are proportional to the PCO_2 and not affected by the [SID] or the [ATOT]. Stewart (1983) emphasized that "the behaviour of $[H^+]$ and all the other dependent variables is determined, and completely explained, by their having to obey all six of [the equations]. Nothing less than the whole set of six equations is sufficient."

Plasma and muscle intracellular fluid contain strong ions, carbon dioxide and weak acids in differing concentrations. Compared to the extracellular fluid, the intracellular fluid has a higher potassium concentration and lower concentrations of sodium and chloride, thus making the [SID] higher in the intracellular fluid. The [ATOT], comprised of weak organic acids such as proteins and organic phosphates, is higher in the intracellular fluid. Stewart (1981, 1983) demonstrated mathematically that the intra- and extracellular concentration of hydrogen ions and bicarbonate ions is determined by the [SID], PCO_2 and [ATOT] in each fluid compartment. The hydrogen ion concentration is increased by increasing the PCO_2 or [ATOT], or by decreasing the [SID]. The bicarbonate concentration is increased by increasing the PCO_2 or [SID], or decreasing the [ATOT]. Thus the regulation of the intra- or extracellular hydrogen ion concentration cannot be examined simply by measuring changes in pH or

bicarbonate concentration since these measures say nothing about the reason for the change.

1.10 PURPOSE OF THIS THESIS

The existence of lactic acid in exercising muscle has been known since the work of Berzelius in the early 1800's. The lactic acid content of muscle and blood was shown to increase with increasing severity of exercise. The increase in the intra- and extracellular lactic acid concentration was considered the primary cause of the acidosis of heavy exercise. Hydrogen ions have been implicated as one of the factors responsible for the development of fatigue during heavy exercise. If hydrogen ion accumulation is responsible for fatigue then removal of excess hydrogen ions from the intracellular compartment of muscle has to occur to maintain a normal acid-base balance and prevent or slow the fatigue process.

Changes in the plasma concentration of the lactate anion has been used to estimate the output of hydrogen ions from muscle during exercise or recovery. It has been debated as to whether hydrogen ions are released in excess of lactate ions or whether hydrogen ion and lactate release are coupled. In addition, hydrogen ion release from cells was shown to be associated with the coupled transmembrane movement of ions into and out of the cell, either via a sodium/hydrogen exchanger or a chloride/bicarbonate exchanger.

As reviewed above, the quantitative study of acid-base behaviour in a solution can be made if the PCO_2, the concentration of strong ions and the concentration of weak acids are known for that solution. This approach implies that the increase in intracellular hydrogen ion concentration during maximal exercise and the subsequent removal of hydrogen ions during recovery are mediated through changes in the PCO_2, the strong ion difference ([SID]) and the concentration of weak acids ([ATOT]). Stewart has shown this to be true by means of mathematical modelling, but this approach has not been physiologically tested.

The work in this thesis has examined the changes in the acid-base status of the body during and immediately following 30 s of maximal cycle ergometer exercise. The main objectives were to provide quantitative data of the responses to the acidosis of short-term exercise in muscle and in the circulating plasma, to examine these acid-base changes in terms of changes in PCO_2, strong ions and weak acids, to determine the role played by the lungs and inactive tissue in the recovery of acid-base balance, and to examine the fate of lactate after heavy exercise.

The following chapters describe an experimental approach to the study of a severe acidosis that accompanies heavy leg exercise through the measurement of the acid-base variables in femoral venous

blood, arterial blood and a deep arm vein, and of CO_2 output by the lungs.

2 METHODS

2.1 INTRODUCTION

This thesis examined the acid-base changes associated with 30 s of maximal exercise. The acidosis which accompanied the exercise was analysed with respect to changes in PCO_2, the strong ion difference ([SID]) and the concentration of weak acids ([ATOT]). This thesis was also concerned with the whole body regulation of acid-base balance, in particular, the role played by the inactive muscle and the lung in restoring acid-base balance to normal levels. As it was difficult to examine all the aspects of this topic in a single experiment, the work was divided into three parts. Part A examined the magnitude of the acid load developed in the active quadriceps muscle during 30 s of maximal exercise and the subsequent removal of the acid from the muscle during recovery. Femoral venous and arterial blood were sampled and analysed for changes in PCO_2 and strong ions. Changes in tissue PCO_2 and strong ions were estimated using data from the femoral vein and from previously published data (Jones et al., 1985). In Part B, recovery from the short-term exercise was studied to determine the contribution of the lung and inactive tissue as potential sites for removing the acid load from the body. The forearm muscle was used as being representative of inactive tissue in the

body. Arterial and deep forearm venous blood were sampled to examine the contribution of the inactive forearm muscle to the recovery process. Ventilation and gas exchange were measured to determine the pulmonary contribution to the recovery process. In Part C, muscle biopsies were obtained from the quadriceps femoris muscle (vastus lateralis) and analysed for intracellular concentrations of specific strong ions to determine whether strong ion movements occurred during maximal exercise and recovery and whether these changes contributed to the acidosis of heavy exercise and to the recovery of intracellular acid-base balance. Although the study was divided in this way, comparability of data between subjects was established by performance indices obtained in all the studies.

2.2 SUBJECTS

The subjects were healthy male students and employees of McMaster University. Three subjects, age 33 ± 7 yrs (mean ± SD), height 182 ± 3 cm, weight 78 ± 8 kg, participated in Part A (subjects 1,2 and 6 in Table 1). Six subjects, age 30 ± 5 yrs, height 181 ± 2 cm, weight 79 ± 8 kg, participated in Part B (subjects 1-6 in Table 1). Six subjects, age 25 ± 6 yrs, height 181 ± 5 cm, 81 ± 7 kg, participated in Part C (subjects 7-12 in Table 1).

The experimental protocol and possible risks involved in the experiment were outlined to each subject before obtaining a written

Table 1. Summary of subject characteristics.

Subject	Age, yrs	Height, cm	Weight, kg
1	27	181	75.0
2	40	185	86.5
3	27	180	70.0
4	26	180	83.5
5	29	182	87.0
6	31	180	72.0
7	20	185	79.5
8	22	185	78.0
9	20	188	89.0
10	19	178	91.0
11	19	179	79.0
12	23	170	73.0

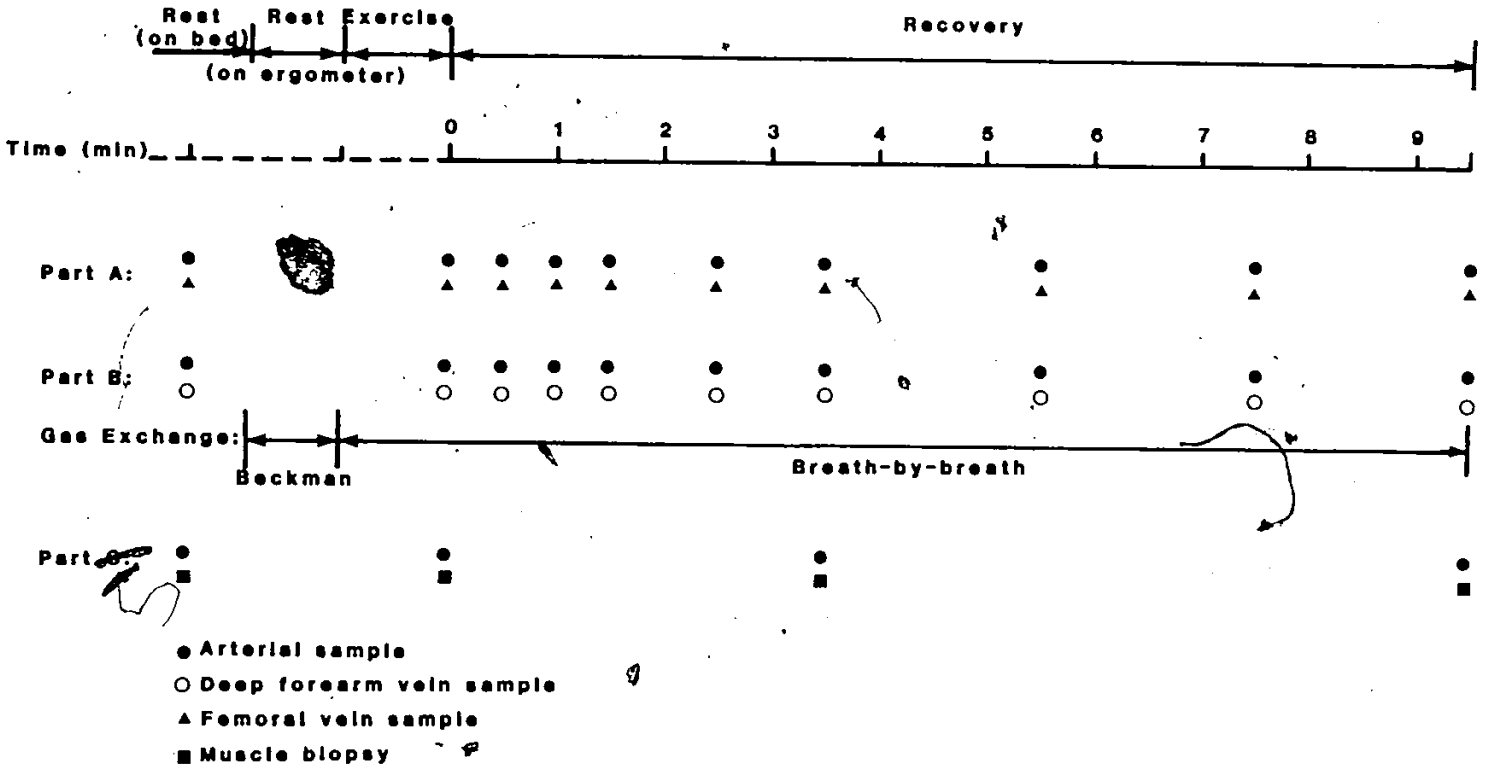
consent to participate. The experimental protocol and all procedures were approved by McMaster University's ethics committee.

2.3 GENERAL PROTOCOL

The studies were performed in the morning. Diet was not controlled but the subjects were advised to eat only a light breakfast on the morning of the experiment. Whenever the subject performed a second experiment on a different day he was told to eat a comparable meal on each occassion.

An outline of the experimental protocol is presented in Figure 1. Prior to the exercise test the subject rested supine on a bed for 30 min before taking the resting control blood samples (Parts A,B,C) and biopsies (Part C). The subject then moved to the constant-velocity cycle ergometer (McCartney et al., 1983), the saddle height was adjusted for optimal leg extension, the torso was stabilised on the saddle with a restraining harness wrapped around the hips and the feet were secured to the pedals with toe clips and tape. The subject sat quietly on the ergometer and specific instructions were given regarding the exercise protocol. Ventilation and gas exchange data were collected for 5-10 min (Part B). After all resting measurements were completed, the motor of the ergometer was started and the subject was allowed to catch up to the predetermined speed of 100 rpm before exertin[illegible]al force; the time taken was

Figure 1. Outline of the experiment protocol for Parts A, B and C.


Rest (on bed)
Rest
Exercise (on ergometer)
Recovery
Time (min)
0
1
2
3
4
5
6
7
8
9
Part A:
Part B:
Gas Exchange:
Beckman
Breath-by-breath
Part C:
Arterial sample
Deep forearm vein sample
Femoral vein sample
Muscle biopsy

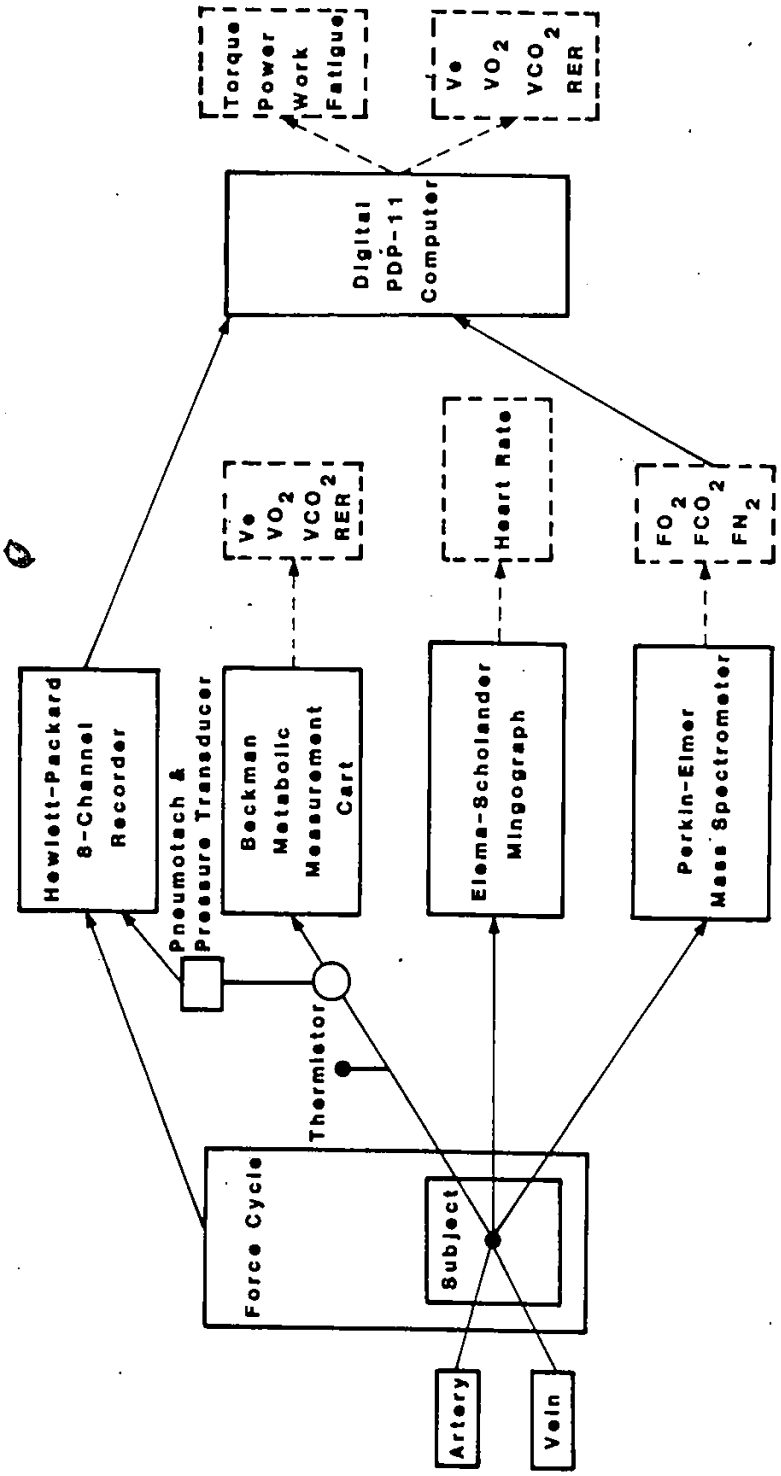
approximately 5 s. Immediately upon attaining the correct speed the subject was instructed to pedal maximally for 30 s. The subject was encouraged verbally throughout the 30 s exercise. At the completion of the exercise test the subject sat quietly on the ergometer for a 10 min recovery period. Heart rate was monitored continuously throughout exercise and recovery using a V5 ECG lead placement. Blood samples (Parts A,B,C) and muscle biopsies (Part C) were taken immediately post-exercise and at specific times during the 10 min recovery. Ventilation and gas exchange were monitored continuously during exercise and throughout recovery (Part B). A schematic representation of the experimental setup is presented in Figure 2.

2.4 MEASUREMENT OF FORCE OUTPUT

The 30 s of maximal exercise was performed on a constant-velocity cycle ergometer (McCartney et al., 1983). The modified ergometer consisted of a 3-hp DC electric motor connected in series to a DC regenerative controller. This system maintained pedal speed constant despite the maximal efforts exerted by the subject. A unidirectional clutch housed in the front sprocket assembly allowed the subject to start and stop pedalling immediately. An emergency stop button was attached to the handlebars within easy reach of both investigator and subject.

Forces applied to the pedal cranks were recorded continuously

Figure 2. Schematic representation of experimental set-up used in Parts A, B and C. Gas exchange was measured in Part B only.


Torque
Power
Work
Fatigue
Ve
VO_2
VCO_2
RER
Digital PDP-11 Computer
Ve
VO_2
VCO_2
RER
Heart Rate
FO_2
FCO_2
FN_2
Hewlett-Packard 8-Channel Recorder
Pneumotach & Pressure Transducer
Beckman Metabolic Measurement Cart
Elema-Scholander Mingograph
Perkin-Elmer Mass Spectrometer
Thermistor
Force Cycle
Subject
Artery
Vein

by strain gauges attached to the leading and trailing edges of the pedal cranks. Force data were transmitted via brass slip-rings from a strain gauge, Wheatstone bridge system to a Hewlett-Packard 7700 Series Recorder and to a laboratory computer (Digital Equipment PDP 11). The computer sampled force data at 10 ms intervals and integrated the force data with respect to time. Calculations of peak torque, average torque, peak power, average power and work for each leg during each pedal stroke were made by the computer (McCartney et al., 1983). Data were smoothed by continuous averaging of three successive pedal revolutions. The fatigue index was calculated as the percentage decline in power from the maximum value to that recorded at the end of the 30 s test (McCartney, Heigenhauser and Jones, 1983). The ergometer was calibrated statically immediately before each test; baseline for each pedal was established at a point 90° from top dead centre, the pedal was locked into place and a weight was placed on the pedal. The torque that was generated was sampled by the laboratory computer and its value was used in the calculation of the various performance variables.

2.5 MEASUREMENT OF GAS EXCHANGE

In Part B, ventilation and gas exchange were measured at rest, throughout exercise and recovery. The subject breathed through a low-resistance, low dead-space nonrebreathing valve (Hans Rudolph no. 2700). Expired gas was passed through flexible, low-resistance tubing

and analysed for volume and gas concentration by one of two methods. Resting measurements were made as 15 s averages using a Beckman Metabolic Measurement Cart Horizon System (SensorMedics) (Norton, 1982; Jones, 1984). Exercise and recovery measurements were made breath-by-breath using an open circuit gas analysis system. The use of two systems for measuring gas exchange was necessary because the computerised breath-by-breath system could sample for only 10 min.

2.5.1 Metabolic Measurement Cart

The Metabolic Measurement Cart is a microprocessor-controlled system for measurement of gas exchange during rest and exercise. The system is operated by an INTEL 8085A microprocessor which controls the transducers, sensors and sampling equipment.

Expired air was passed through a jewel-mounted turbine incorporating electro-optical detectors to measure gas volume. Expired gas concentrations, temperature and pressure were measured in a fixed volume mixing chamber. The oxygen concentration was measured with a temperature-controlled, fast-response, polarographic sensor similar to the Beckman OM-11 Oxygen Analyzer. Carbon dioxide was measured with a dual-beam nondispersive infrared optical system with a pneumatic detector, similar to the Beckman LB-2 Medical Gas Analyzer. Temperature was measured with a Yellow Springs 440088 transducer and pressure with a Honeywell 140PC transducer. Calibration procedures

for pressure, volume, temperature and gas concentrations were built into the system and controlled by the microprocessor. Volume calibration required delivery of a fixed volume (1.0 l) at varying flow rates with a manually driven pump. Gas calibration was made with standard calibration gases previously analysed for concentration of oxygen and carbon dioxide by the Lloyd-Haldane technique. Calibration of the instrument was performed immediately prior to the test.

2.5.2 Breath-by-breath system

During exercise and recovery expired gas was passed through low resistance tubing and air flow was measured by a pneumotachograph (Fleisch no. 3) with a differential pressure transducer (Hewlett-Packard 270). Signals from the pressure transducer were passed to a chart recorder (Hewlett-Packard 7700 Series Recorder) and a laboratory computer (Digital Equipment PDP 11) sampled the flow signal every 50 ms. Breath temperature was measured with a thermometer placed approximately 1 m upstream from the pneumotachograph. A probe was placed in the mouthpiece and expired gas was sampled by a mass spectrometer (Perkin Elmer MGA-1100) at a flow of 60 ml/min; signals from the mass spectrometer were sampled by the computer every 50 ms. Validation data for the breath-by-breath system are presented in Appendix A and show that ventilation, CO_2 output and O_2 intake were measured to 0.9 l/min (± 95% confidence), 35 ml/min and 42 ml/min, respectively.

The entire system was calibrated immediately before each test. A calibration curve for air flow was constructed by the computer; ten steady-state rates of flow ranging from 0-300 l/min were introduced into the expiratory circuit by means of a rotameter/power vaccum system and sampled by the computer. Signals from the pneumotachograph and transducer were compared to the flow calibration curve to calculate flow for each sampling period. A calibration curve for gas concentration was constructed with four standard reference gases which had previously been analysed by the Lloyd-Haldane technique. Concentrations of oxygen, carbon dioxide and nitrogen in each breath sample were calculated by comparing the output from the mass spectrometer to the calibration curve constructed by computer.

The flow signals from the pneumotachograph and pressure transducer were converted to individual breath data by the computer. The time taken for a single breath corresponded to the total time between two periods of zero flow lasting approximately 250 ms. Expiratory time during a breath corresponded to periods of changing positive flow. Expired volume was obtained by integrating the positive flow signal with respect to time for each sampling period and summing the values to obtain the total volume for each breath. Minute ventilation corresponding to each breath was calculated using the volume/breath ratio and the time/breath ratio.

Signals from the pressure transducer and mass spectrometer were adjusted by the computer for the temporal delay between air flow measurements and gas analysis. The concentration of oxygen and carbon dioxide in each sample were integrated with respect to time and summed to obtain a volume of oxygen and carbon dioxide for each breath. Minute values of oxygen intake and carbon dioxide output corresponding to each breath were calculated using the volume/breath ratio and the time/breath ratio.

2.6 ANALYTICAL METHODS

2.6.1 Arterial and venous catheterisation

Catheters were placed into the artery and vein while the subject rested supine on a bed. Local anesthesia was first achieved with subcutaneous admininstration of 2% Lidocaine Hydrochloride (Astra Pharmaceutical). A percutaneous teflon catheter (20 gauge, 4-5 cm Deseret Angiocath) was introduced into the brachial or radial artery at the point of the arterial pulse (found by palpation). A percutaneous teflon catheter (18 gauge, 5 cm Deseret Angiocath) was introduced retrograde into a deep forearm vein. The femoral venous catheter (14 gauge, 30 cm Deseret Cut-Down Catheter) was introduced surgically using aseptic techniques. An incision was made in the skin distal to the inguinal ligament. The femoral vein was located by palpating the femoral arterial pulse and the catheter was passed

through a point slightly medial to the pulse, retrogradely into the femoral vein. The patency of the arterial and venous catheters was maintained with a saline (0.9% sodium chloride) drip. The pressure in the arterial line was maintained in excess of 250 mm Hg with a pressure cuff placed around the saline bag. All catheters were secured to the skin with tape to prevent them from becoming accidently dislodged from the vessels.

2.6.2 Blood sampling

Blood was sampled according to the protocol outlined in Figure 1. Blood was drawn simultaneously from the artery and vein. At rest and later in recovery, approximately 1 ml of blood was first drawn from the catheter to clear the dead space of saline and blood. However during the first 2 min recovery when samples were drawn every 30 s, the catheters remained open and blood allowed to drip slowly from the line thus obviating the need to clear the dead space.

Approximately 6 ml of blood was drawn into a heparinised glass syringe and immediately divided into two portions. The blood remaining in the glass syringe was mixed, cleared of air bubbles, capped and stored on ice for analysis of blood gases, pH and electrolyte concentrations. The second portion of blood was transferred into tubes containing ethylenediaminetetraacetic acid (EDTA), mixed and stored on ice for analysis of lactate concentration.

2.6.3 Analysis of blood gases and pH

Plasma pH, PCO_2 and PO_2 were measured by electrodes (Corning 178 pH/Blood Gas Analyzer). The pH and blood gas analysers are routinely checked in this laboratory with tonometered blood samples. The mean difference between duplicate analyses on blood samples was 0.5 ± 0.4 mm Hg (mean ± SD) for carbon dioxide and 0.9 ± 1.3 mm Hg for oxygen. The mean difference between duplicate pH measurements was 0.004 ± 0.005 units. Hemoglobin concentration and oxygen saturation were measured photometrically (Radiometer OSM2 Hemoximeter). All measurements were made at 37° C. The analysers were calibrated immediately before and throughout the period of analysis with known standards. The hydrogen ion concentration was calculated as the antilog -pH. Plasma bicarbonate concentration was calculated from pH and PCO_2 (Siggaard-Andersen, 1963).

The hydrogen ion concentration was also calculated from the PCO_2, strong ion difference ([SID]) and concentration of weak acid ([ATOT]) according to the following equation (Stewart, 1981):

$$[H^+]^4 + (Ka + [SID])\,[H^+]^3 + (Ka\,([SID] - [ATOT]) - (Kc \times PCO_2 + K'w))\,[H^+]^2 - (Ka\,(Kc \times PCO_2 + K'w) + K3 \times Kc \times PCO_2)\,[H^+] - Ka \times K3 \times Kc \times PCO_2 = 0.$$

The PCO_2 was measured and the [SID] was calculated as the difference between the sum of the measured concentrations of sodium, potassium, calcium and the sum of the measured concentrations of chloride and lactate. The resting [ATOT] was taken as 20 mEq/l (Stewart, 1981) and the change in [ATOT] after maximal exercise was estimated from changes in the hemoglobin concentration. The constants were those used in Stewart (1981, 1983): $Ka = 3 \times 10^{-7}$ Eq/l, $Kc = 2.46 \times 10^{-11}$ $(Eq/l)^2/mm$ Hg, $K3 = 6 \times 10^{-11}$ Eq/l, and $K'w = 4.4 \times 10^{-14}$ $(Eq/l)^2$. The equation was solved by computer using an Applesoft BASIC version of the programme listed in Stewart (1981).

2.6.4 Analysis of plasma electrolyte concentrations

Plasma sodium, potassium and ionised calcium concentrations were measured by ion-selective electrodes (Radiometer KNA1 Sodium-Potassium Analyzer; Radiometer ICA1 Ionized Calcium Analyzer). Measurements were made at 37° C. Plasma chloride concentration was measured by titration (Buchler-Cotlove Chloridometer Model 4-2008). All analysers were calibrated immediately before and throughout the period of analysis using known standards. The mean difference between duplicate measurements were: sodium, 0.4 ± 0.6 mmol/l; potassium, 0.02 ± 0.04 mmol/l; calcium, 0.005 ± 0.005 mmol/l; chloride, 1.2 ± 1.0 mmol/l.

2.6.5 Measurement of plasma lactate concentration

The second portion of the sample containing blood and EDTA were centrifuged and the separated plasma was stored frozen. An aliquot of plasma was removed, deproteinised in cold 6% (w/v) perchloric acid and centrifuged. The acidified supernatant was analysed for lactate concentration by a fluorometric enzyme technique (Lowry and Passonneau, 1972). The mean difference between duplicate lactate measurements was 0.21 ± 0.20 mmol/.l

2.6.6 Muscle biopsy procedure

Muscle biopsies were obtained from the lateral portion of the quadriceps femoris muscle (vastus lateralis). The technique was similar to that described by Bergström (1962), but modified by applying suction to the needle at the time of the biopsy. All instruments were cleaned and sterilised before use.

The subject rested supine on the bed and the skin and outer layer of subcutaneous tissue were anesthetised with local administration of 2% Lidocaine Hydrochloride (Astra Pharmaceutical). The skin and subcutaneous tissue were punctured with a scalpel blade. The biopsy needle was introduced into the muscle, suction was applied and the biopsy was taken. The needle was rapidly removed from the muscle and immediately placed in liquid nitrogen. The muscle sample

was removed from the needle under liquid nitrogen, wrapped in foil and stored in liquid nitrogen for future analysis. Pressure was maintained over the incision to minimize excessive bleeding. The incision was sutured and protected with tape.

The biopsies taken prior to the exercise and at 4 and 10 min recovery were taken while the subject rested on the bed. The biopsy taken immediately post-exercise was taken with the subject seated on the cycle ergometer. The elapsed time between introducing the needle into the muscle and placing the needle in liquid nitrogen was approximately 5 s.

2.6.7 Measurement of intramuscular ion concentrations by neutron activation analysis

Tissue preparation : The frozen muscle samples were finely ground under liquid nitrogen and visible fat and connective tissue were removed. The muscle sample was freeze-dried to a powder in polyethylene vials and weighed to determine the muscle wet weight/dry weight ratio. The biopsy dry weight ranged from 10-30 mg.

Neutron activation analysis : The technique of neutron activation analysis is based on the formation of radioactive isotopes occurring when stable isotopes are irradiated with neutrons. In the reaction with thermal neutrons, a neutron is captured and an isotope

of the same element as the target element is formed except that it has an atomic weight which is one mass unit higher than the original element. If the new isotope is radioactive it can be detected and counted.

Vials containing the freeze-dried tissue were carried to the nuclear reactor core (McMaster University Nuclear Reactor) via pneumatic lines and irradiated with thermal neutrons for 10 s, then delivered back for detection and counting of isotopic activity of the desired elements (sodium, potassium, calcium, magnesium and chloride). The delay time for transport of the vial from the reactor core, transfer of the irradiated tissue into tared, unirradiated vials and positioning of the tissue on the detector for counting was approximately 100 s. The tissues were transferred from the contaminated vials to fresh vials. The irradiated tissue was counted for 10 min using a lithium/germanium detector coupled to a Canberra multichannel analyzer (Series 40 or Series 90).

The content of element "X" in the muscle was determined by comparing the irradiation intensity of element "X" in the muscle sample to the irradiation intensity of element "X" in the standard (citrus leaves, National Bureau of Standards #1572) according to the following equation:

$$Wx/Ws = Cx/Cs$$

where Wx is the amount of the element in the sample, Ws is the amount of the element in the standard, Cx is the radioactivity of the element in the sample and Cs is the radioactivity of the element in the standard. The content of "X" was calculated by computer; the irradiation time, half-life of the isotope, delay and counting times of the sample and standard, and weight of the sample and standard were accounted for by the computer program. The extracellular content of "X" was calculated from the plasma value of "X" corrected for water content and Donnan factor (0.96 for sodium, potassium and chloride, and 0.92 for calcium and magnesium). The extracellular water content was assumed to be 0.39 ml/g d.w. at rest, 0.58 ml/g d.w. immediately post-exercise, 0.53 ml/g d.w. at 4 min recovery and 0.50 ml/g d.w. at 10 min recovery (Sahlin et al., 1978b; Sjøgaard, Adams and Saltin, 1985). The intracellular content of "X" was the difference between the total muscle content and the extracellular content of "X". A sample calculation is presented in Appendix B.

2.7 STATISTICS

All values are reported as the mean ± SD. An analysis of variance was used to compare performance measurements in Parts A, B and C. The Student Newman-Keuls post Hoc test was used to compare the means if a significant F ratio was obtained. A paired t-test was used to compare pre-exercise and recovery blood measurements. The paired

t-test was also used to compare arterial and venous blood measurements at specific times in recovery. Statistical significance was accepted at $p<0.05$.

3 RESULTS

This thesis examined the acidosis associated with short-term, maximal exercise and recovery of acid-base balance back towards control levels. The development and subsequent resolution of the acidosis was examined in terms of changes in the strong ions in the working tissue, and changes in CO_2 and strong ions across the working and nonworking muscle, and in the lung. It was impossible to examine all these aspects in one experiment, thus it was necessary to divide the thesis into three studies examining the acidosis of heavy exercise in the working muscle (quadriceps muscle) and femoral venous and arterial blood (Part A), recovery of the acidosis across the lung and inactive muscle (Part B) and the importance of strong ion movements in acid-base balance during heavy exercise (Part C). Maximal exercise was performed on a constant-velocity cycle ergometer for 30 s duration. Performance was assessed during each 30 s exercise test, thereby making it possible to compare measurements between the different parts of the thesis.

3.1 PERFORMANCE VARIABLES DURING 30 s MAXIMAL CYCLE ERGOMETER EXERCISE

During the 30 s maximal exercise, no differences were observed

between any of the performance variables in Parts A and B (Table 2). The maximal peak power output generated during one complete revolution of the pedals was 1575 ± 338 W (mean ± SD). The maximal average power output maintained throughout the 30 s of exercise was 967 ± 224 W. The total work performed during the exercise in Part B was 21.0 ± 4.8 kJ. The fatigue index represents the decline in peak power (or torque) that occurred during the 30 s of exercise. The fatigue index for maximum peak power output and maximal average power output was 50± 10% and 49 ± 8%, respectively.

3.2 PULMONARY GAS EXCHANGE ASSOCIATED WITH SHORT-TERM MAXIMAL EXERCISE

Ventilation ($\dot{V}E$), O_2 intake ($\dot{V}O_2$) and CO_2 output ($\dot{V}CO_2$) were measured at rest and during exercise and recovery to assess the lungs' contribution in regulating acid-base balance (Part B). Breath-by-breath data for a single subject are presented in Figure 3 and mean data for all the subjects at discrete times during recovery are shown in Figure 4. Resting $\dot{V}O_2$ was 343 ± 37 ml/min and increased over 7 times during the 30 s of exercise; the $\dot{V}O_2$ at the end of exercise was 2687 ± 253 ml/min (Fig. 4). The total volume of O_2 used during the 30 s of exercise (ie. the cumulative O_2 from each breath) was 908 ± 117 ml. The $\dot{V}O_2$ began to decrease immediately upon completion of the exercise; the half-time (t1/2) for recovery of $\dot{V}O_2$ (ie. the time taken to decrease to 50% of the peak $\dot{V}O_2$ - rest $\dot{V}O_2$

Table 2. Summary of performance data during 30 s of maximal exercise.

	Part A (n=3)	Part B (n=6)	Part C (n=6)
max. peak torque, N.m	127 ±19	149 ±30	166 ±22
max. average torque, N.m	80 ±9	91 ±20	106 ±12
max. peak power, W	1330 ±209	1575 ±338	1799 ±265
max. average power, W	845 ±100	967 ±224	1143 ±142
total work, kJ	18.6* ±1.2	21.0 ±4.8	25.9* ±1.9
fatigue index, %:			
peak torque	45 ±6	49 ±9	41 ±16
peak power	46 ±6	50 ±10	43 ±16
average power	48 ±6	49 ±8	51 ±11

values are mean ± SD

* significant difference between Part A and Part C ($p < 0.05$)

Figure 3. Ventilation, CO_2 output and O_2 intake of a single subject (6) at rest, during maximal exercise and recovery.

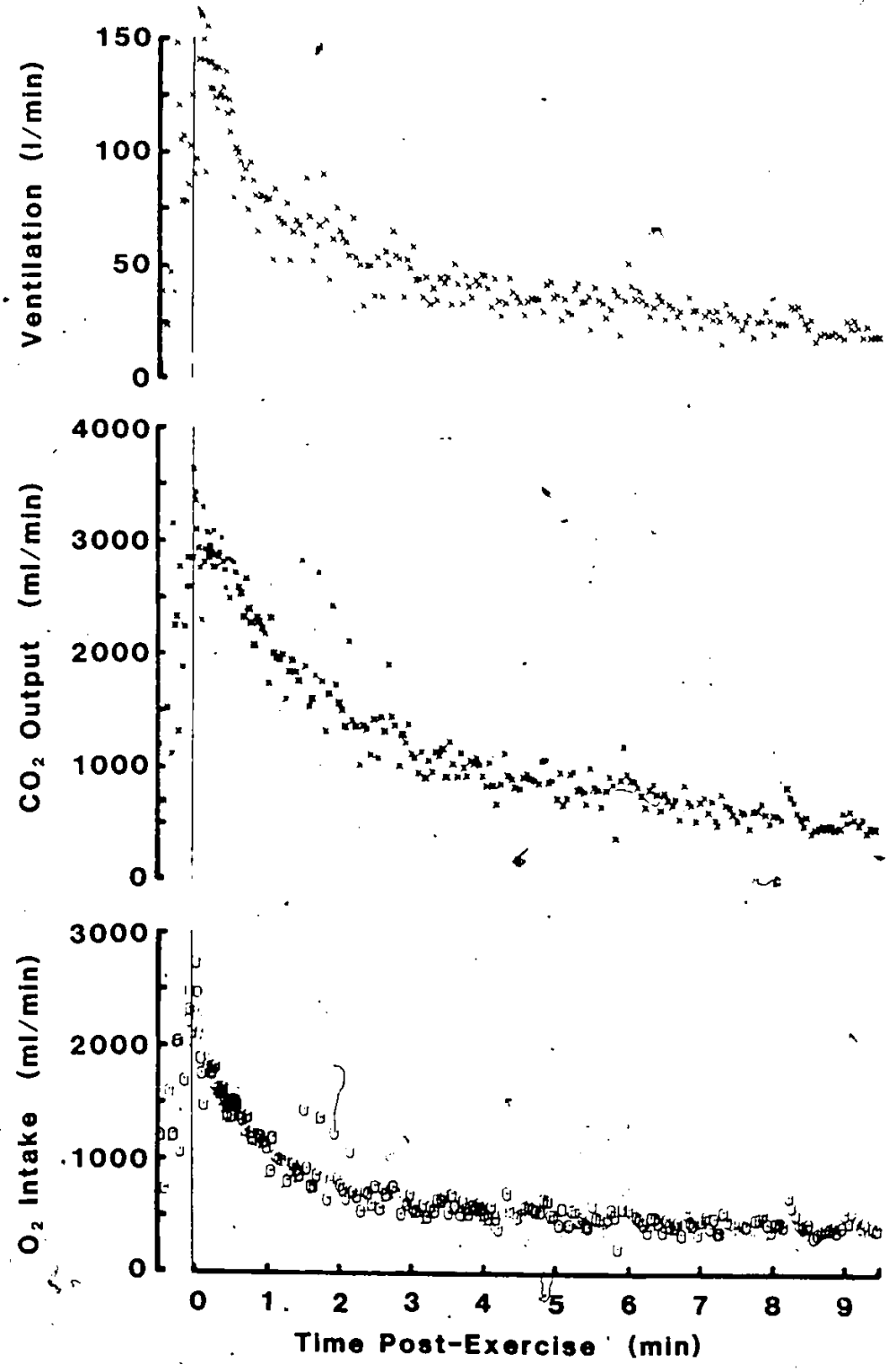
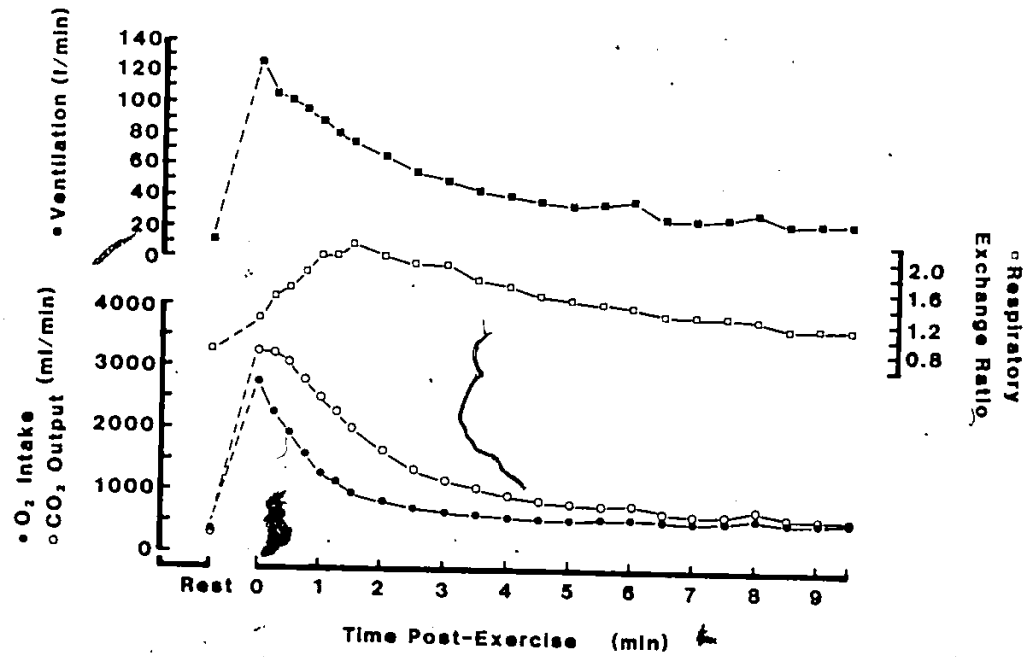

Ventilation (l/min)
150
100
50
0
CO_2 Output (ml/min)
4000
3000
2000
1000
0
O_2 Intake (ml/min)
3000
2000
1000
0
0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

Figure 4. Ventilation, CO_2 output, O_2 intake and respiratory exchange ratio at rest and during recovery from maximal exercise (n=6).


• Ventilation (l/min)
140
120
100
80
60
40
20
0
• O2 Intake
◦ CO2 Output (ml/min)
4000
3000
2000
1000
0
▫ Respiratory Exchange Ratio
2.0
1.6
1.2
0.8
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

difference) was 47 ± 4 s. The $\dot{V}O_2$ at 10 min recovery (502 ± 106 ml/min) was still elevated above rest levels.

The $\dot{V}CO_2$ was 273 ± 43 ml/min at rest and increased to 3238 ± 195 ml/min at the end of 30 s exercise. After exercise, the $\dot{V}CO_2$ decreased at a slower rate compared to the $\dot{V}O_2$; the t1/2 for the recovery of $\dot{V}CO_2$ was 105 ± 15 s. The $\dot{V}CO_2$ at 10 min recovery (550 ± 105 ml/min) was still elevated above rest levels. The slower fall in $\dot{V}CO_2$ compared to $\dot{V}O_2$ contributed to the increase in the respiratory exchange ratio (RER) in the first few minutes of recovery. The RER was 0.79 ± 0.06 at rest and increased to 1.22 ± 0.13 immediately post-exercise and 2.15 ± 0.20 at 1.5 min of recovery. The RER decreased for the remainder of the recovery (Fig. 4).

The $\dot{V}E$ was 10 ± 1 l/min at rest and increased to 124 ± 17 l/min at the end of 30 s exercise (Fig. 4). The t1/2 for recovery of $\dot{V}E$ was 107 ± 19 s. The $\dot{V}E$ at the end of the 10 min recovery (23 ± 5 l/min) was greater than rest levels.

3.3 ACID-BASE CHANGES ACROSS THE ACTIVE MUSCLE DURING AND AFTER MAXIMAL EXERCISE (PART A)

During maximal leg cycling exercise an acid load develops in the active muscle. A generalised acidosis develops in the whole body as the acid is removed from the intra- to the extracellular

compartment.

3.3.1 Hydrogen ion concentration across the active muscle

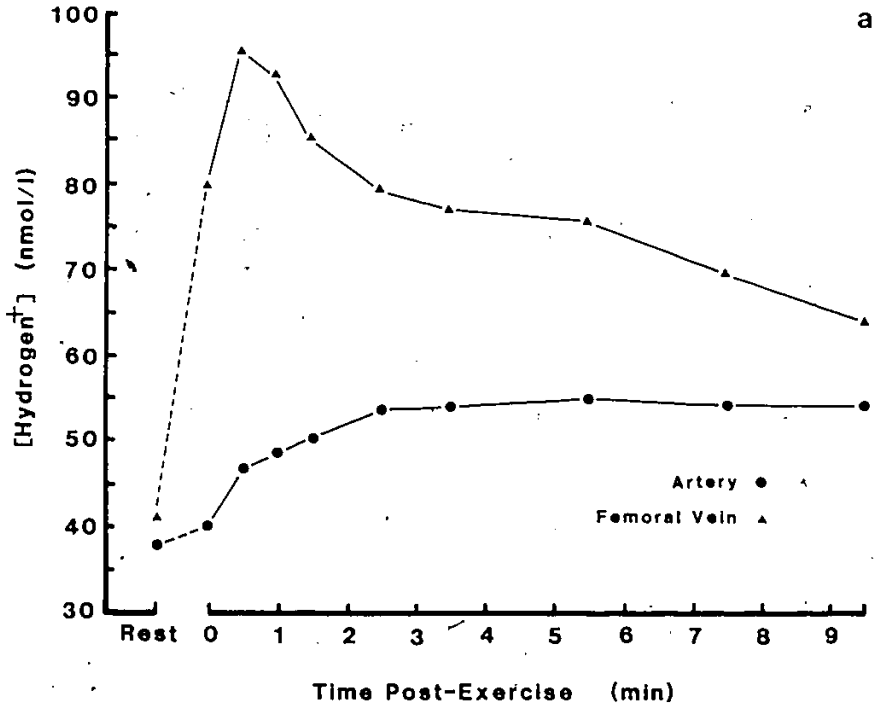
At rest the hydrogen ion concentration ($[H^+]$) in the femoral vein was 41.2 ± 3.5 nmol/l (pH 7.39 ± 0.04). After exercise the femoral venous $[H^+]$ increased to 95.3 ± 11.7 nmol/l (pH 7.02 ± 0.05) (Table 3, Fig. 5a). The $[H^+]$ began to decrease in all subjects after 1 min recovery (Table 3, Fig. 5a). Normal $[H^+]$ was not achieved during the course of the recovery; the femoral venous $[H^+]$ was 63.5 ± 3.6 nmol/l (pH 7.20 ± 0.03) at 10 min.

The arterial $[H^+]$ was 37.8 ± 2.1 nmol/l (pH 7.42 ± 0.04) at rest. After maximal exercise the arterial $[H^+]$ gradually increased and reached 53.6 ± 1.9 nmol/l (pH 7.27 ± 0.02) at 10 min recovery (Table 3, Fig. 5a). The arterial acidosis was less marked than that found in the femoral vein; the venous-arterial $[H^+]$ difference ranged from 48.5 ± 9.2 nmol/l at 30 s recovery to 9.9 ± 1.8 nmol/l at 10 min recovery (Table 4).

The $[H^+]$ of the intra- and extracellular compartments were examined with respect to changes in the PCO_2, strong ion difference and concentration of weak acids.

Figure 5 a. Arterial and femoral venous hydrogen ion concentrations (nmol/l) at rest and during recovery from maximal exercise (n=3).

b. Arterial and forearm venous hydrogen ion concentrations (nmol/l) at rest and during recovery from maximal exercise (n=6).


a
100
90
80
70
60
50
40
30
[Hydrogen+] (nmol/l)
Artery
Femoral Vein
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

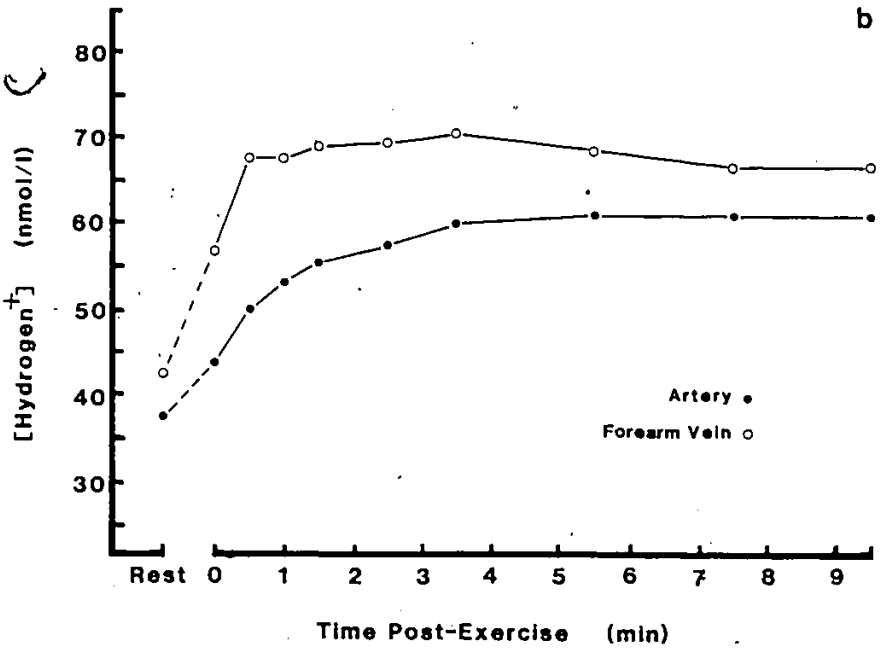

b
80
70
60
50
40
30
[Hydrogen+] (nmol/l)
Artery
Forearm Vein
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

Table 3. Dependent (H^+, HCO_3^-) and independent (PCO_2, SID, ATOT) variables in the femoral vein and artery at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Femoral Vein:										
H^+, nmol/l	41.2 ±3.5	79.8* ±11.7	95.3* ±11.5	92.6* ±17.1	85.3* ±15.5	79.0* ±10.0	76.4* ±7.7	74.9* ±5.8	69.0* ±4.1	63.5* ±3.6
PCO_2, mm Hg	45.8 ±4.8	99.6* ±22.0	105.8* ±17.7	85.6* ±20.9	67.5 ±18.6	53.9 ±9.7	47.7 ±8.2	47.2 ±7.7	43.2 ±6.3	39.8* ±4.9
SID, mEq/l	52.3 ±8.1	54.5 ±4.7	51.8 ±5.6	48.9 ±4.6	41.7 ±1.8	42.8* ±3.7	40.3* ±3.7	41.0* ±3.7	41.4* ±6.1	41.3* ±3.4
ATOT, mEq/l**	20	23	22	22	22	22	22	21	21	20
HCO_3^-, mmol/l	27.3 ±0.9	30.2 ±2.8	27.5 ±2.4	22.6* ±1.5	19.3* ±1.6	16.7* ±1.0	15.3* ±1.2	15.4* ±1.3	15.4* ±1.5	15.4* ±1.5
Artery:										
H^+, nmol/l	37.8 ±2.1	40.1 ±2.1	46.8* ±3.3	48.6* ±3.1	50.3* ±2.2	53.6* ±2.8	53.8* ±2.0	54.4* ±1.4	53.8* ±2.0	53.6* ±1.9
PCO_2, mm Hg	41.0 ±1.9	35.3 ±2.1	36.5* ±2.0	33.4* ±3.4	31.6* ±2.8	30.6* ±2.3	29.9* ±1.4	29.2* ±3.6	29.8* ±3.4	31.5* ±3.2
SID, mEq/l	48.4 ±1.2	46.3 ±2.1	44.2* ±1.5	41.8* ±1.3	39.4 ±5.6	41.9* ±2.7	41.8* ±0.8	40.3* ±2.8	41.6 ±3.2	40.3* ±2.5
ATOT, mEq/l**	20	22	22	22	22	22	22	22	21	22
HCO_3^-, mmol/l	26.6 ±1.7	21.7* ±0.7	19.2* ±0.9	16.9* ±1.3	15.5* ±1.0	14.1* ±1.1	13.6* ±0.4	13.2* ±1.3	13.6* ±1.0	14.5* ±1.1

values are mean ± SD

* significantly different from rest ($p < 0.05$)

** estimated from Table 7.3 (Stewart, 1981) and from the mean % change in femoral venous and arterial hemoglobin concentration.

Table 4. Femoral venous-arterial differences for selected acid-base variables at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
H^+, nmol/l	3.3* ±1.4	39.7* ±13.1	48.5* ±9.2	44.0* ±14.1	35.0* ±13.6	25.4* ±7.2	22.6* ±5.7	20.6* ±4.7	15.1* ±2.8	9.9* ±1.8
PCO_2, mm Hg	4.9 ±5.6	64.3* ±22.2	69.4* ±16.1	52.2* ±18.8	35.9* ±17.8	23.3* ±9.8	17.8* ±7.1	18.0* ±5.9	13.4* ±2.9	8.3* ±2.8
SID, mEq/l	3.9 ±8.8	8.2* ±3.8	7.6* ±4.3	7.1* ±3.5	4.7 ±3.5	0.9 ±1.8	-1.5 ±3.5	0.7 ±1.1	-0.2 ±3.2	0 ±1.1
HCO_3^-, mmol/l	0.7 ±2.6	8.5* ±2.2	8.3* ±1.5	5.7* ±1.4	3.8 ±2.0	2.6 ±2.0	1.6 ±1.3	2.2* ±1.1	1.8* ±0.6	1.0 ±1.0

values are mean ± SD

* significant difference between femoral vein and artery ($p < 0.05$)

3.3.2 Plasma PCO_2 across the active muscle

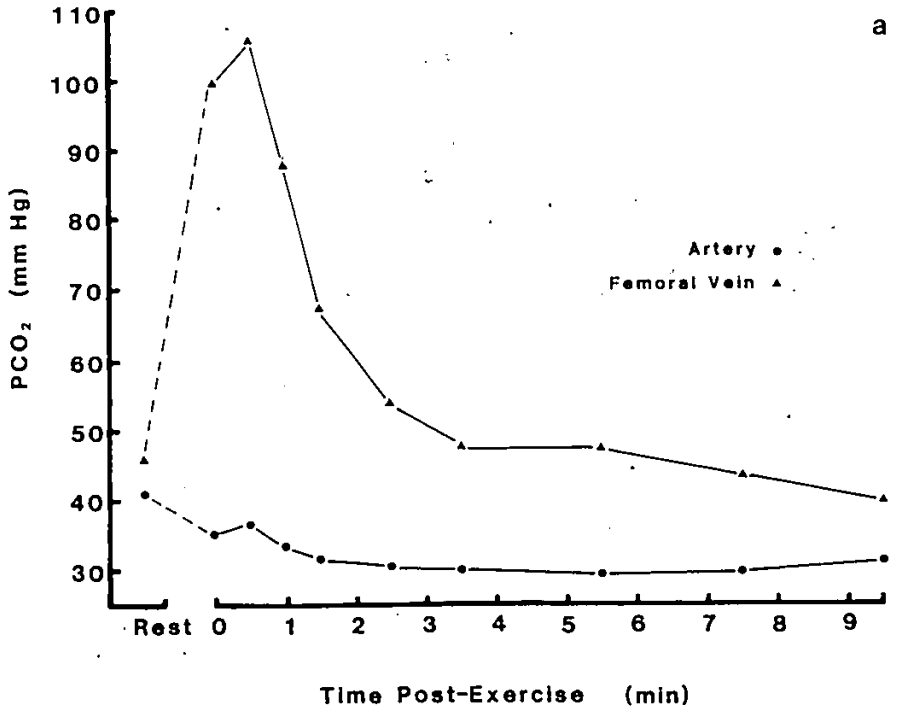
The femoral venous PCO_2 was 45.8 ± 4.8 mm Hg at rest. CO_2 output from muscle increased during and immediately after maximal exercise (Table 4); the PCO_2 increased to 105.8 ± 17.7 mm Hg at 30 s recovery (Tables 3, Fig. 6a). The PCO_2 began decreasing by 1 min recovery and was reduced below resting levels by the end of the recovery (Table 3, Fig. 6a). The arterial PCO_2 was less than resting levels for most of the recovery period (Table 3, Fig. 6a).

3.3.3 Strong ion difference across the active muscle

The strong ion difference ([SID]) is the difference between the concentration of strong basic cations and the concentration of strong acid anions. The plasma [SID] was calculated as $(([Na^+] + [K^+] + [Ca^{2+}]) - ([Cl^-] + [\text{lactate}]))$. The femoral venous [SID] was 52.3 ± 8.1 mEq/l at rest and decreased rapidly after exercise, reaching steady levels (approximately 41 ± 4 mEq/l) by 2 min recovery (Table 3, Fig. 7a). The arterial [SID] was 48.4 ± 1.2 mEq/l at rest and decreased below resting levels by 30 s recovery (Table 3, Fig. 7a). The arterial and femoral venous [SID] values were similar after 2 min of recovery (Table 4, Fig. 7a). The decrease in the plasma [SID] after exercise was due to an increase in the concentration of strong anions relative to the concentration of strong cations.

Figure 6 a. Arterial and femoral venous PCO_2 (mm Hg) at rest and during recovery from maximal exercise (n=3).

b. Arterial and forearm venous PCO_2 (mm Hg) at rest and during recovery from maximal exercise (n=6).


a
110
100
90
80
70
60
50
40
30
PCO_2 (mm Hg)
Artery •
Femoral Vein ▲
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

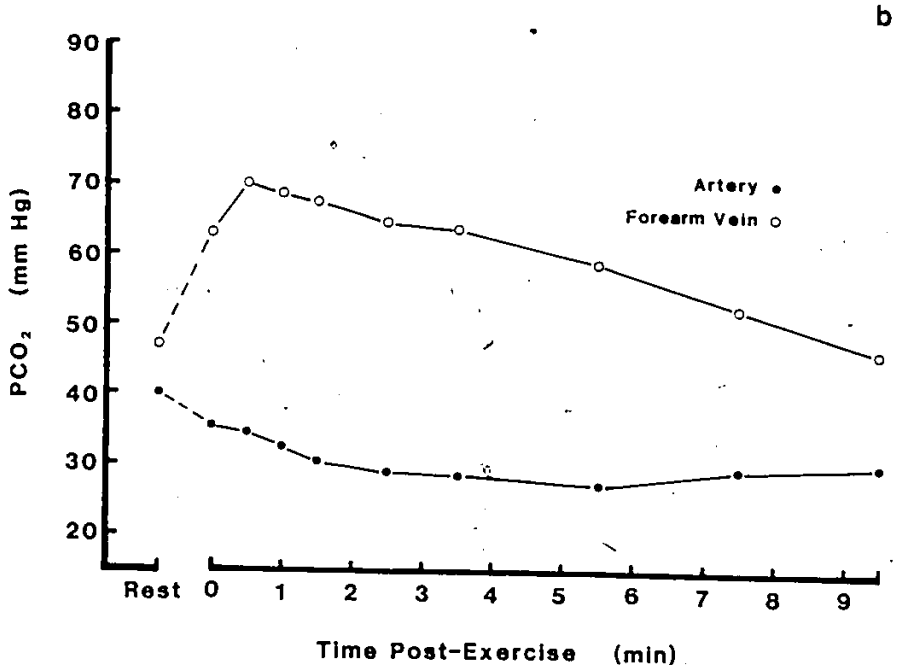
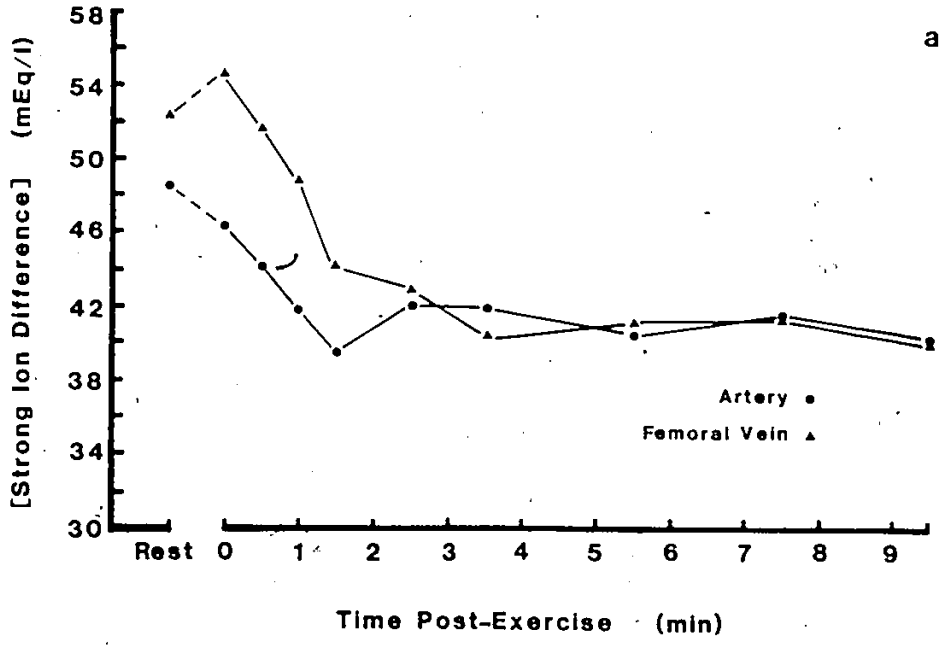
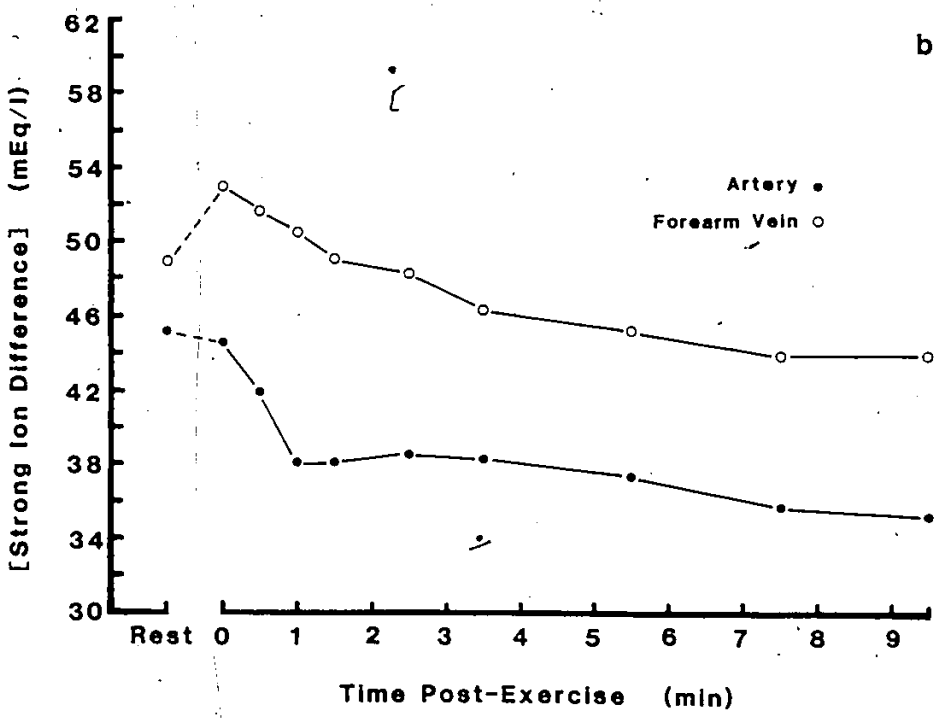

b
90
80
70
60
50
40
30
20
PCO_2 (mm Hg)
Artery •
Forearm Vein ○
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

Figure 7 a. Arterial and femoral venous strong ion difference (mEq/l) at rest and during recovery from maximal exercise (n=3).

b. Arterial and forearm venous strong ion difference (mEq/l) at rest and during recovery from maximal exercise (n=6).


a
[Strong Ion Difference] (mEq/l)
58
54
50
46
42
38
34
30
Artery •
Femoral Vein ▲
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)


b
[Strong Ion Difference] (mEq/l)
62
58
54
50
46
42
38
34
30
Artery •
Forearm Vein ○
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

3.3.4 Strong ion concentrations in the artery and femoral vein

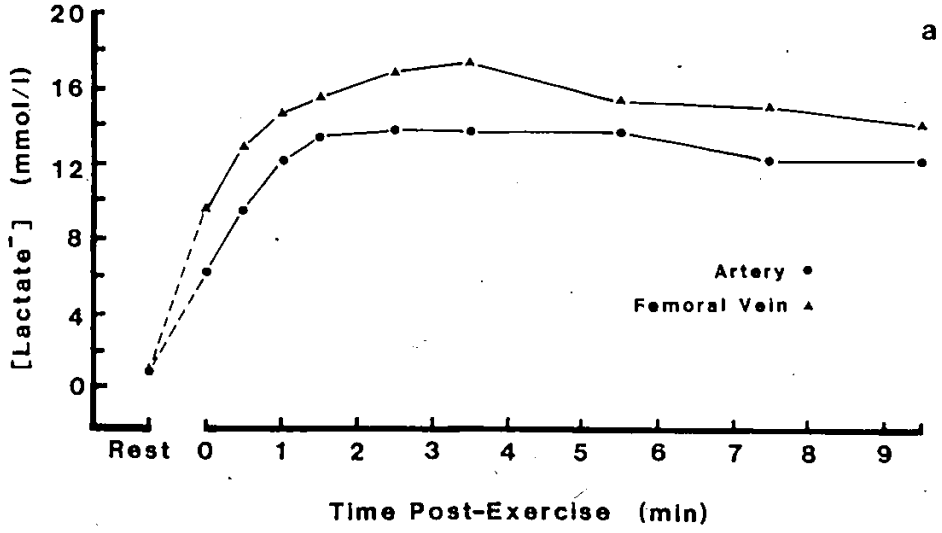
The calculation of [SID] is based on the plasma concentration of strong ions. However, when estimating ion movements across the muscle the venous and arterial ion concentrations should first be corrected for the decrease in plasma volume that occurs with heavy exercise. The ion concentrations measured in the femoral vein and artery are presented in Table 5 and the mean values corrected for changes in plasma volume are presented in Table 6. The fv-a differences for measured and corrected values are presented in Tables 7 and 8, respectively.

Lactate : The femoral venous and arterial lactate concentration ([lactate]) increased after exercise (Table 5 and 6, Fig. 8a); lactate levels averaged 15.0 ± 1.0 mmol/l and 13.1 ± 1.1 mmol/l in the femoral vein and artery, respectively. The fv-a [lactate] difference was positive throughout recovery and represented a net release of lactate from the muscle into the extracellular space (Table 7 and 8).

Sodium : The femoral venous sodium concentration ($[Na^+]$) increased from 138 ± 2 mmol/l at rest to 150 ± 3 mmol/l immediately post-exercise (Table 5, Fig. 9a). The $[Na^+]$ decreased for the remainder of recovery, reaching resting levels after 5 min. The arterial $[Na^+]$ was 137 ± 1 mmol/l at rest and increased to 144 ± 2

Figure 8 a. Arterial and femoral venous lactate concentrations (mmol/l) at rest and during recovery from maximal exercise (n=3). Values have not been corrected for the decrease in plasma volume.

b. Arterial and forearm venous lactate concentrations (mmol/l) at rest and during recovery from maximal exercise (n=6). Values have not been corrected for the decrease in plasma volume.


a
20
16
12
8
4
0
[Lactate⁻] (mmol/l)
Artery •
Femoral Vein ▲
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

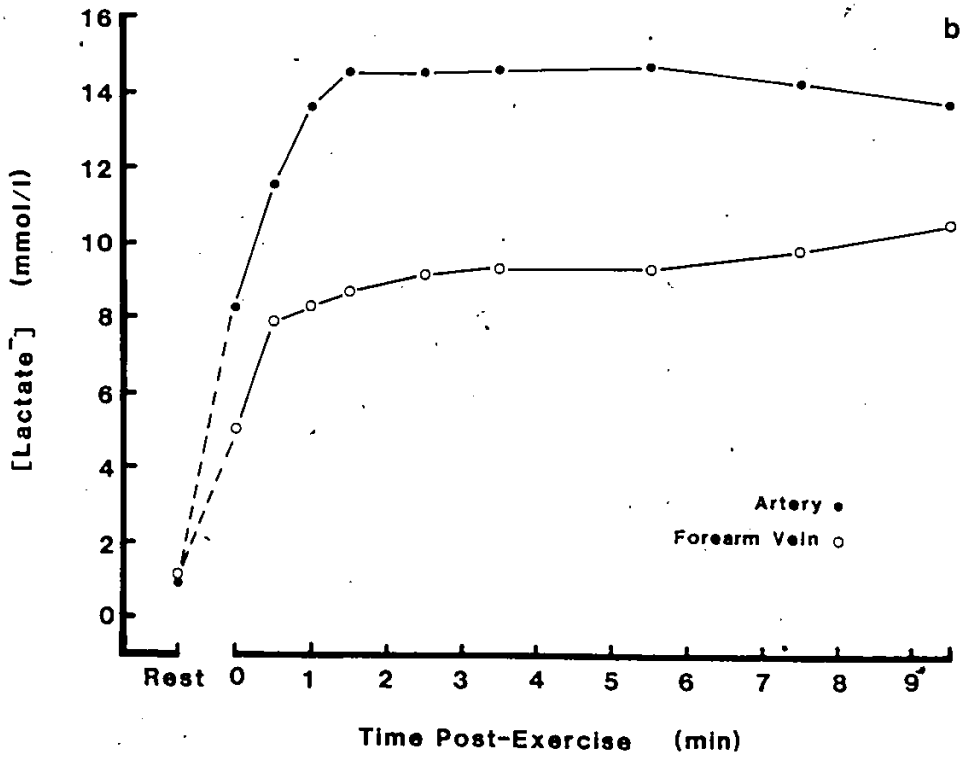

b
16
14
12
10
8
6
4
2
0
[Lactate⁻] (mmol/l)
Artery •
Forearm Vein ○
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

Table 5. Plasma strong ion (lactate, Na^+, K^+, Ca^{2+}, Cl^-) concentrations in the femoral vein and artery at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Femoral Vein:										
lactate, mmol/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
Na^+, mmol/l	138 ±2	150* ±3	149* ±2	147* ±2	145* ±1	143 ±2	141 ±1	139 ±1	138 ±1	138 ±1
K^+, mmol/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+}, mmol/l	1.11 ±0.04	1.35* ±0.03	1.33* ±0.03	1.28* ±0.02	1.22* ±0.03	1.21* ±0.02	1.16 ±0.05	1.16 ±0.04	1.17 ±0.04	1.13 ±0.04
Cl^-, mmol/l	92 ±6	97 ±3	94 ±4	92 ±5	93 ±7	91 ±5	91 ±6	90 ±5	89 ±6	91 ±3
Artery:										
lactate, mmol/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
Na^+, mmol/l	137 ±1	144* ±2	145* ±1	144* ±1	143* ±1	141* ±1	141* ±1	139* ±1	138 ±1	138 ±1
K^+, mmol/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+}, mmol/l	1.11 ±0.04	1.21* ±0.08	1.22* ±0.06	1.19* ±0.07	1.17* ±0.06	1.16* ±0.04	1.17* ±0.04	1.15 ±0.04	1.14* ±0.05	1.14* ±0.04
Cl^-, mmol/l	94 ±2	101 ±2	100 ±1	98 ±1	97 ±5	93 ±3	92 ±3	92 ±1	91 ±2	92 ±2

values are mean ± SD

* significantly different from rest ($p < 0.05$)

Table 6. Plasma strong ion concentrations in the femoral vein and artery at rest and during recovery from maximal exercise (corrected for changes in plasma volume)*.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Femoral Vein:										
lactate, mmol/l	1.0	8.5	12.0	13.5	14.6	15.5	16.3	15.0	14.2	13.9
Na^+, mmol/l	138	132	137	134	134	132	131	135	129	135
K^+, mmol/l	5.4	6.9	6.3	5.6	5.3	4.9	4.8	5.0	5.0	5.1
Ca^{2+}, mmol/l	1.11	1.19	1.22	1.17	1.13	1.11	1.07	1.12	1.09	1.10
Cl^-, mmol/l	92	85	86	84	86	84	84	87	84	89
plasma volume, %Δ**	-	-13.5	-8.8	-9.4	-7.5	-8.8	-8.2	-3.6	-6.9	-2.2
Artery:										
lactate, mmol/l	0.9	5.7	8.7	11.2	12.4	12.8	12.8	12.6	11.5	11.4
Na^+, mmol/l	137	131	132	132	132	130	131	127	130	128
K^+, mmol/l	4.5	6.3	5.7	5.2	4.8	4.4	4.3	4.1	4.3	4.3
Ca^{2+}, mmol/l	1.11	1.10	1.11	1.11	1.08	1.07	1.09	1.05	1.07	1.06
Cl^-, mmol/l	94	92	91	90	90	85	85	84	86	86
plasma volume, %Δ**	-	-9.8	-9.8	-8.5	-8.5	-8.5	-7.9	-9.8	-6.5	-7.9

values are mean data for 3 subjects

* all values were corrected for change in plasma volume according to the following formula:
$[x]_t/(1 - (([Hb]_{rest}/[Hb]_t) - 1))$

** % change in plasma volume calculated as:
$(([Hb]_{rest}/[Hb]_t) - 1) \times 100$

Table 7. Femoral venous-arterial difference for plasma strong ions (lactate, Na^+, K^+, Ca^{2+}, Cl^-) at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
lactate, mmol/l	0.1 ±0.1	3.4 ±3.2	3.5 ±2.4	2.6 ±2.5	2.2 ±2.5	3.0* ±1.4	3.7 ±2.8	1.7 ±2.2	2.9 ±0.5	1.8 ±0.3
Na^+, mmol/l	1.0 ±1.0	6.0 ±5.3	4.3* ±2.1	3.3* ±1.5	1.3* ±1.2	1.7 ±1.2	0.7 ±0.6	0.3 ±0.6	0 ±1.0	0 ±0
K^+, mmol/l	0.9 ±0.7	1.0 ±0.6	0.6 ±0.7	0.5 ±0.9	0.3 ±0.6	0.6 ±0.6	0.5 ±0.6	0.7 ±0.6	0.7 ±0.5	0.5 ±0.5
Ca^{2+}, mmol/l	0 ±0.08	0.14 ±0.10	0.10* ±0.04	0.09 ±0.06	0.01 ±0.03	0.05 ±0.05	-0.01 ±0.07	0.01 ±0.02	0.03 ±0.04	-0.01 ±0.05
Cl^-, mmol/l	-2.0 ±7.8	-4.3* ±2.5	-6.0 ±4.0	-5.7 ±3.8	-4.7 ±3.1	-1.7 ±2.5	-1.0 ±5.3	-1.3 ±4.0	-2.0 ±4.6	-1.3 ±1.5

values are mean ± SD

* significant difference between femoral vein and artery ($p < 0.05$)

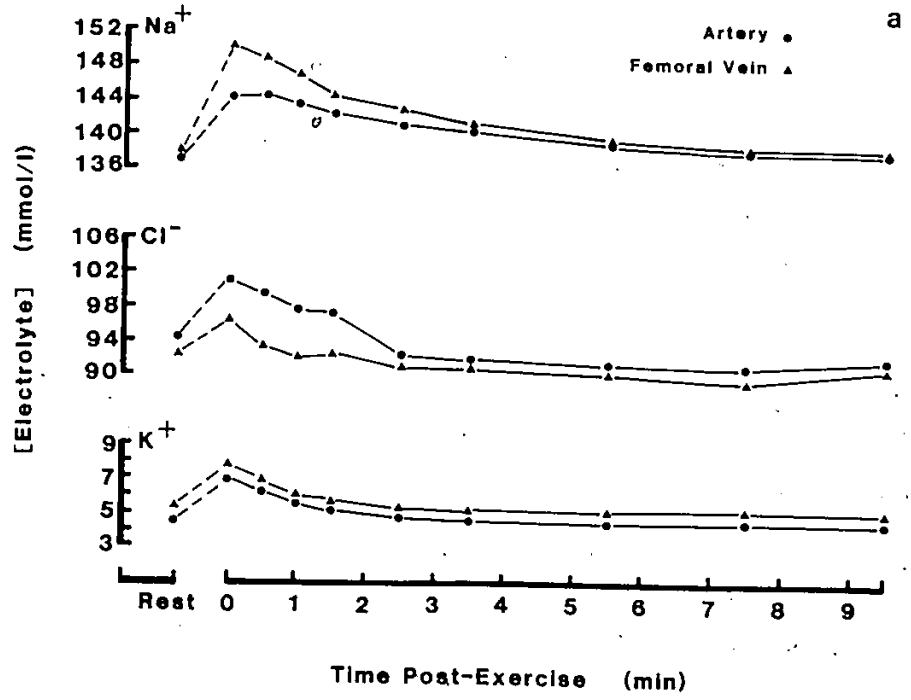
Table 8. Femoral venous-arterial differences for plasma strong ions at rest and during recovery from maximal exercise (corrected for changes in plasma volume).

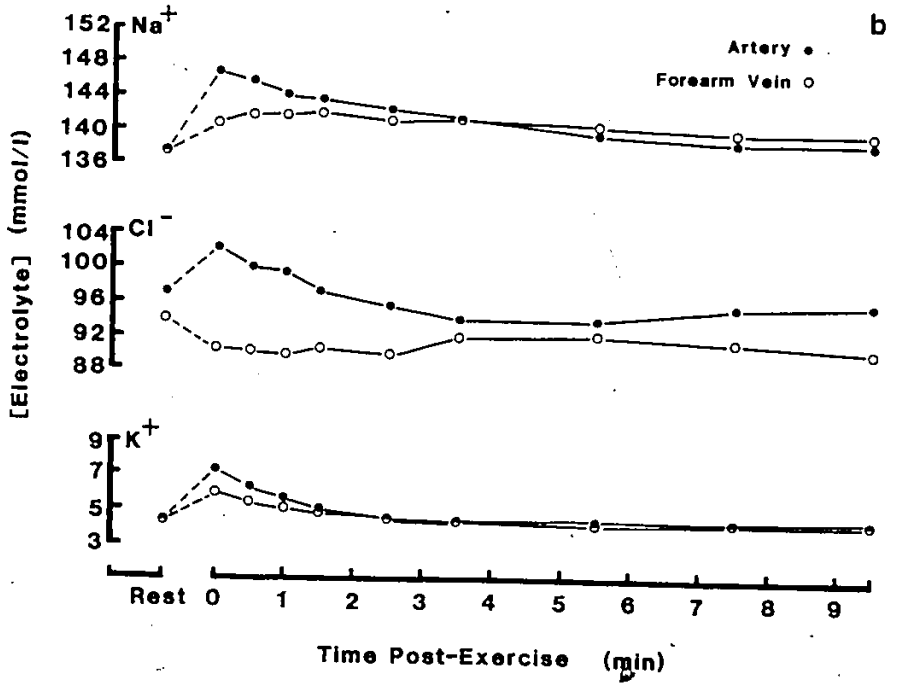
	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
lactate, mmol/l	0.1	2.8	3.3	2.3	2.2	2.7	3.5	2.4	2.7	2.5
Na^+, mmol/l	1.0	1.0	5.2	2.0	2.9	1.3	0.1	7.9	-0.4	7.2
K^+, mmol/l	0.9	0.6	0.6	0.4	0.5	0.5	0.5	0.9	0.7	0.8
Ca^{2+}, mmol/l	0	0.09	0.11	0.06	0.05	0.04	-0.02	0.07	0.02	0.04
Cl^-, mmol/l	-2.0	-7.0	-5.0	-6.0	-4.0	-1.0	-1.0	3.0	-2.0	3.0

values are mean data for 3 subjects

Figure 9 a. Arterial and femoral venous Na^+, K^+ and Cl^- concentrations (mmol/l) at rest and during recovery from maximal exercise (n=3).

b. Arterial and forearm venous Na^+, K^+ and Cl^- concentrations (mmol/l) at rest and during recovery from maximal exercise (n=6).


a
Artery •
Femoral Vein ▲
152
148
144
140
136
Na+
106
102
98
94
90
Cl−
9
7
5
3
K+
[Electrolyte] (mmol/l)
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)


b
Artery •
Forearm Vein ○
152
148
144
140
136
Na+
104
100
96
92
88
Cl−
9
7
5
3
K+
[Electrolyte] (mmol/l)
Rest 0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

mmol/l after maximal exercise (Table 5, Fig. 9a). The arterial [Na^+] decreased and reached resting levels after 7 min of recovery (Fig. 9a).

The femoral venous and arterial [Na^+] decreased when corrected for changes in plasma volume (Table 6). The fv-a [Na^+] difference was variable but positive throughout recovery indicating that sodium was being released from the working muscle (Table 8).

Potassium : The resting potassium concentration ([K^+]) was higher in the femoral vein (5.4 ± 1.1 mmol/l) than in the artery (4.5± 0.4 mmol/l). The [K^+] increased during maximal exercise; peak values occurred immediately after exercise and were 7.8 ± 1.2 mmol/l and 6.9± 1.0 mmol/l in the femoral vein and artery, respectively (Table 5, Fig. 9a). During recovery the [K^+] decreased and reached resting levels after 1-2 min.

An increase in the plasma [K^+] was observed even after correcting for the decrease in plasma volume (Table 6). The fv-a [K^+] difference was small but positive throughout recovery indicating a net release of potassium from the muscle into the extracellular space (Table 8).

Ionised Calcium : At rest the concentration of ionised calcium ([Ca^{2+}]) was 1.11 ± 0.04 mmol/l in the both the femoral vein

and artery. After 30 s maximal exercise the $[Ca^{2+}]$ increased to 1.35 ± 0.03 mmol/l in the femoral vein and 1.21 ± 0.08 mmol/l in the artery (Table 5). The $[Ca^{2+}]$ decreased during recovery; resting levels were reached after 3 min in the femoral vein, but the arterial $[Ca^{2+}]$ remained above resting levels for the entire recovery.

The $[Ca^{2+}]$ increased when corrected for the change in plasma volume (Table 6). The fv-a $[Ca^{2+}]$ difference demonstrated a small increase in calcium across the active muscle (Table 8).

Chloride : The femoral venous chloride concentration ($[Cl^-]$) was 92 ± 6 mmol/l at rest and did not change significantly after maximal exercise (Table 5, Fig. 9a). The arterial $[Cl^-]$ increased from 94 ± 2 mmol/l at rest to 101 ± 2 mmol/l immediately post-exercise (Table 5, Fig. 9a). The $[Cl^-]$ decreased to resting levels after 1 min of recovery.

The corrected $[Cl^-]$ decreased in the femoral vein and artery after exercise (Table 6); the fv-a $[Cl^-]$ was negative and represented a net uptake of chloride by the active muscle (Table 8).

3:3.5 Bicarbonate concentration across the active muscle

The femoral venous bicarbonate concentration ($[HCO_3^-]$) was 27.3± 0.9 mmol/l at rest. The $[HCO_3^-]$ began decreasing by 1 min recovery and

reached steady levels (15.4 ± 1.2 mmol/l) by 3 min recovery (Table 3, Fig. 10a). The arterial [HCO_3^-] was 26.6 ± 1.7 mmol/l at rest and decreased immediately after exercise, reaching steady levels (14.1 ± 1.1 mmol/l) after 3 min recovery (Table 3, Fig. 10a).

3.4 ACID-BASE CHANGES ACROSS THE INACTIVE MUSCLE DURING AND AFTER MAXIMAL EXERCISE (PART B)

Maximal cycling exercise produces a generalised acidosis in the body as the intracellular acidosis is corrected. The inactive tissue may play a role in regulating the whole body acid-base balance. The forearm muscle was studied to determine the role played by the inactive tissue in the recovery process.

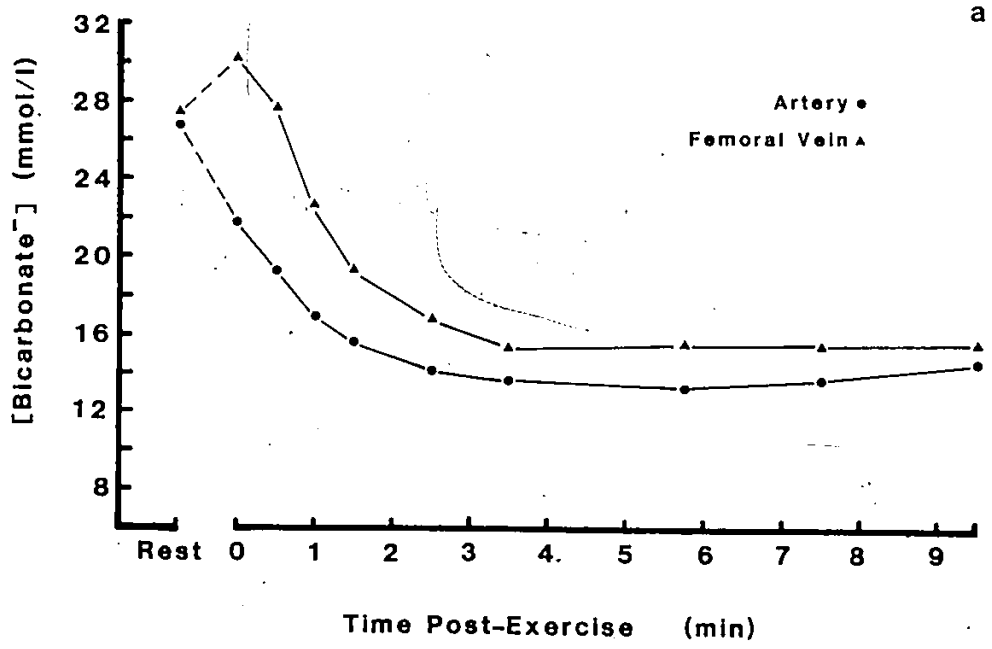
3.4.1 Hydrogen ion concentration across the inactive muscle

The arterial [H^+] was 37.7 ± 2.2 nmol/l (pH 7.43 ± 0.03) at rest. An acidosis developed in the artery after maximal leg exercise; the [H^+] reached steady levels (60.8 ± 10.9 nmol/l) after 3 min recovery (Table 9, Fig. 5b).

The forearm venous [H^+] was 42.5 ± 2.9 nmol/l (pH 7.37 ± 0.03) at rest. The venous [H^+] increased rapidly and reached steady levels (68.3 ± 15.6 nmol/l) by 1 min recovery (Table 9, Fig. 5b). The venous [H^+] was higher than arterial values throughout the recovery (Table

Figure 10 a. Arterial and femoral venous bicarbonate concentrations (mmol/l) at rest and during recovery from maximal exercise (n=3).

b. Arterial and forearm venous bicarbonate concentrations (mmol/l) at rest and during recovery from maximal exercise (n=6).


a
Artery •
Femoral Vein ▲
[Bicarbonate⁻] (mmol/l)
32
28
24
20
16
12
8
Rest
0
1
2
3
4
5
6
7
8
9
Time Post-Exercise (min)

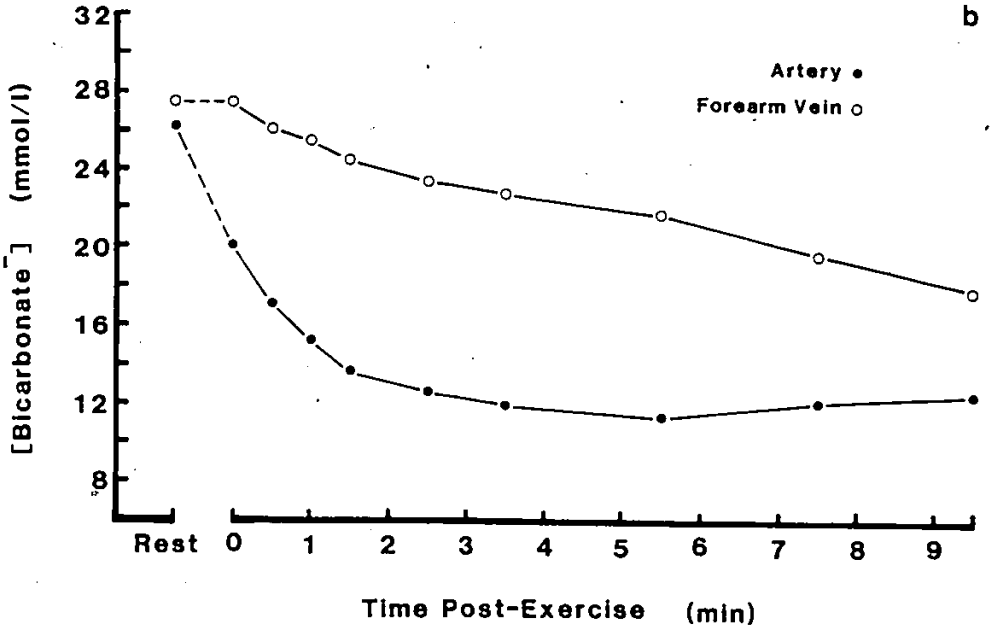

b
Artery •
Forearm Vein ○
[Bicarbonate⁻] (mmol/l)
32
28
24
20
16
12
8
Rest
0
1
2
3
4
5
6
7
8
9
Time Post-Exercise (min)

Table 9. Dependent (H^+, HCO_3^-) and independent (PCO_2, SID, ATOT) variables in the forearm vein and artery at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min							
		0	-0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Forearm Vein:										
H^+, nmol/l	42.5 ±2.9	57.1* ±9.5	67.7* ±17.6	67.7* ±15.8	69.1* ±16.8	69.5* ±16.9	70.6* ±17.3	68.4* ±16.5	66.8* ±15.5	66.7* ±18.9
PCO_2, mm Hg	47.1 ±4.3	63.1* ±7.5	69.9* ±10.7	68.8* ±9.9	67.4* ±10.3	64.6* ±10.0	63.4* ±8.5	58.4* ±4.8	52.0 ±7.6	46.1 ±4.1
SID, mEq/l	48.9 ±3.2	53.0* ±1.8	51.7 ±4.9	50.5 ±4.2	49.1 ±1.9	48.3 ±1.9	46.3* ±1.5	45.2* ±3.4	43.9* ±3.3	43.9* ±6.1
ATOT, mEq/l**	20	23	23	23	23	23	23	23	22	22
HCO_3^-, mmol/l	27.4 ±2.2	27.4 ±2.4	26.1 ±3.7	25.5* ±2.8	24.5* ±3.2	23.4* ±3.3	22.8* ±3.7	21.7* ±3.5	19.6* ±3.3	17.7* ±3.4
Artery:										
H^+, nmol/l	37.7 ±2.2	43.8* ±4.9	50.3* ±4.5	53.4* ±6.8	55.8* ±8.4	57.8* ±7.9	60.1* ±9.9	61.1* ±11.5	61.1* ±11.6	61.1* ±13.4
PCO_2, mmol/l	39.9 ±2.5	35.3* ±1.9	34.5* ±2.0	32.6* ±2.0	30.5* ±2.5	29.0* ±2.0	28.5* ±2.4	26.9* ±2.5	29.2* ±2.3	29.8* ±1.4
SID, mEq/l	45.2 ±2.2	44.5 ±2.1	41.8* ±2.8	38.0* ±2.6	38.1* ±4.2	38.5* ±3.4	38.3* ±5.1	37.3* ±3.6	35.7* ±5.1	35.3* ±2.5
ATOT, mEq/l**	20	22	22	22	22	22	22	22	22	22
HCO_3^-, mmol/l	26.1 ±1.2	20.0* ±1.5	17.1* ±1.8	15.2* ±1.8	13.6* ±1.9	12.6* ±2.1	11.9* ±2.2	11.3* ±2.4	12.1* ±2.2	12.4* ±2.3

values are mean ± SD

* significantly different from rest ($p < 0.05$)

** estimated from Table 7.3 (Stewart, 1981) and from the mean % change in forearm vein and arterial hemoglobin concentration.

10).

3.4.2 Plasma PCO_2 across the inactive muscle

Arterial PCO_2 was 39.9 ± 2.5 mm Hg at rest and decreased to 35.3 ± 1.9 mm Hg immediately after maximal exercise (Table 9, Fig. 6b). The arterial PCO_2 was less than resting levels throughout recovery.

The forearm venous PCO_2 was 47.1 ± 4.3 mm Hg at rest and increased to 69.9 ± 10.7 mm Hg at 30 s recovery (Table 9, Fig. 6b). The venous PCO_2 decreased slowly for the remainder of recovery, reaching resting levels after 7 min recovery. The PCO_2 was higher in the vein than in the artery throughout recovery (Table 10).

3.4.3 Strong ion difference across the inactive muscle

At rest the [SID] was 45.2 ± 2.2 mEq/l in the artery and 48.9± 3.2 mEq/l in the forearm vein. The arterial [SID] decreased following maximal leg exercise (Table 9, Fig. 7b). The forearm venous [SID] 48.9 ± 3.2 increased to 53.0 ± 1.8 mEq/l immediately after exercise and then decreased for the remainder of the recovery. The [SID] was higher in the forearm vein than in the artery during the entire recovery (Table 10, Fig. 7b).

Table 10. Forearm venous-arterial differences for selected acid-base variables at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
H^+, nmol/l	4.8* ±1.5	13.4* ±5.2	16.4* ±13.8	14.1* ±9.7	13.3* ±9.1	11.7* ±9.3	10.5* ±8.5	7.3* ±5.7	5.7* ±4.3	5.6* ±5.7
PCO_2, mm Hg	7.2* ±4.8	27.9* ±6.9	34.8* ±12.0	36.2* ±8.8	36.9* ±9.4	35.6* ±10.3	35.0* ±8.3	31.5* ±7.0	22.8* ±7.2	16.3* ±5.1
SID, mEq/l	3.8* ±3.6	8.5* ±3.1	9.9* ±4.4	12.6* ±6.7	11.0* ±3.3	10.2* ±1.8	7.7* ±6.5	7.4* ±1.5	9.0* ±8.5	6.4* ±4.3
HCO_3^-, mmol/l	1.3 ±2.3	7.5* ±1.4	9.1* ±2.4	10.5* ±1.5	11.3* ±1.6	11.2* ±1.5	11.3* ±1.9	10.8* ±2.0	7.5* ±2.1	5.2* ±2.0

values are mean ± SD

* significant difference between forearm vein and artery ($p < 0.05$)

The decrease in the [SID] indicated that after maximal leg exercise the concentration of strong anions increased relative to the concentration of strong cations. In addition, the strong anion concentration was higher in the artery than in the forearm vein.

3.4.4 Strong ion concentrations in the artery and forearm vein

The measured concentration of strong ions is presented in Table 11. These values were corrected for the decrease in plasma volume and are presented in Table 12. The forearm venous-arterial difference for the measured and corrected values are presented in Tables 13 and 14, respectively.

Lactate : The arterial [lactate] was 0.9 ± 0.2 mmol/l at rest. After maximal exercise the [lactate] increased, reaching steady levels (14.2 ± 1.7 mmol/l) after 1 min recovery (Table 11, Fig. 8b). The forearm venous [lactate] was 1.1 ± 0.2 mmol/l at rest. The venous [lactate] increased immediately following maximal exercise and continued to increase gradually throughout the recovery (Table 11 and 12, Fig. 8b). The venous [lactate] was less than the arterial concentration throughout recovery indicating that lactate was being taken up by the inactive muscle (Table 14).

Sodium : The resting [Na^+] was similar in the artery (137 ± 2 mmol/l) and forearm vein (138 ± 2 mmol/l) at rest. After maximal leg

Table 11. Plasma strong ion (lactate, Na^+, K^+, Ca^{2+}, Cl^-) concentrations in the forearm vein and artery at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Forearm Vein:										
lactate, mmol/l	1.1 ±0.2	5.0* ±2.3	7.9* ±3.7	8.3* ±2.8	8.7* ±3.0	9.1* ±2.7	9.3* ±3.4	9.3* ±2.9	9.8* ±2.0	10.5* ±2.9
Na^+, mmol/l	138 ±2	141* ±1	142* ±2	142* ±1	142* ±1	141* ±1	142* ±1	141* ±1	140* ±1	139 ±1
K^+, mmol/l	4.3 ±0.3	5.9* ±0.4	5.4* ±0.2	5.1* ±0.2	4.9* ±0.3	4.5* ±0.2	4.4 ±0.4	4.3 ±0.5	4.4 ±0.4	4.4 ±0.4
Ca^{2+}, mmol/l	1.08 ±0.05	1.21* ±0.08	1.24* ±0.12	1.21* ±0.08	1.20* ±0.07	1.17* ±0.07	1.18* ±0.09	1.17* ±0.10	1.13* ±0.06	1.12 ±0.09
Cl^-, mmol/l	93 ±3	91* ±4	90 ±4	90 ±4	91* ±3	90* ±2	92 ±3	92 ±3	92 ±4	90* ±5
Artery:										
lactate, mmol/l	0.9 ±0.2	8.3* ±1.9	11.5* ±1.4	13.6* ±1.3	14.5* ±1.3	14.5* ±1.7	14.6* ±1.8	14.7* ±1.7	14.2* ±2.0	13.7* ±2.2
Na^+, mmol/l	137 ±2	146* ±1	145* ±2	144* ±1	143* ±1	142* ±1	141* ±1	140* ±1	139 ±1	138 ±1
K^+, mmol/l	4.3 ±0.3	7.2* ±0.8	6.3* ±0.9	5.6* ±0.7	5.1* ±0.6	4.5 ±0.6	4.4 ±0.6	4.4 ±0.5	4.4 ±0.5	4.4 ±0.5
Ca^{2+}, mmol/l	1.11 ±0.05	1.22* ±0.09	1.22* ±0.10	1.22* ±0.09	1.22* ±0.11	1.18* ±0.09	1.15* ±0.08	1.14* ±0.06	1.13 ±0.07	1.14* ±0.06
Cl^-, mmol/l	97 ±3	102* ±4	100 ±5	100* ±3	97 ±5	96 ±2	94 ±5	94 ±2	96 ±3	96 ±1

values are mean ± SD

* significantly different from rest ($p < 0.05$)

Table 12. Plasma strong ion concentrations in the forearm vein and artery at rest and during recovery from maximal exercise (corrected for changes in plasma volume)*.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Forearm Vein:										
lactate, mmol/l	1.1	4.4	6.9	7.3	7.7	8.0	8.2	8.2	8.3	9.6
Na^+, mmol/l	138	125	123	125	125	125	124	124	125	128
K^+, mmol/l	4.3	5.2	4.7	4.5	4.3	4.0	3.8	3.8	4.0	4.0
Ca^{2+}, mmol/l	1.08	1.07	1.08	1.06	1.06	1.03	1.04	1.03	1.01	1.03
Cl^-, mmol/l	93	81	79	80	80	80	81	81	82	83
plasma volume, %Δ**	-	-12.7	-14.9	-13.2	-13.2	-13.2	-13.8	-13.2	-11.5	-9.0
Artery:										
lactate, mmol/l	0.9	7.4	10.4	12.3	13.1	13.1	13.4	13.4	13.1	12.6
Na^+, mmol/l	137	131	131	130	129	128	129	127	128	127
K^+, mmol/l	4.3	6.5	5.7	5.0	4.6	4.1	4.0	4.0	4.1	4.1
Ca^{2+}, mmol/l	1.11	1.09	1.10	1.10	1.10	1.06	1.05	1.04	1.04	1.04
Cl^-, mmol/l	97	92	90	90	88	86	86	86	88	88
plasma volume, %Δ**	-	-11.6	-11.0	-11.0	-11.0	-11.0	-9.1	-9.7	-8.5	-9.1

values are mean data for 6 subjects

* all values were corrected for change in plasma volume according to the following formula:
$[x]_t/(1 - (([Hb]_{rest}/[Hb]_t) - 1))$

** % change in plasma volume calculated as:
$(([Hb]_{rest}/[Hb]_t) - 1) \times 100$

Table 13. Forearm venous-arterial differences for plasma strong ions (lactate, Na^+, K^+, Ca^{2+}, Cl^-) at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
lactate, mmol/l	0.2 ±0.3	-3.3* ±1.5	-3.7* ±3.3	-5.3* ±1.8	-5.7* ±2.2	-5.4* ±1.5	-5.2* ±2.1	-5.5* ±1.7	-4.4* ±1.1	-3.2* ±0.8
Na^+, mmol/l	0.2 ±0.8	-5.6* ±1.7	-3.8* ±1.3	-2.2* ±1.5	-1.4* ±1.1	-1.2 ±1.3	0 ±1.0	1.0 ±0	1.2 ±1.3	1.2 ±0.4
K^+, mmol/l	0 ±0.5	-1.3* ±1.0	-0.8 ±1.0	-0.5 ±0.7	-0.2 ±0.7	0 ±0.6	0 ±0.5	-0.1 ±0.6	0 ±0.5	0 ±0.6
Ca^{2+}, mmol/l	-0.03* ±0.02	-0.01 ±0.80	0.02 ±0.11	-0.02 ±0.08	-0.01 ±0.06	0 ±0.05	0.03 ±0.05	0.03 ±0.10	0 ±0.06	-0.02 ±0.07
Cl^-, mmol/l	-4.0* ±3.4	-11.7* ±3.3	-9.4* ±3.6	-9.7* ±4.3	-6.3* ±2.7	-5.7* ±2.4	-2.0 ±6.0	-1.8 ±2.8	-4.2 ±6.2	-5.5* ±5.7

values are mean ± SD

* significant difference between forearm vein and artery ($p < 0.05$)

Table 14. Forearm venous-arterial differences for plasma strong ions at rest and during recovery from maximal exercise (corrected for changes in plasma volume).

	Rest	Time Post-Exercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
lactate, mmol/l	0.2	-3.0	-3.5	-5.0	-5.4	-5.1	-5.2	-5.2	-4.8	-3.0
Na^+, mmol/l	0.2	-6.2	-7.6	-4.5	-3.7	-3.5	-5.3	-3.1	-2.4	1.2
K^+, mmol/l	0	-1.2	-1.0	-0.5	-0.3	-0.1	-0.2	-0.2	-0.1	0
Ca^{2+}, mmol/l	-0.03	-0.02	-0.02	-0.04	-0.03	-0.02	-0.02	-0.01	-0.03	-0.02
Cl^-, mmol/l	-4.0	-11.2	-11.4	-10.3	-7.4	-6.6	-5.3	-4.2	-6.1	-5.0

values are mean data for 6 subjects

exercise, the $[Na^+]$ increased in both the artery and vein (Table 11, Fig. 9b). The $[Na^+]$ decreased to resting levels by 10 min recovery.

The corrected $[Na^+]$ decreased below resting levels after exercise in the forearm vein and artery (Table 12). The v-a $[Na^+]$ difference was negative throughout recovery indicating that sodium was taken up by the inactive muscle (Table 14).

Potassium : The arterial and forearm venous $[K^+]$ were similar at rest (4.3 ± 0.3 mmol/l). Immediately after maximal exercise the $[K^+]$ increased; the $[K^+]$ was higher in the artery (7.2 ± 0.8 mmol/l) than in the vein (5.9 ± 0.4 mmol/l) (Tables 11, Fig. 9b). The $[K^+]$ decreased in recovery and reached resting levels by 3 min of recovery (Table 11).

The corrected plasma $[K^+]$ increased immediately after maximal exercise and decreased to below resting levels by the end of recovery (Table 12). The negative v-a $[K^+]$ difference indicated that potassium was taken up by the inactive muscle (Table 14).

Ionised Calcium : The resting $[Ca^{2+}]$ was 1.11 ± 0.05 mmol/l and 1.08 ± 0.05 mmol/l in the artery and forearm vein, respectively. Immediately after maximal exercise the $[Ca^{2+}]$ increased in both the artery and vein (Table 11). During recovery the $[Ca^{2+}]$ slowly decreased towards resting levels (Table 11).

The venous and arterial corrected [Ca^{2+}] were similar to resting levels immediately after maximal exercise but began to decrease later in recovery (Table 12). The v-a [Ca^{2+}] difference was small but negative during recovery indicating that the [Ca^{2+}] decreased across the inactive forearm muscle (Table 14).

Chloride : The resting arterial [Cl^-] was 97 ± 3 mmol/l. The [Cl^-] increased immediately after maximal exercise but returned to resting levels by 1.5 min recovery (Table 11, Fig. 9b). The resting venous [Cl^-] was 93 ± 3 mmol/l. The venous [Cl^-] tended to decrease after maximal exercise, returning to resting levels by 3 min recovery (Table 11, Fig. 9b).

The corrected [Cl^-] decreased after maximal exercise in both the forearm vein and artery (Table 12). The v-a [Cl^-] difference indicated that chloride was taken up by the inactive muscle throughout recovery (Table 14).

3.4.5 Bicarbonate concentration across the inactive muscle

The arterial [HCO_3^-] was 26.1 ± 1.2 mmol/l at rest and decreased immediately after maximal exercise (Table 9, Fig. 10b). A relatively steady [HCO_3^-] (12 ± 2 mmol/l) was reached after 3 min recovery. The forearm venous [HCO_3^-] was 27.4 ± 2.2 mmol/l at rest and

remained steady for the first 30 s recovery (Table 9, Fig. 10b). The venous [HCO_3^-] began decreasing by 1 min recovery and continued to fall for the remainder of the recovery. The venous [HCO_3^-] was higher than the arterial [HCO_3^-] throughout the recovery (Table 10).

3.5 THE CONCENTRATION OF STRONG IONS IN THE ACTIVE MUSCLE DURING AND AFTER MAXIMAL EXERCISE (PART C)

3.5.1 Performance variables during 30 s maximal exercise

Performance during the 30 s of maximal cycle ergometer exercise was assessed and compared to the performance of the subjects in Parts A and B. The subjects in this section (subjects 7-12 in Table 1) performed more work during the 30 s of exercise than the three subjects in Part A (subject 1,2,6 in Table 1); the total work performed was 25.9 ± 1.9 kJ in Part C and 18.6 ± 1.2 kJ in Part A. There were no other differences in performance between any of the groups indicating that the exercise conditions were similar for the three groups.

3.5.2 Arterial acid-base and strong ion concentrations after maximal exercise

Arterial blood was sampled for acid-base (hydrogen ions, PCO_2, [SID]) and strong ion (lactate, sodium, potassium, calcium and

chloride) concentrations. These variables were similar to those obtained at comparable times in Parts A and B and indicate that the response to maximal exercise was similar in the three groups (compare Table 15 with Tables 3, 5, 9, 11).

3.5.3 Fluid volume changes in the active muscle after maximal exercise

Maximal exercise is associated with fluid shifts from the plasma into the interstitial and intracellular compartments of the active muscle. The wet weight/dry weight (w.w./d.w.) ratio of muscle biopsy samples was calculated to determine the effect of 30 s of maximal exercise on the fluid volume change in muscle. The w.w./d.w. ratio was 4.08 ± 0.32 mg w.w./mg d.w. at rest and increased after maximal exercise; the w.w./d.w. ratio was 4.30 ± 0.20 mg w.w./mg d.w., 4.43 ± 0.26 mg w.w./mg d.w. and 4.41 ± 0.20 mg w.w./mg d.w. at 0, 3.5 and 9.5 min recovery, respectively. Therefore the water content of the quadriceps muscle increased from 0.75 ml/gm w.w. at rest to 0.77 ml/gm w.w. throughout exercise.

3.5.4 Intracellular strong ion concentrations in active muscle after maximal exercise

The intracellular strong ion content and concentration were measured in the active quadriceps femoris muscle (vastus lateralis) to

Table 15. Acid-base variables and strong ion concentrations in the artery at rest and during recovery from maximal exercise (Part C).

	Rest	Time Post-Exercise, min 0	3.5	9.5
H^+, nmol/l	36.0 ±0.8	54.3 ±4.6	66.6 ±10.0	66.8 ±10.6
PCO_2, mm Hg	38.9 ±1.9	37.2 ±2.1	29.8 ±2.4	29.4 ±3.0
SID, mEq/l	50.6 ±1.4	44.6 ±2.0	40.2 ±2.4	38.8 ±0.6
HCO_3^-, mmol/l	26.6 ±1.2	17.0 ±2.6	11.3 ±2.5	11.2 ±3.0
lactate, mmol/l	0.8 ±0.2	14.2 ±2.4	17.5 ±2.6	16.4 ±2.6
Na^+, mmol/l	138 ±1	148 ±1	143 ±1	140 ±1
K^+, mmol/l	4.2 ±0.4	6.5 ±0.3	3.8 ±0.1	3.9 ±0.2
Ca^{2+}, mmol/l	1.12 ±0.03	1.26 ±0.03	1.20 ±0.05	1.18 ±0.05
Cl^-, mmol/l	93 ±1	99 ±1	92 ±1	91 ±1

values are mean ± SD

determine whether ion exchange occurs between the intra- and extracellular compartment during maximal exercise and recovery. Ion exchange could change the intracellular [SID] and thus affect the intracellular acid-base status of the muscle.

The intracellular strong ion contents of resting muscle were: sodium, 26 ± 5 umol/gm d.w.; potassium, 347 ± 57 umol/gm d.w.; calcium, 6.1 ± 1.5 umol/gm d.w.; magnesium, 80.0 ± 8.6 umol/gm d.w.; and chloride, 28 ± 16 umol/gm d.w.. The intracellular strong ion concentrations in resting muscle were: sodium, 9 ± 3 mmol/l; potassium, 124 ± 16 mmol/l; calcium, 1.1 ± 0.3 mmol/l; magnesium, 14.3 ± 1.0 mmol/l; and chloride, 10 ± 6 mmol/l. No significant changes were observed in strong ion contents or concentrations following 30 s maximal exercise (Tables 16 and 17).

3.6 SUMMARY

Maximal exercise lasting 30 s produces an acid load within the muscle that may impair the metabolic and contractile processes in the muscle and lead to fatigue. In this study the acidosis of heavy exercise and the recovery of normal acid-base balance was examined with respect to changes in PCO_2, strong ions and weak acids; that is, those variables responsible for regulating the intra- and extracellular $[H^+]$. This section summarises the main changes occurring in the independent variables during maximal exercise and

Table 16. Intracellular strong ion content (μEq/gm d.w.) in the quadriceps femoris muscle at rest and during recovery from maximal exercise.

	Rest	Time Post- Exercise, min		
		0	3.5	9.5
Na^+	26 ±5 (2)	44 ±6 (2)	47 ±27 (3)	6 ±8 (3)
K^+	347 ±57	342 ±56	357 ±63	344 ±54
Ca^{2+}	6.1 ±1.5 (4)	6.1 ±1.8 (6)	7.7 ±3.4 (6)	5.8 ±1.0 (5)
Mg^{2+}	80.0 ±8.6 (4)	74.6 ±11.0 (6)	79.5 ±21.3 (6)	80.9 ±9.4 (5)
Cl^-	28 ±16 (4)	58 ±50 (4)	56 ±51 (6)	21 ±13 (5)

values are mean ± SD

values in parentheses denotes number of samples used in the analysis

Table 17. Intracellular strong ion concentration (mmol/l i.c. water) and intracellular strong ion difference (mEq/l i.c. water) in the quadriceps femoris muscle at rest and during recovery from maximal exercise.

	Rest	Time Post-Exercise, min 0	3.5	9.5
Na^+	9 ±3 (2)	16 ±4 (2)	17 ±11 (3)	2 ±3 (3)
K^+	124 ±16 (4)	126 ±22 (6)	123 ±13 (6)	118 ±15 (5)
Ca^{2+}	1.1 ±0.3 (4)	1.1 ±0.3 (6)	1.3 ±0.5 (6)	1.0 ±0.1 (5)
Mg^{2+}	14.3 ±1.0 (4)	13.7 ±1.8 (6)	13.6 ±3.0 (6)	13.9 ±1.5 (5)
Cl^-	10 ±6 (4)	17 ±17 (5)	20 ±19 (6)	7 ±4 (5)
lactate*	3	46	-	15
[SID]	151	109	115**	128

values are mean ± SD

values in parentheses denotes number of samples used in the analysis

* estimated from muscle lactate concentration presented in Table 18 and wet weight/dry weight ratio measured in muscle biopsies in this study

** assumes an intracellular lactate concentration of 35 mmol/l i.c. water

recovery, and describes how these changes affect the intra- and extracellular [H^+].

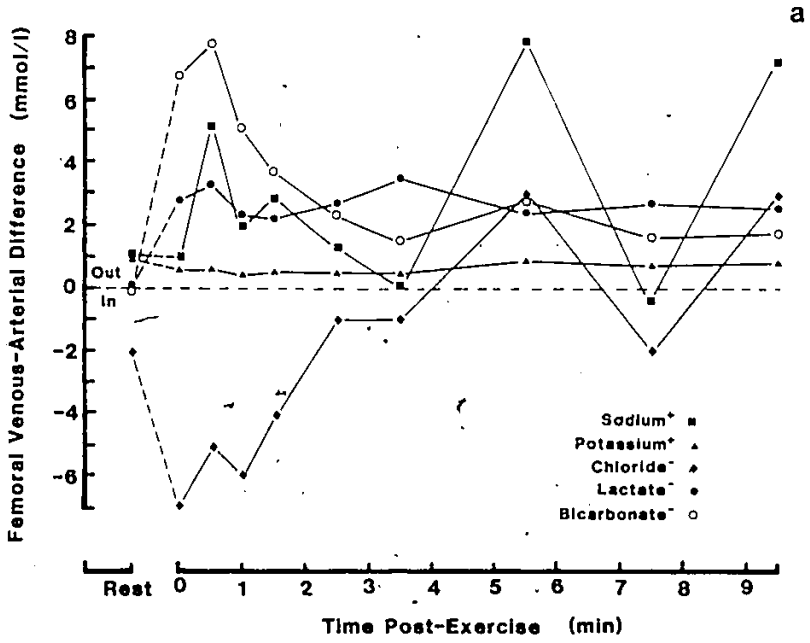
3.6.1 The acid load in the active muscle

An increase in intracellular [H^+] occurs when the intracellular PCO_2 or weak acid concentration ([ATOT]) increase, or when the [SID] decreases. The muscle wet weight/dry weight ratio increased during the 30 s of maximal exercise indicating that the fluid content of the tissue increased. When the strong ion concentrations were corrected for changes in plasma volume, the fv-a concentration differences indicated that sodium, potassium, calcium and lactate were released from the muscle and chloride was taken up by the muscle immediately after maximal exercise (Fig. 11a). However, the intracellular concentration of sodium, potassium, magnesium, calcium or chloride did not change during exercise or recovery (Table 17). The intramuscular [lactate] was not measured in this study but previous studies in which I was a co-investigator demonstrated that the muscle [lactate] increased to approximately 30 mmol/kg w.w. (Tables 18 and 19) or approximately 45 mmol/l i.c. water. This increase in the intracellular [lactate] would decrease the intracellular [SID] by 45 mEq/l and raise the intracellular [H^+].

Phosphate compounds in the muscle contribute to the [ATOT]. The intracellular [ATOT] was not measured in this study but previous

Figure 11 a. Femoral venous-arterial difference for strong ions and bicarbonate at rest and during recovery from maximal exercise. Strong ion concentrations were corrected for the decrease in plasma volume.

b. Forearm venous-arterial difference for strong ions and bicarbonate at rest and during recovery from maximal exercise. Strong ion concentrations were corrected for the decrease in plasma volume.


a
Femoral Venous-Arterial Difference (mmol/l)
8
6
4
2
0
-2
-4
-6
Out
In
Sodium+
Potassium+
Chloride-
Lactate-
Bicarbonate-
Rest
0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

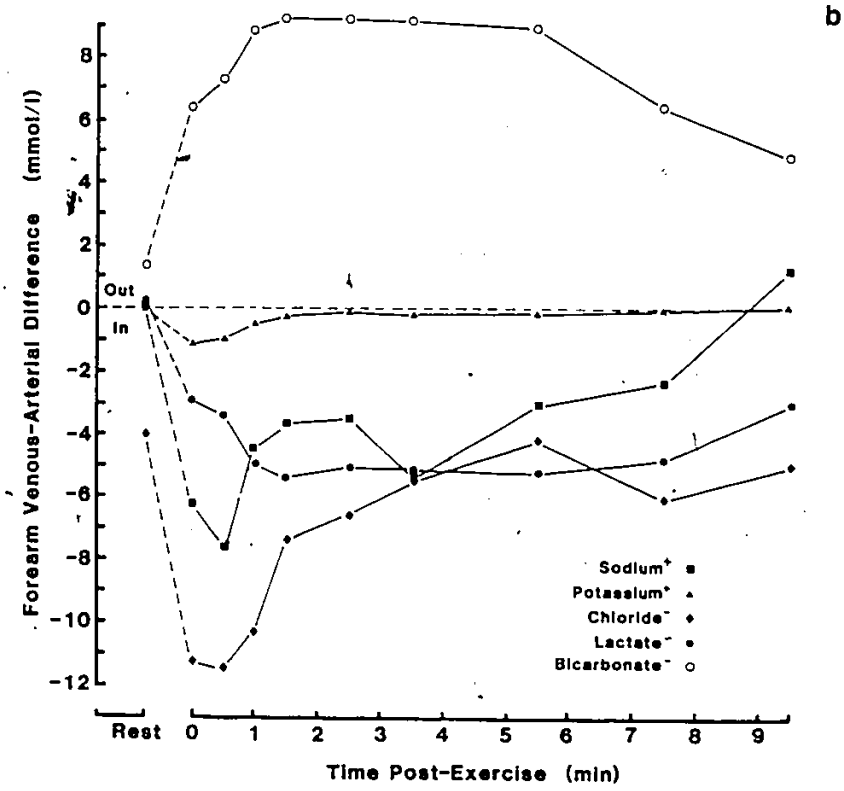

b
Forearm Venous-Arterial Difference (mmol/l)
8
6
4
2
0
-2
-4
-6
-8
-10
-12
Out
In
Sodium+
Potassium+
Chloride-
Lactate-
Bicarbonate-
Rest
0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

Table 18. Muscle metabolite concentrations (mmol/kg w.w.) at rest and during recovery from maximal exercise (pedal speed = 60 rpm).

Metabolite	Rest	Time Post-Exercise, min 0	10
ATP	4.9 ±1.1	3.1 ±1.3	4.0 ±0.8
CP	16.2 ±1.9	6.4 ±3.0	15.4 ±2.9
glucose-6-phosphate	0.15 ±0.07	4.80 ±1.14	0.28 ±0.24
fructose-6-phosphate	0.05 ±0.01	1.19 ±0.62	0.14 ±0.16
fructose 1,6-bisphosphate	0.22 ±0.15	2.01 ±0.83	0.31 ±0.17
lactate	1.8 ±0.6	31.0 ±4.3	10.5 ±2.2

values are mean ± SD

data taken from Table 1 (Jones, et al., 1985)

Table 19. Muscle metabolite concentrations (mmol/kg w.w.) at rest and after a single bout of maximal exercise (pedal speed = 100 rpm).

Metabolite	Rest	Immediately Post-Exercise
ATP	5.2 ±0.4	3.1 ±0.3
CP	14.3 ±0.7	4.2 ±0.9
glucose-6-phosphate	0.52 ±0.11	6.86 ±0.28
fructose-6-phosphate	0.12 ±0.03	1.23 ±0.11
fructose-1,6-bisphosphate	0.27 ±0.06	1.22 ±0.46
lactate	1.4 ±0.3	28.9 ±2.7
glycogen	85.8 ±9.4	67.6 ±2.8

values are mean ± SD

data taken from Table 1 (McCartney, et al., 1985)

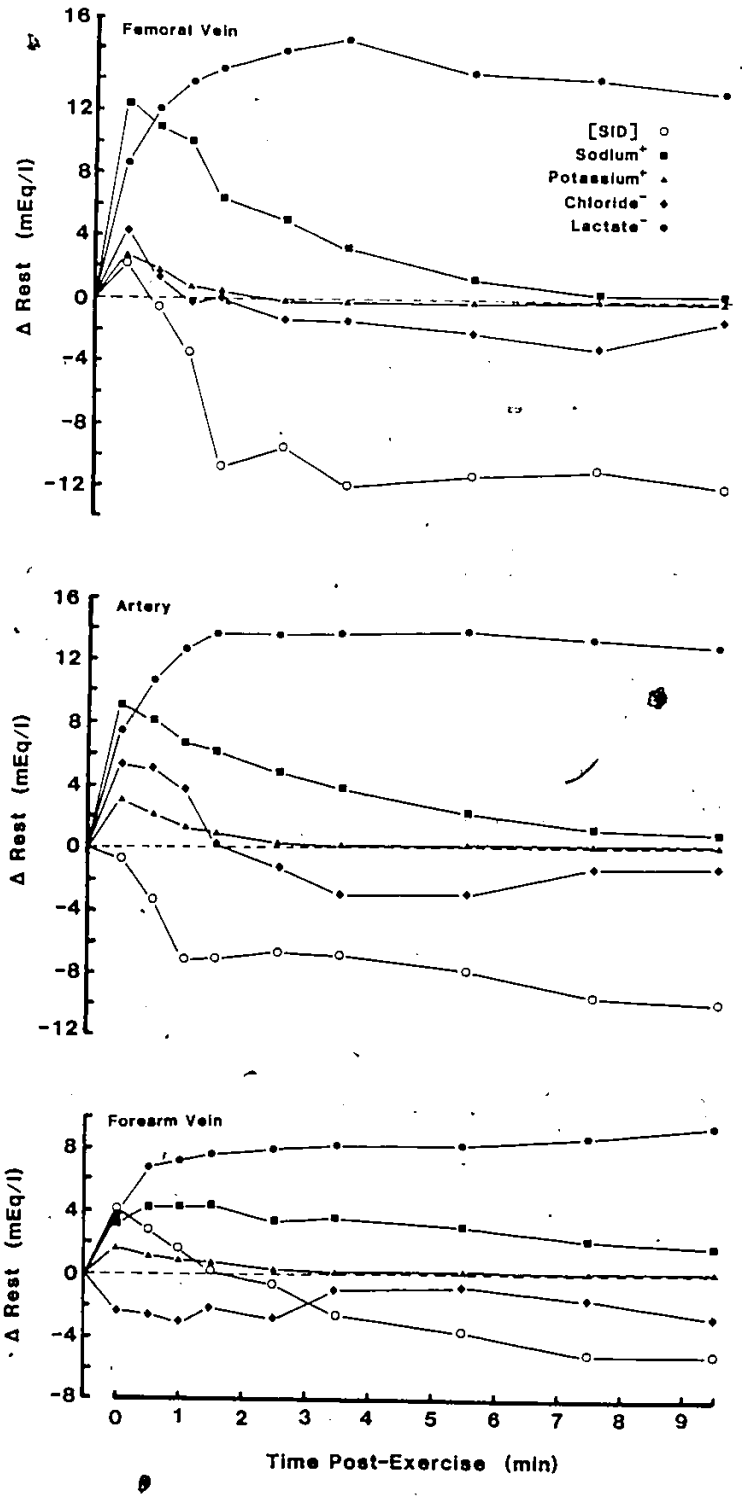
studies have demonstrated that ATP and CP decrease and hexose phosphates (glucose-6-P, fructose-6-P, fructose-1,6-bisP) increase during 30 s exercise (Tables 18 and 19). It cannot be determined to what extent these changes actually affect the [ATOT] or $[H^+]$ since the total phosphate concentration may not change.

3.6.2 Acid-base balance across the active tissue

Following 30 s of maximal exercise there were large changes in the plasma concentration of various acid-base variables. The fv-a concentration difference for hydrogen ions and bicarbonate increased after heavy exercise indicating an "apparent efflux" of these ions from the active muscle. CO_2 output from the muscle occurred rapidly; The femoral venous PCO_2 increased briefly after exercise (Fig. 6a). The increase in femoral venous PCO_2 produced a respiratory acidosis in the plasma.

The plasma [SID] was calculated from the plasma concentration of strong ions. Strong ion exchange across the muscle occurs more slowly than CO_2 exchange. Lactate was released from the muscle throughout recovery (Fig. 11a) and caused the femoral venous and arterial [lactate] to increase (Fig. 8a). The increase in the plasma [lactate] was the main factor responsible for the decrease in the femoral venous and arterial [SID] after heavy exercise (Fig. 12). The decrease in the plasma [SID] produced a metabolic acidosis in the

Figure 12. Recovery-rest differences for femoral venous, arterial and forearm venous strong ion concentrations and strong ion differences.


Femoral Vein
Artery
Forearm Vein
[SID]
Sodium+
Potassium+
Chloride−
Lactate−
Δ Rest (mEq/l)
16
12
8
4
0
-4
-8
-12
0 1 2 3 4 5 6 7 8 9
Time Post-Exercise (min)

plasma after exercise.

The plasma volume decreased after maximal exercise (Table 7) as fluid moved from the plasma into the muscle. The [ATOT] was not measured in this study but the decrease in plasma volume would tend to increase the protein concentration (as determined by the increase in the hemoglobin concentration) and thus increase the [ATOT] (Table 3). An increase in [ATOT] also contributed to a metabolic acidosis in the plasma.

3.6.3 Recovery of whole body acid-base balance

As the $[H^+]$ is mediated by changes in PCO_2, [SID] and [ATOT], recovery of normal acid-base balance requires removal of CO_2 to reduce the PCO_2, removal of lactate to increase the [SID] and increase the plasma volume to lower the [ATOT]. CO_2 output increased during exercise and was maintained during the early part of recovery (Fig. 3 and 4). The $\dot{V}CO_2$ decreased as CO_2 efflux from the muscle reduced. The efficiency with which the lungs removed the excess CO_2 was demonstrated by the fv-a PCO_2 difference across the lung; the arterial PCO_2 was reduced below resting levels after exercise (Fig. 6).

Strong ion exchange occurred across the inactive forearm muscle; the v-a concentration difference demonstrated that sodium, potassium, chloride and lactate were taken up by the forearm muscle

(Fig. 11b). Although the increase in the forearm venous [lactate] was mainly responsible for the fall in the venous [SID] (Fig. 12), lactate uptake by the forearm maintained a higher [SID] in the forearm vein compared to the artery.

4 DISCUSSION

4.1 INTRODUCTION

It has been known since the early 1800's that heavy exercise leads to a rapid production of lactic acid, although the magnitude of this acidosis has only recently been appreciated. Also, the mechanisms controlling muscle contraction and the associated metabolism have become better understood, and the extent to which decreases in pH may impair both contraction and metabolism have been emphasised. Although the control of muscle pH in heavy exercise assumes great importance, our understanding of the quantitative contributions of different processes is very limited. The extent of buffering within muscle, of removal of CO_2 and lactate and the extrusion of hydrogen ions have been debated for many years but largely in the absence of quantitative data. Recently Stewart (1981) has re-examined the concepts underlying the acid-base behaviour of biological solutions and has argued that the $[H^+]$ of a solution should be considered a dependent variable. He has demonstrated using physicochemical principles that the $[H^+]$ of a solution is determined by the independent variables PCO_2, the strong ion difference ([SID]) and the concentration of weak acids ([ATOT]), and a set of equations which govern the solution. Although this approach is theoretically

valid and has been shown mathematically to conform to theory, this approach has not been used to examine acid-base behaviour under physiological conditions. Therefore, the work in this thesis had as its main objectives: to provide quantitative data of the responses to a massive acidosis due to short-term, maximal exercise; to examine the acid-base changes in terms of changes in CO_2, strong ions and weak acids; to determine the importance of the lungs and inactive tissue as sites for regulating acid-base balance after exercise, and to determine the fate of lactate after maximal exercise.

4.2 MAXIMAL CYCLE ERGOMETER EXERCISE

Constant-velocity cycle ergometer exercise lasting only 30 s was used to generate an intracellular acid load. This protocol was shown previously to raise the muscle lactate content to 30 mmol/kg w.w. (Jones et al., 1985; McCartney et al., 1985). Work, power and torque were measured to assess performance during the 30 s of exercise. The heavy nature of the exercise was reflected in the peak and average power output, and the total work generated during the exercise test; the average power output and the total work performed were in excess of 800 W and 18 kJ, respectively. All performance variables were similar to those reported previously using a comparable exercise protocol (McCartney, Heigenhauser and Jones, 1983; Jones et al., 1985).

The O_2 intake ($\dot{V}O_2$) increases linearly with increasing intensity of exercise and thus can also be used to evaluate the heavy nature of the exercise task. The $\dot{V}O_2$ increased more than 7 fold above rest levels during the 30 s of exercise. Preliminary studies demonstrated that the $\dot{V}O_2$ at the end of 30 s exercise represents approximately 60% of the individual's maximal aerobic capacity.

The onset of heavy exercise is associated with an increased turnover of ATP, a breakdown of CP and an activation of oxidative phosphorylation and glycolysis. The energy required during the exercise test was calculated to determine whether the increase in $\dot{V}O_2$ could sustain energy production through aerobic metabolism or whether glycolysis was also necessary. The total work performed in Part B, 21 kJ, required approximately 5 kcal of energy (0.239 kcal/kJ) or an equivalent of 5 l O_2 (5 kcal/l O_2 x 20% efficiency (Pugh, 1974)). The $\dot{V}O_2$ measured at the end of the exercise was approximately 2700 ml/min but only approximately 910 ml O_2 (cumulative $\dot{V}O_2$) were actually used during the exercise. The O_2 stored in the body is small (25 ml O_2/kg) (Cherniack and Longobardo, 1970) and could contribute a maximum of 2000 ml O_2. Complete utilisation of phosphagen (ATP + CP = 21 mmol/kg w.w. (Table 18)) in working muscle represents approximately 1000 ml O_2 (2.4 ml O_2/mmol phosphagen (Margaria, 1976)). The O_2 requirements of exercise exceed the maximum O_2 available (3.9 l O_2) by approximately 1100 ml O_2, thus requiring that glycolysis also be activated for energy production.

4.3 INTRACELLULAR ACIDOSIS IN THE WORKING MUSCLE AFTER MAXIMAL EXERCISE

At the onset of maximal exercise phosphorylase is rapidly activated, leading to maximal flux down the glycolytic pathway; the maximal rate of phosphorylase activity measured in vitro is approximately 50 mmol glucose/kg/min (Newsholme and Start, 1974). The flux through glycolysis is greater than can be accommodated through the pyruvate dehydrogenase reaction into the citric acid cycle (Ward et al., 1982) and leads to an increase in glycolytic intermediates and lactate. The intracellular acidosis which accompanies heavy exercise is generally attributed to an elevated lactic acid production. Maximal cycle ergometer exercise raises the intramuscular [lactate] (Hermansen and Osnes, 1972; Sahlin et al., 1976; Sahlin et al., 1978a; Jacobs et al., 1982; Jacobs et al., 1983; Boobis, Williams and Wootton, 1983) and lowers the intramuscular pH as determined in muscle homogenates (Hermansen and Osnes, 1972; Sahlin et al., 1976). The exercise protocol used in this study was also expected to produce an intracellular acidosis. Maximal exercise similar to that used in this study increased the intramuscular [lactate] to 30 mmol/kg w.w. (Table 18 and 19).

Although the intramuscular [H^+] was shown to be related to the tissue [lactate] (Sahlin et al., 1976; Sahlin et al., 1978a), Stewart

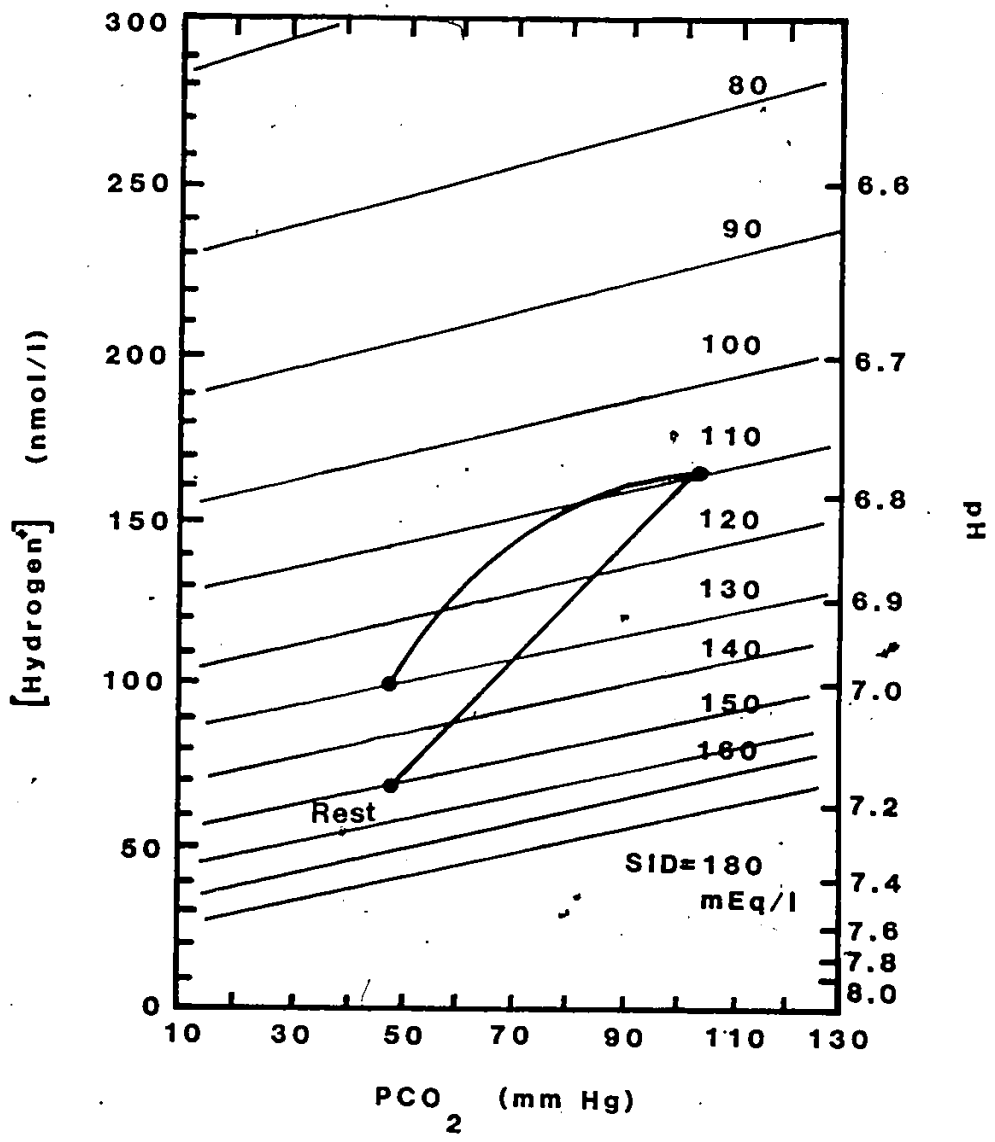
(1981) argued that the $[H^+]$ of the intra- and extracellular fluids is determined by the relationship between the independent variables, PCO_2, [SID] and [ATOT], within each fluid compartment. Stewart (1981) demonstrated that the $[H^+]$ of a solution increased when the PCO_2 or [ATOT] increased, or when the [SID] decreased. In this study, the acid load produced during 30 s of maximal exercise was examined with respect to changes in the intracellular PCO_2, [SID] and [ATOT]. The relationship between the estimated intracellular $[H^+]$ and the estimated intracellular PCO_2, [ATOT] and [SID] is presented in Figure 13.

4.3.1 Intracellular weak acid concentration in the active muscle after maximal exercise

Weak acids are only partially dissociated in solution; the molecules of the parent acid (HA) and the products of dissociation (H^+, A^-) exist together in solution. According to Stewart (1981) weak acids have dissociation constants ranging between 10^{-4}-10^{-12} Eq/l (pKa 4.0-12.0). The total concentration of weak acid ([ATOT]) is defined as the sum of [HA] and $[A^-]$ (Stewart, 1981). The tissue [ATOT] consists mainly of proteins and phosphates.

The [ATOT] of resting muscle is unknown but was assumed to be approximately 200 mEq/l (Stewart, 1981). The phosphagens (ATP and CP) and hexose phosphates (glucose-6-phosphate (glucose-6-P),

Figure 13. Relationship between the intracellular PCO_2, strong ion difference and hydrogen ion concentration in the quadriceps femoris muscle at rest and during recovery from maximal exercise. The intracellular PCO_2 was estimated from the femoral venous PCO_2; the intracellular strong ion difference was estimated from the muscle strong ion concentration and Stewart (1981).


300
250
200
150
100
50
0
[Hydrogen+] (nmol/l)
80
90
100
110
120
130
140
150
160
Rest
SID=180
mEq/l
6.6
6.7
6.8
6.9
7.0
7.2
7.4
7.6
7.8
8.0
pH
10
30
50
70
90
110
130
PCO2 (mm Hg)

fructose-6-P, fructose-1,6-bisP) are weak acids and contribute to the [ATOT] (Hultman and Sahlin, 1980). These weak acids cannot cross the cell membrane and must be metabolised within the cell. Maximal exercise reduces the phosphagen concentration and increases the concentration of the hexose phosphates (Table 18 and 19); the concentration of phosphagen and hexose phosphates returned to near resting levels by 10 min recovery (Table 18). Although the concentration change of individual metabolites may be quite large, the total phosphate concentration (ie. [phosphagens] + [hexose phosphates]) changes very little; the total phosphate concentration was approximately 21 mmol/kg w.w. at rest, 17 mmol/kg w.w. after maximal exercise and 20 mmol/kg w.w. at the end of recovery (Table 18). Thus the intracellular [ATOT] may not change during exercise or recovery and will not contribute to the change in intracellular $[H^+]$.

The pKa does not directly contribute to the [ATOT] but affects the relationship between the weak acid (HA) and its conjugate base (A^-). Protein buffering is largely attributable to the imidazole groups of histidine (pKa 6.4-7.0) with a lesser contribution from N-terminal amino groups (pKa 7.4-7.9) (Hultman and Sahlin, 1980). For physiological purposes it is necessary to use a single "effective" pKa to account for all buffering in the physiological range (Stewart, 1980); a pKa of 6.82 and 6.52 were used for intra- and extracellular fluids, respectively (Stewart, 1981, 1983). Any factor tending to change the average pKa of the intracellular fluid will affect the

acid-base status within the cell; for example, the pKa of water, the imidazole groups of histidine and the CO_2-bicarbonate buffer system change with temperature (Rahn, Reeves and Howell, 1975; Reeves, 1976). An increase in pKa of the cell tends to decrease the $[H^+]$ of the cell. NMR studies demonstrated that CP (pKa 4.5) breakdown to inorganic phosphate (pKa 6.8) at the onset of exercise is associated with a transient alkaline pH shift within the muscle (Mole et al., 1985) and that the rapid resynthesis of CP after exercise (Harris et al., 1976) would be expected to acidify the tissue. Formation of hexose phosphates (pKa 6.1-6.3) and breakdown of ATP (pKa 7.0) may also contribute to changes in the average pKa of the cell. The combined effect of these changes on the intracellular pKa cannot be determined with any certainty.

4.3.2 Intracellular PCO_2 in the active muscle after maximal exercise

Maximal exercise causes an increase in CO_2 production within the muscle. The muscle membrane is highly permeable to CO_2 and the CO_2 formed within the working muscle rapidly equilibrates with the extracellular fluid. Aerobic CO_2 production in the muscle probably ceases at the completion of exercise but CO_2 efflux from the muscle will continue for some time until the CO_2 gradient between the intra- and extracellular spaces decreases and the intracellular PCO_2 has returned to resting levels.

Few studies have estimated the muscle PCO_2 during heavy exercise. Sahlin and coworkers (1978) used the Krogh tissue model to calculate the theoretical PCO_2 in muscle after maximal exercise. They determined that the tissue PCO_2 was close to the arithmetic mean of the arterial and femoral venous PCO_2. They argued that during exercise the high density of open capillaries in muscle would diminish the CO_2 gradient between muscle and capillary blood and prevent the tissue CO_2 from significantly rising. This model was tested during steady-state conditions in brain tissue and was found to accurately reflect the PCO_2 as measured by electrode (Gleichmann et al., 1962; Ponten and Siesjö, 1966). However brain tissue, unlike skeletal muscle, maintains a relatively constant CO_2 production. This relationship may not exist in tissues like skeletal muscle where the metabolic rate may increase more than 7 fold. In addition, a higher venous PCO_2 relative to the intracellular PCO_2 requires that the CO_2 diffuse out of the muscle against a substantial concentration gradient. Thus, the femoral venous PCO_2 may be more representative of the intracellular PCO_2 after maximal exercise.

The intracellular PCO_2 was not measured but was assumed to equal the femoral venous PCO_2. This being the case, the intracellular PCO_2 would increase from approximately 45 mm Hg at rest to 105 mm Hg after 30 s of maximal exercise. The increase in intracellular $[H^+]$ associated with this increase in PCO_2 was approximately 24 nmol/l, or approximately 25% of the total increase in $[H^+]$ (Fig. 13). Changing

the intracellular PCO_2 at constant [SID] (110 mEq/l) and [ATOT] (200 mEq/l) changes the intracellular $[H^+]$ approximately 0.4 mmol/l/mm Hg (calculated according to equations for the intracellular $[H^+]$ in Appendix B2). Although the PCO_2 is important in regulating the $[H^+]$ of biological fluids, the intracellular fluid appears to be relatively protected against excessive increases in PCO_2 that could occur during heavy exercise. In addition, excessive increases in intracellular PCO_2 are prevented since the elevated muscle blood flow provides an infinite sink for CO_2 ; blood with a high PCO_2 is delivered to the lungs and blood with low PCO_2 returns to the muscle. During recovery CO_2 diffuses from inside the muscle and reduces the intracellular PCO_2 to resting levels and the intracellular $[H^+]$ decreases by 24 mmol/l. This is a relatively fast process and is probably complete by 3 minutes recovery (Fig. 6a and 13).

4.3.3 Intracellular strong ion difference in the active muscle after maximal exercise

Strong ions are completely dissociated in biological fluids; that is, there are no undissociated parent molecules in solution. The important strong ions in biological solutions are sodium (Na^+), potassium (K^+) and chloride (Cl^-). Lactate being almost completely dissociated at physiological pH (pKa 3.7) is also considered an important strong anion, especially during heavy exercise. Other strong ions such as calcium (Ca^{2+}), magnesium (Mg^{2+}), and sulphate

(SO_4^{2-}) occur in low concentrations. The strong ion difference ([SID]) is defined as the difference between the sum of the strong cation concentrations and the sum of the strong anion concentrations. The intracellular [SID] was calculated as ($[Na^+ + K^+ + Ca^{2+} + Mg^{2+}] - [Cl^- + lactate]$).

Muscle biopsies were taken from the quadriceps femoris muscle to determine the effects of 30 s of maximal leg exercise on the intracellular strong ion concentrations. The intracellular strong ion concentrations for sodium, potassium, calcium, magnesium and chloride did not change during or after the exercise. However, the estimated muscle [lactate] increased to approximately 30 mmol/kg w.w. (Tables 18 and 19); the corresponding intracellular [lactate] was approximately 45 mmol/l i.c. water (assuming that the wet weight/dry ratio was 4.3 and the intracellular water/total muscle water ratio was 0.85 (Bergström, Guarnieri and Hultman, 1971; Sahlin et al., 1978a; Sjøgaard and Saltin, 1982; Sjøgaard, Adams and Saltin, 1985)). The increase in the intracellular [lactate] was responsible for the decrease in the intracellular [SID] (Table 17); the [SID] decreased from approximately 150 mEq/l at rest to approximately 110 mEq/l after maximal exercise. The estimated decrease in [SID] increased the intracellular [H^+] approximately 75 mmol/l, or 75% of the total increase in the intracellular [H^+] (Fig. 13).

Thus the intracellular [H^+] increased from 70 nmol/l (pH 7.15)

at rest to 170 mmol/l (pH 6.77) after maximal exercise (Fig. 13). The calculated pH at rest was somewhat higher than the pH (6.90-7.10) reported in homogenates of resting muscle (Hermansen and Osnes, 1972; Sahlin et al., 1976; Sahlin et al., 1978b). The pH measured in muscle homogenates after heavy exercise ranged between 6.4 and 6.6 (Hermansen and Osnes, 1972; Sahlin et al., 1976; Sahlin et al., 1978b), lower that the intracellular pH calculated after exercise in this study.

The intracellular [SID] increased to approximately 130 mEq/l after 10 min of recovery (Table 17) and lowered the intracellular [H^+] by approximately 45 mmol/l (Fig. 13). The [SID] increased primarily because of the decrease in the intracellular [lactate] as no changes occurred in the intracellular concentration of the other strong ions (Table 17). Thus the production and removal of lactate from muscle are important processes contributing to the intracellular acid-base status during and following maximal exercise.

The constancy of the intracellular concentrations of sodium, potassium, magnesium and chloride was unexpected since previous studies reported increases of 10-80% for intracellular [Na^+], decreases of 13-20% for intracellular [K^+], and decreases of 13-20% for intracellular [Mg^{2+}] (Sahlin et al., 1978b; Sjøgaard, 1983; Sjøgaard, Adams and Saltin, 1985). An apparent intracellular [SID] calculated from the strong ions concentrations (not including chloride or lactate) reported in these studies was shown to decrease

approximately 10-15% after heavy exercise. In addition, the femoral venous-arterial (fv-a) strong ion differences demonstrated that sodium and potassium were released from the active muscle and chloride was taken up by the active muscle immediately after exercise (Table 8, Fig. 11). Factors contributing to the differences in intracellular strong ion behaviour between this study and previous studies include use of a different exercise protocol (30 s cycling vs 6 min cycling (Sahlin et al., 1978), 3 x 3 min cycling (Sjøgaard and Saltin, 1982; Sjøgaard, 1983), one-legged, dynamic knee extensions (Sjøgaard, Adams and Saltin, 1985)) or differences in the muscle [lactate] leading to changes in ion fluxes. In addition methodological errors may have hidden real changes in the intracellular concentration of strong ions.

Sources of error in determining the intracellular strong ion concentrations : The intracellular concentrations of sodium, potassium, magnesium, calcium and chloride were analysed in muscle biopsy tissue using neutron activation analysis. This method was used previously to study the ion content of resting skeletal muscle (Bergström, 1962). A large range of mean values have been reported for the intracellular strong ion concentrations of resting skeletal muscle: sodium, 4-20 mmol/l; potassium, 150-170 mmol/l; magnesium, 14-16 mmol/l; chloride, 4 mmol/l (Bergström, 1962; Graham, 1967; Bergström, Guarnieri and Hultman, 1971; Sahlin et al., 1978b; Sjøgaard, 1983). The large range reported for intracellular values may be attributed to the different methods of analysis (neutron

activation, atomic absorption spectrophotometry) and to the method of estimating the fluid volume of the intracellular and interstitial space in muscle (chloride space, inulin space). In general the resting sodium, magnesium and chloride are similar to reported values, while the potassium concentration is lower than reported values (Table 17).

Interpretation of the tissue strong ion data must be made with caution because of the large variability displayed in their measurement. Potential sources of error in neutron activation analysis include variation in the flux density of the neutrons and counting errors. Variations in flux density would not be a factor due to the short irradiation time. Counting errors are a major source of error and include errors in determining the sample and standard peak and background. The error within a single determination is proportional to the square root of the total number of counts in the sample. Elements counted with a low peak/background ratio will have a greater error. In this thesis the maximum counting error for a single determination was less than 13% for calcium (mean error, 10.1%), but less than 5% for the remaining ions; the mean errors were sodium, 0.6%; potassium, 1.7%; magnesium, 2.3%; chloride, 0.6%. Therefore, the errors associated with the analytical methods were small for all ions except for calcium.

Contamination of the muscle biopsy samples with blood will

introduce errors in the measurement of the ion contents and the estimation of the water content. Steps were taken to minimise excessive bleeding but it was not completely prevented. Blood in the sample will raise the content of those ions that exist in low concentration in muscle but in high concentration in the plasma (ie. sodium and chloride) and reduce the content of those ions that exist in high concentration in the muscle but in low concentration in the plasma (ie. potassium). In addition, the tissue wet weight/dry weight ratio will be overestimated if the muscle sample is contaminated with blood.

The water content of the intra- and extracellular space of rested and exercised muscle was not measured but was estimated from reported values. The interstitial fluid volume must be known so that the ion content can be converted to an ion concentration. The water content of the muscle increases during heavy exercise. The water distributes in both the interstitial and intracellular compartments (Bergström, Guarnieri and Hultman, 1971; Sahlin et al., 1978; Sjøgaard and Saltin, 1982; Sjøgaard, Adams and Saltin, 1985); the interstitial water content increases relatively more than the intracellular water content (Sjøgaard and Saltin, 1982). The inability to determine changes in muscle water content, especially during heavy exercise of the type used in this study, may introduce a measurement error.

4.3.4 Summary of intracellular acid-base changes after maximal exercise

The intracellular $[H^+]$ of the quadriceps femoris muscle was calculated at rest and after 30 s of maximal cycle ergometer exercise using the approach of Stewart (1981). The intracellular $[H^+]$ at rest and after exercise was determined by the intracellular PCO_2 (estimated from the femoral venous PCO_2), the intracellular [SID] (calculated from measured strong ion levels) and the intracellular [ATOT] (estimated from Stewart, 1981). The intracellular $[H^+]$ of resting skeletal muscle was approximately 70 nmol/l (pH 7.15) (PCO_2, 45 mm Hg; [SID], 152 mEq/l; [ATOT], 200 mEq/l). This was somewhat higher than the pH range (6.90-7.10) reported for homogenates of resting muscle (Hermansen and Osnes, 1972; Sahlin et al., 1976; Sahlin et al., 1978b).

An intracellular acid load was produced during 30 s of maximal cycle ergometer exercise as the intracellular $[H^+]$ increased from 70 nmol/l at rest to 170 nmol/l (pH 6.77) immediately after exercise (Fig. 13). Approximately 25% (or 25 nmol/l) of the total change in $[H^+]$ was accounted for by the increase in PCO_2 and 75% (or 75 nmol/l) was due to the decrease in [SID] (Fig. 13). The intracellular $[H^+]$ calculated in this study (ie. 170 nmol/l) was lower than values reported for muscle homogenates ($[H^+]$ 220-390 nmol/l; pH 6.4-6.6) after heavy exercise (Hermansen and Osnes, 1972; Sahlin et al., 1976;

Sahlin et al., 1978b) and lower than the $[H^+]$ (295 nmol/l; pH 6.53) calculated according to the relationship between the [lactate] and pH of muscle homogenates (Sahlin et al., 1976). These differences are even greater when considering that the homogenate PCO_2 will be low and probably not representative of the intracellular PCO_2 after exercise. The reason for the differences between the $[H^+]$ calculated in this study and the $[H^+]$ calculated from pH measurements of muscle homogenates is unknown The discrepancy could be attributed to an inappropriate choice of value for the intracellular pKa or [ATOT], or movement of intracellular strong ions may have taken place and the actual [SID] may have decreased more than was measured. The quantitative resolution of this problem awaits further study.

The process of neutralising the acid load began immediately after exercise. CO_2 production decreases after exercise and diffusion of CO_2 from the muscle lowered the intracellular PCO_2. The process of eliminating the excess CO_2 from muscle was probably complete by 3 min recovery Lactate was shown to contribute to the intracellular acidosis through its effect on the [SID] and thus removing lactate from inside the cell is important for recovery of normal intracellular acid-base balance. Lactate disappearance in muscle occurs more slowly than CO_2 removal; the intramuscular [lactate] was still elevated after 10 min recovery. The changes in PCO_2 and [SID] occurring during recovery decreased the intracellular $[H^+]$ to approximately 100 nmol/l (pH 7.00). Removal of CO_2 and lactate from the muscle established a

plasma acidosis (Fig. 14, 15 and 16) that must be neutralised before whole body acid-base balance can be considered normal. The remaining discussion deals with the recovery from the acute acidosis of maximal exercise and examines mechanisms available for removing the acid load through metabolism of lactate, exchange of other strong ions and excretion of CO_2.

4.4 FATE OF LACTATE DURING RECOVERY FROM MAXIMAL EXERCISE

The intracellular [SID] was shown to significantly affect the intracellular [H^+] (Section 4.3.3). The [SID] changes reported in this study were due primarily to changes in the intracellular [lactate]. Clearly the regulation of the intracellular [lactate] is an important process in acid-base control. The lactate produced in the muscle during maximal exercise was removed from the previously active muscle during recovery by a number of processes, including metabolic removal in the active muscle, and diffusion into the circulation and transport to lactate metabolising tissues. The circulation is important in this regard as it removes lactate away from the active muscle, thus maintaining a diffusion gradient between the intra- and extracellular fluids, and transports it to tissues in the body where it can be metabolised or excreted.

4.4.1 Blood flow in the quadriceps muscle at rest and during recovery from maximal exercise

Resting muscle blood flows ranging between 2-7 ml/100 gm/min have been reported using xenon 133 (Lassen, Lindbjerg and Munck, 1964; Grimby, Häggendal and Saltin, 1967; Suzuki and Bonde-Petersen, 1983) and venous occlusion plethysmography (Matsui, Kitamura and Miyamura, 1978). The resting blood flow in skeletal muscle was assumed to be approximately 3 ml/100 gm/min (Lassen, Lindbjerg and Munck, 1964; Grimby, Häggendal and Saltin, 1967).

Blood flow to the working muscle increases during exercise. Muscle blood flows ranging between 30-80 ml/100 gm/min have been reported during heavy exercise (Grimby, Häggendal and Saltin, 1967; Pirnay et al., 1972), however flows as high as 290 ml/100 gm/min were calculated after maximal dynamic knee extensions (Sjøgaard, Adams and Saltin, 1985). Few studies have examined the blood flow in muscle during recovery from exercise. Muscle blood flows between 10-15 ml/100 gm/min were reported 10 min after maximal running (Matsui, Kitamura and Miyamura, 1978; Suzuki and Bonde-Petersen, 1983) or cycling exercise (Matsui, Kitamura and Miyamura, 1978).

The muscle blood flow, calculated (Appendix B6) at the end of 30 s of exercise, was approximately 88 ml/100 gm/min. The blood flow was calculated using the Fick equation and assuming that: 1) the $\dot{V}O_2$

measured at the end of exercise was similar for Parts A and B, 2) that the extra $\dot{V}O_2$ above that found at rest was used by the working muscle and 3) that approximately 15 kg of muscle was active during exercise. The blood flow calculated at the end of exercise was assumed to stay elevated during the first minute of recovery. The blood flow calculated for the end of the 10 min recovery was approximately 10 ml/100 gm/min assuming that approximately 50% of the extra VO_2 was used by the previously active muscle.

The muscle blood flows estimated for the quadriceps muscle at rest and during recovery from maximal exercise were used to calculate the strong ion exchange between the muscle and the plasma.

4.4.2 Metabolism of lactate in the previously active quadriceps femoris muscle during recovery from maximal exercise

Approximately 450 mmol of lactate (30 mmol/kg w.w. x 15 kg w.w. active muscle) were produced in the working muscle during 30 s of maximal exercise and only about 150 mmol of lactate (10 mmol/kg w.w. x 15 kg w.w. muscle) remained in the msucle after 10 min recovery; the rate of lactate disappearance from the previously active muscle was approximately 2 mmol/kg w.w./min (30 mmol/min). A similar rate of disappearance was calculated from data in previous studies (Sahlin et al., 1976; [illegible] et al., 1978b; Sjøgaard, Saltin and Adams, 1985), but was higher [illegible] the value of 0.7 mmol/kg w.w./min reported by

Hermansen and Vaage (1977). The reason for the discrepancy is unknown since the muscle [lactate] was similar after exercise. Immediately after exercise a large [lactate] gradient existed between the intracellular and extracellular compartments and favoured the diffusion of lactate from the muscle into the plasma; the intracellular-femoral venous [lactate] gradient was 2 mmol/l at rest and 35 mmol/l immediately after exercise. After 10 min of recovery the [lactate] gradient had dissipated and the lactate was in equilibrium throughout the body; the intracellular-femoral venous [lactate] gradient was approximately 1 mmol/l at the end of recovery. Calculations based on the average rate of lactate removal (30 mmol/min) and the average fv-a [lactate] difference (2.7 mmol/l) show that an average muscle blood flow of 11 l/min (75 ml/100 gm/min) would be required to account for lactate removal by the circulation. This represents approximately 85% of the estimated muscle blood flow at the completion of 30 s of exercise (see Section 4.4.1). It is unlikely that the average muscle blood flow would be this high during the 10 min of recovery, thus diffusion of lactate from the muscle cannot completely explain the decrease in muscle [lactate].

Conversion to glycogen : The glycogen content of the working muscle decreases during 30 s maximal exercise (Table 19). During recovery glycogen is resynthesised from glucose, lactate or other glycolytic intermediates. Hermansen and Vaage (1977) observed that during recovery from exercise muscle glycogen was resynthesised at a

rate of 0.51 mmol glucosyl U/kg w.w./min. Lactate accounted for approximately 50% of the total amount of glycogen resynthesised in the muscle, while glycolytic intermediates and glucose accounted for approximately 20% and 5%, respectively. If glycogen resynthesis occurred at a similiar rate in this study then approximately 75 mmol of lactate (0.51 mmol glucosyl U/kg w.w./min x 2 mmol lactate/mmol glucosyl U x 15 kg x 10 min x 50%), or 25% of the initial lactate load would be removed via this mechanism.

Oxidation of lactate : The average fv-a differences for [lactate] and O_2 content were both approximately 2.7 mmol/l. The average RQ across the leg during recovery was approximately 2.0. The similarity between lactate output and O_2 intake implies that for every mole of lactate that diffused out of the muscle, 1/3 mole of lactate was potentially oxidised to CO_2 and water. Thus, approximately 25% of the lactate disappearing from muscle, but not converted into glycogen, may have been oxidised and the remaining 75% may have diffused into the circulation. An accurate measure of muscle blood flow is required to substantiate this conclusion.

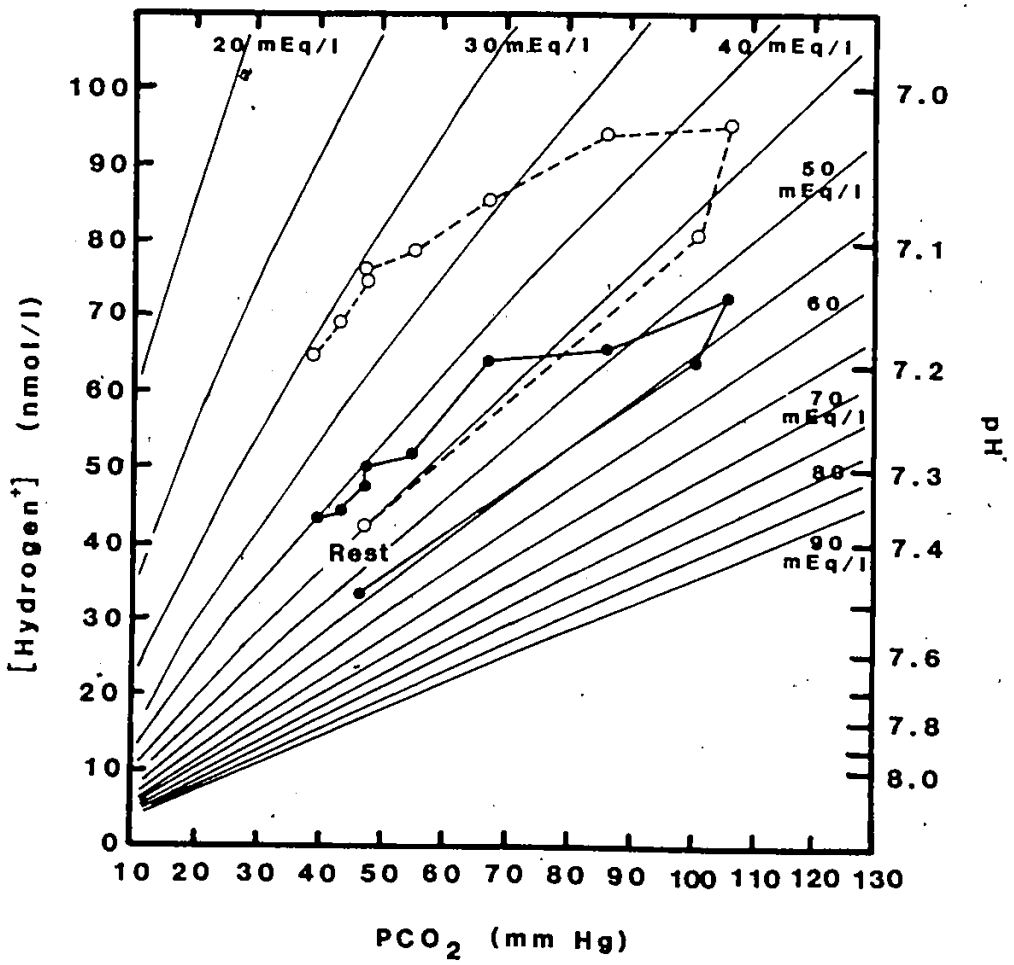
Thus, of the 300 mmol of lactate disappearing from muscle during recovery, approximately 75 mmol (25%) may have been converted into glycogen, 55 mmol (20-25%) could have been oxidised and 170 mmol (55-60%) could have diffused from the muscle. Hermansen and Vaage (1977) estimated that less than 15% of the lactate produced during

exercise was oxidised and only 10% diffused into the circulation. In their study the recovery fv-a [lactate] difference was only 0.5 mmol/l, even though the arterial [lactate] was 10-20 mmol/l.

4.4.3 Lactate release across the previously active quadriceps femoris muscle during recovery from maximal exercise

Lactate release from the active muscle is an important mechanism for reducing the intracellular acid load, but by doing so, establishes an extracellular acidosis (Fig. 14). The total acid load in the blood is comprised of a respiratory (CO_2) component and a metabolic ($nonCO_2$) component. Titrating whole blood with strong ions provides a measure of the $nonCO_2$ acid in the blood. The importance of lactate in producing the metabolic acid load can be determined by comparing the lactate output across the muscle with the $nonCO_2$ acid output. Lactate output from resting muscle was less than 1 umol/100 gm/min but increased to approximately 290 umol/100 gm/min after maximal exercise. The $nonCO_2$ acid output (fv-a base deficit difference x muscle blood flow) was approximately 280 umol/100 gm/min after exercise. The similarity of lactate output and $nonCO_2$ acid output supports the concept that lactate output is responsible for the metabolic or nonrespiratory acid load in blood. This is contrary to previous reports that have demonstrated that the $nonCO_2$ acid output across the muscle exceeded the lactate output during exercise and recovery. Benadé and Heisler (1978) demonstrated that following

Figure 14. Relationship between the femoral venous PCO_2, strong ion and hydrogen ion concentration at rest and during recovery from maximal exercise. ● plotted using the measured femoral venous PCO_2 and strong ion difference; ○ plotted using the measured femoral venous PCO_2 and hydrogen ion concentration.


20 mEq/l
30 mEq/l
40 mEq/l
50 mEq/l
60
70 mEq/l
80
90 mEq/l
Rest
[Hydrogen+] (nmol/l)
100
90
80
70
60
50
40
30
20
10
0
7.0
7.1
7.2
7.3
7.4
7.6
7.8
8.0
pH
10 20 30 40 50 60 70 80 90 100 110 120 130
PCO2 (mm Hg)

electrical stimulation of the isolated rat diaphragm and frog sartorius muscle the acid output/lactate output ratio was 14 and 50, respectively. Stainsby and coworkers reported that acid output across an electrically stimulated isolated dog gastrocnemius-plantaris muscle preparation was 4.7 and 11 times greater than lactate output during steady-state (Barbee, Stainsby and Chirtel, 1983) and nonsteady-state contractions (Chirtel, Barbee and Stainsby, 1984), respectively. The differences may be related to lower muscle lactate levels in the previous studies due to longer duration, less intense electrically-induced contractions. The exchange of other strong ions was not examined in the previous studies and may have accounted for the greater acid output in these studies.

Lactate release from muscle continued throughout recovery but at a lower rate; the lactate output was approximately 20 umol/100 gm/min at 10 min recovery. Once in the plasma the lactate is transported to the liver and inactive tissue where it can be metabolised and removed.

4.4.4 Blood flow in the inactive forearm muscle during recovery from maximal leg exercise

Blood flow is important for delivery of the lactate to the inactive tissues and for removal of CO_2 from the inactive muscle. The resting blood flow in the inactive tissue was assumed to be 3 ml/100

gm/min and similar to the resting flow in leg tissue. The blood flow in the inactive forearm decreases during leg exercise but increases above resting levels after exercise (Bishop et al., 1957; Blair et al., 1961; Bevegård and Shepherd, 1966). Blair and coworkers (1957) demonstrated that the blood flow to the inactive forearm may increase 60-70% above resting levels after relatively heavy leg exercise. I have assumed that the blood flow to the inactive forearm increases to approximately 5 ml/100 gm/min immediately after maximal leg exercise and that blood flow returned to resting levels (3 ml/100 gm/min) by the end of recovery. The blood flow was used to estimate the flux of strong ions across the inactive forearm muscle.

4.4.5 Metabolism of lactate in the inactive tissues of the body during recovery from maximal exercise

Lactate is the only strong ion that can be metabolised in the body. Liver, adipose tissue, heart, kidney and inactive skeletal muscle are capable of metabolising lactate. Ahlborg, Hagenfeldt and Wahren (1976) estimated that during 30 min of lactate infusion into a resting individual, approximately 10% of the total amount of lactate infused was removed by the liver, 20% by the heart, adipose tissue and kidney and 35% remained in the distribution volume of the lactate and 35% was removed by inactive muscle. The inactive muscle may remove as much as 50% of the lactate infused into the body since the lactate distributed throughout the body will eventually be metabolised.

Lactate exchange across the liver, adipose tissue, heart and kidney was not examined, but lactate uptake by the forearm increased from -1 umol/100 gm/min at rest to 15-25 umol/100 gm/min during recovery. Thus the rate of lactate removal by inactive tissue was approximately 2.2 mmol/min (assuming: average body weight, 80 kg; weight of inactive muscle, 20% body weight; average v-a [lactate] difference, -4.5 mmol/l; blood flow to the inactive tissues, 3 ml/100 gm/min). Total lactate production in the working muscle was approximately 450 mmol (Section 4.4.2) and approximately 60%, or 270 mmol, was released into the circulation (Section 4.4.2). Approximately 135 mmol of lactate were taken up by the inactive muscle assuming that 50% of the plasma lactate was removed by the inactive muscle. Thus the inactive muscle is an important site for lactate removal and in doing so, increases the plasma [SID] and decreases the plasma $[H^+]$.

In the inactive muscle the lactate can be converted to glycogen (Hermansen and Vaage, 1977; Brooks and Gaesser, 1980) or other metabolic intermediates (Jorfeldt, 1970; Brooks and Gaesser, 1980), oxidised to CO_2 and water (Jorfeldt, 1970; Hubbard, 1973; Brooks and Gaesser, 1980) or converted to fat. Conversion to glycogen is unlikely because glycogen breakdown probably did not occur in the inactive muscle. Oxidation of 1 mole of lactate requires 3 moles of O_2. The average v-a [lactate] difference during the 10 min of recovery was approximately -4.5 mmol/l. Oxidation of the total amount of lactate requires an a-v O_2 content difference of 13.5 mmol/l; the

average a-v O_2 content difference measured across the forearm was 6.2 mmol/l. Thus only about 45% of the lactate taken up by the forearm could be oxidised. Synthesis of 1 mole of fat (ie. palmitate) requires 9 moles of lactate and 4 moles of O_2, and 11 moles of CO_2 are produced (RQ 2.8) (McGilvery, 1979). An average respiratory quotient of 1.6 was calculated for the recovery period. Thus the elevated CO_2 output across the inactive muscle suggests that fat synthesis may occur in the inactive tissue.

As lactate is released from the previously active muscle the plasma [lactate] increased and the plasma [SID] decreased. The fall in the [SID] increased the $[H^+]$ in the femoral vein (Fig. 14), artery (Fig. 15) and deep forearm vein (Fig. 16). The inactive muscle was an important site for recovery of acid-base balance as the [lactate] was lower and the [SID] was higher in the venous blood leaving the inactive muscle compared to the arterial blood. Strong ions in addition to lactate also contribute to the [SID] and acid-base balance of the body. The effect of these ions will be considered in the following section.

4.5 FATE OF STRONG IONS OTHER THAN LACTATE

The concentration of strong ions is important in determining the [SID] of the intracellular fluid and the plasma. The [SID] is a

Figure 15. Relationship between the arterial PCO_2, strong ion difference and hydrogen ion concentration at rest and during recovery from maximal exercise. ● plotted using the measured arterial PCO_2 and strong ion difference; ○ plotted using the measured arterial PCO_2 and hydrogen ion concentration.

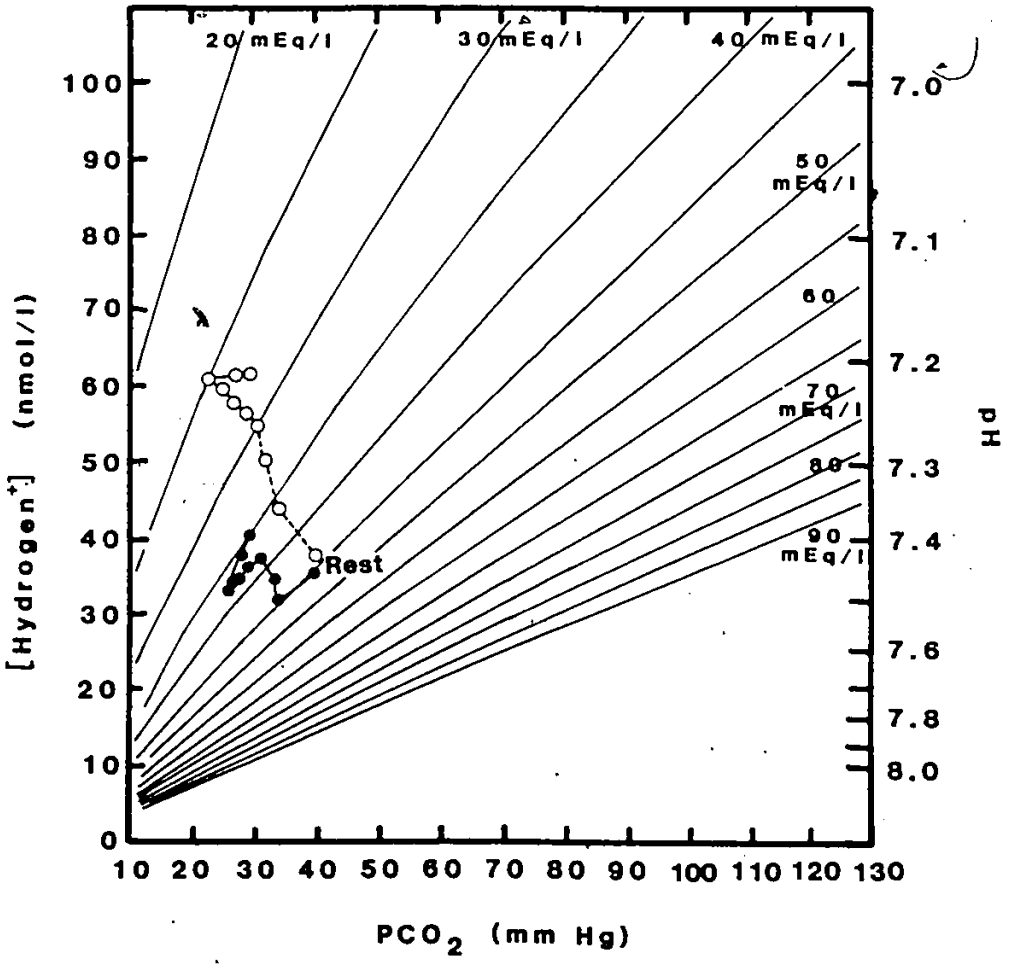
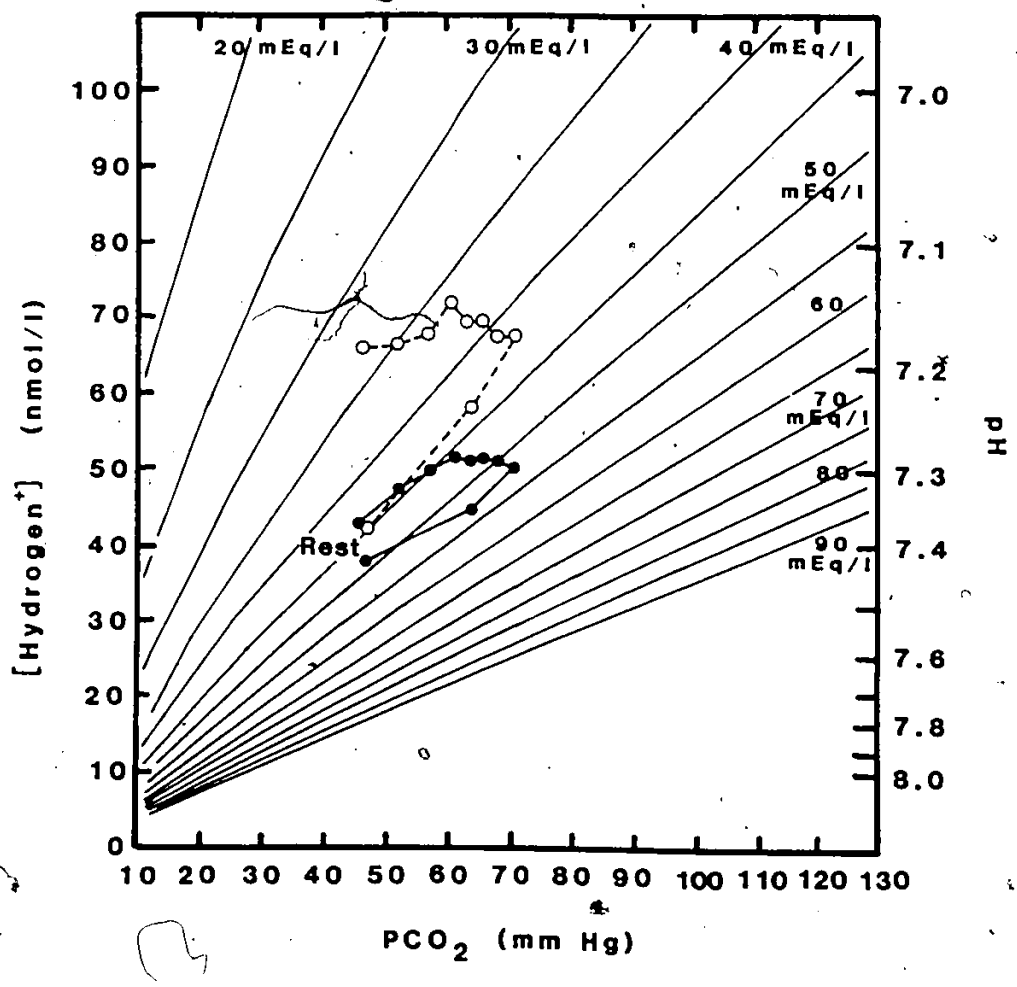

20 mEq/l
30 mEq/l
40 mEq/l
50 mEq/l
60
70 mEq/l
80
90 mEq/l
Rest
[Hydrogen+] (nmol/l)
pH
PCO2 (mm Hg)
100
90
80
70
60
50
40
30
20
10
0
7.0
7.1
7.2
7.3
7.4
7.6
7.8
8.0
10 20 30 40 50 60 70 80 90 100 110 120 130

Figure 16. Relationship between the forearm venous PCO_2, strong ion difference and hydrogen ion concentration at rest and during recovery from maximal exercise. ● plotted using the measured forearm venous PCO_2 and strong ion difference; o plotted using the measured forearm venous PCO_2 and hydrogen ion concentration.


20 mEq/l
30 mEq/l
40 mEq/l
50 mEq/l
60
70 mEq/l
80
90 mEq/l
Rest
[Hydrogen+] (nmol/l)
100
90
80
70
60
50
40
30
20
10
0
7.0
7.1
7.2
7.3
7.4
7.6
7.8
8.0
pH
10 20 30 40 50 60 70 80 90 100 110 120 130
PCO2 (mm Hg)

measure of the concentration difference between strong cations and anions and will be affected by both strong ion and fluid movements across the muscle membrane. Although the concentration of strong ions did not change in the working muscle during exercise or recovery; strong ion exchange did occur across the active and inactive muscle as demonstrated by the v-a strong ion concentration differences. The v-a differences were calculated after the plasma concentration of each strong ion was corrected for the decrease in plasma volume (Tables 6 and 12).

4.5.1 Plasma volume change after maximal exercise

The concentration of strong ions is a measure of the number of moles of strong ion dissolved in the fluid volume, and as such, a change in strong ion concentration could be due to a change in the number of moles of strong ions and/or to a change in the volume. The concentration of most of the plasma strong ions increased during maximal exercise and decreased during recovery (Fig. 12). These changes were partially explained by a decrease in plasma volume that occurred during heavy exercise (Table 6 and 12); the plasma volume decreased approximately 10-15% during the exercise. The plasma volume change was calculated from the blood hemoglobin concentration (Harrison, 1985), assuming that the red cell volume remained constant (van Beaumont, 1973). A decrease in plasma volume was previously demonstrated after exercise; the plasma volume decreased by 15% after

maximal incremental exercise (van Beaumont, 1973; van Beaumont et al., 1973) and 20-25% after maximal intermittent exercise (Hermansen, Orheim and Sejersted, 1984). Fluid movement into the interstitial space can occur because of increases in capillary hydrostatic pressure and surface area, and tissue osmolality caused by an elevated muscle [lactate] and possibly muscle [Na^+] (Mohsenin and Gonzalez, 1984).

Fluid movement into inactive muscle has not been demonstrated; the water content of inactive muscle did not change during either maximal cycle ergometer exercise (Sjøgaard and Saltin, 1982) or one-legged knee extensions (Sjøgaard, Adams and Saltin, 1985). Thus the decrease in plasma volume was attributed to fluid movement into the working quadriceps femoris muscle.

4.5.2 Strong ion exchange across the active quadriceps femoris muscle during recovery from maximal exercise

The fv-a [SID] difference increased immediately after maximal exercise. The arterial [SID] decreased immediately after exercise but the femoral venous [SID] remained at resting levels in the first minute of recovery; the increase in femoral venous [lactate] was balanced mainly by an increase in [Na^+] (Fig. 12). The increase in strong ion concentration occurring during exercise may serve a protective function by preventing the plasma [SID] from decreasing, thus limiting the increase in the plasma [H^+]. The fv-a [SID]

difference decreased after the first minute of recovery as both the femoral venous and arterial [SID] decreased; the femoral venous and arterial [SID] were similar after 3 min recovery (Fig. 7a). The decrease in the plasma [SID] was related to the increase in femoral venous and arterial [lactate] as the concentration of the other strong ions was similar to resting levels (Fig. 12). The decrease in [SID] was primarily responsible for the increase in arterial $[H^+]$ throughout recovery (Fig. 15), and contributed proportionately more to the femoral venous acidosis as the femoral venous PCO_2 decreased below resting levels (Fig. 14).

The increase in concentration of the various strong ions was related to the decrease in the plasma volume. Correcting the strong ion concentration for changes in plasma volume demonstrated that although potassium still increased after exercise, the corrected concentration of sodium and chloride decreased below resting levels immediately after maximal exercise (Table 6). These changes will be examined in terms of strong ion uptake and release by the quadriceps femoris muscle.

Potassium exchange across the active muscle : The femoral venous $[K^+]$ increased approximately 30% immediately after maximal exercise but returned to resting levels during the first minute of recovery (Tables 5 and 8). Elevated potassium levels have been observed during maximal exercise (Bergström, Guarnieri and Hultman,

1971; van Beaumont, 1973; van Beaumont et al., 1973; Tibes et al., 1974; Hermansen, Orheim and Sejersted, 1984; Sjøgaard, 1985; Sjøgaard, Adams and Saltin, 1985). Potassium release from the muscle increased from approximately 3 umol/100 gm/min at rest to 55 umol/100 gm/min after exercise. The increase in the arterial $[K^+]$ after exercise implies that the release of potassium from the quadriceps muscle exceeds the uptake of potassium by other nonworking tissues.

The potassium permeability of nerve and muscle membranes increases during an action potential and potassium is released from the cell. It was previously demonstrated that the amount of potassium released per contraction from electrically stimulated dog and rabbit muscle was approximately 30 nmol/100 gm/impulse (Hník et al., 1976; Hirche, Schumacher and Hagemann,1980). During 30 s of maximal exercise the firing frequency of the quadriceps femoris muscle was probably between 20 and 50 Hz (Marsden, Meadows and Merton, 1971). If the amount of potassium released per impulse is similar to that found in animal muscle, then approximately 20-45 umol K^+/100 gm muscle would be expected to be released from the quadriceps muscle during 30 s of exercise. The total amount of potassium released from the working muscle was approximately 30 umol/100 gm muscle (potassium release/min x 0.5 min). Thus the loss of potassium from exercising muscle may be explained by the potassium released during each action potential. Release of potassium from the working muscle increases the interstitial $[K^+]$ and could decrease the $[K^+]i/[K^+]o$ ratio and

decrease the resting membrane potential. A decrease in resting membrane potential has been implicated as a possible cause of fatigue (Sjøgaard, 1985). If the membrane is constantly depolarised it eventually becomes inactivated, thereby interfering with excitation-contraction coupling.

Potassium release from the quadriceps muscle decreased to resting levels after 10 min recovery (Table 8, Fig. 11a). Sjøgaard, Adams and Saltin (1985) demonstrated that potassium was taken up by muscle after intense dynamic knee extensions. The discrepancy may be related to a much lower muscle lactate concentration (15 mmol/kg w.w.) in their study.

Sodium exchange across the active muscle : The $[Na^+]$ decreased below resting levels after correcting for the decrease in plasma volume (Table 6). Sodium was released from the quadriceps muscle during the early part of recovery (Fig. 11a); sodium output increased from approximately 3 umol/100 gm/min at rest to approximately 200-500 umol/100 gm/min during the first minute of recovery. The arterial $[Na^+]$ decreased even though sodium was released from the quadriceps muscle and implied that sodium was taken up by inactive muscle (Fig. 11b). Sodium loss from the vascular space was previously reported after maximal exercise (van Beaumont et al., 1973; Tibes et al., 1974; Sejersted, Medbo and Hermansen, 1982; Hermansen, Ohneim and Sejersted, 1984).

Calcium exchange across the active muscle : The femoral venous [Ca^{2+}] increased after exercise (Table 8). Calcium does not significantly contribute to the [SID] because it exists in such low concentrations. The plasma [Ca^{2+}] is inversely related to the plasma pH (Nielsen et al., 1977) and is related to a pH-induced reduction in calcium binding to plasma proteins. Release of calcium from the working muscle may also increase the plasma [Ca^{2+}].

Chloride exchange across the active muscle : The femoral venous [Cl^-] decreased during exercise (Fig. 12). Chloride was taken up by the quadriceps muscle during the early part of recovery (Fig. 11a); chloride uptake increased from approximately 5 umol/100 gm/min at rest to approximately 530 umol/100 gm/min during the first minute of recovery (Table 8). In the latter part of recovery the fv-a [Cl^-] difference was variable but suggested that chloride was being released from the quadriceps muscle (Fig. 11a).

Strong ion movements were determined from fv-a concentration differences and indicated that sodium, potassium, calcium and lactate were released and chloride was taken up by the quadriceps muscle during recovery from 30 s of maximal exercise. The work of Boron (1983) and Thomas (1984) suggest that recovery of intracellular acid-base balance involves a stoichiometric exchange of internal chloride with external bicarbonate, or of internal hydrogen ions with

external sodium (Section 1.8). Recovery of intracellular acid-base balance did not appear to involve a stoichiometric exchange of strong ions between the intra- and extracellular fluids (Table 8) as would be predicted by the acid extrusion models. However different methods for acid loading the cell may have contributed to these differences. CO_2 and weak acids (ammonia) are often used to acid load the cell when examining acid extrusion, while lactate production and its effect on the [SID] have a greater physiological importance for intracellular acidification and neutralisation, especially during exercise. Acid loading with strong ions (ie. lactate) instead of weak acids and CO_2 may change the stoichiometric relationship. While previous studies have implied that intracelluar acid-base regulation is dependent upon movement of hydrogen ions or bicarbonate across the cell membrane, only an exchange of strong ions, CO_2 or weak acids is actually required. Movement of strong ions across the cell membrane will make it appear "as if" hydrogen ions have been transported across the membrane, when in fact the [SID] has changed. A change in [SID] will cause the $[H^+]$ and all the other dependent variables to redistribute according to the physicochemical principles governing the system.

4.5.3 Strong ion exchange across the lung

Although the lung's primary function in terms of acid-base balance is the removal of CO_2, strong ion movements may also occur during transit through the lung. The [SID] expected in "idealised"

plasma was calculated according to Stewart (1981) using the following "idealised" values: venous and arterial [ATOT], 20 mEq/l; mixed venous PCO_2, 47 mm Hg; arterial PCO_2, 40 mm Hg; mixed venous $[H^+]$, 42.1 nmol/l; arterial $[H^+]$, 39.8 nmol/l. The [SID] of "idealised" mixed venous and arterial plasma in a nonexercising individual is approximately 45.0 mEq/l and 42.5 mEq/l, respectively. The [SID] decreases approximately 2.5 mEq/l during the transition from idealised mixed venous plasma to idealised arterial plasma. The fv-a [SID] difference at rest was approximately 4 mEq/l and similar to that expected in idealised plasma. The difference may be due to differences between femoral venous and mixed venous blood. The mixed venous blood is composed of blood returning from all tissues in the body, and during exercise the mixed venous [SID] will be comprised mainly of strong ions returning from the working tissues.

Chloride moves out of the red blood cell during the transition from venous to arterial blood ("chloride shift"); the v-a $[Cl^-]$ difference is approximately -1.5 mmol/l (Davenport, 1974). The measured fv-a $[Cl^-]$ difference was approximately -2.0 mmol/l. Strong cations may also move across the red cell membrane and contribute to the decrease in the [SID]; the fv-a strong ion differences were 1.0 mmol/l and 0.9 mmol/l for sodium and potassium, respectively. Although strong ion exchange may occur between the red blood cell and the plasma, this change alone will not significantly alter the acid-base status of the plasma.

4.5.4 Strong ion exchange across the inactive forearm muscle during recovery from maximal exercise

The v-a [SID] difference increased immediately after maximal exercise (Fig. 7b). The increased v-a difference was due to an elevated venous [SID] since the arterial [SID] was similar to resting levels. The [SID] decreased during recovery and contributed to the acidosis in the forearm vein (Fig. 16) and artery (Fig. 15). The venous [SID] was higher than the arterial [SID] throughout recovery (Table 10, Fig. 7b). This difference was attributed to an uptake of lactate and chloride by the inactive tissue (Fig. 12b). The inactive tissue contributes to the recovery of acid-base balance by removing anions; the process is much slower that removing CO_2 and was not complete after 10 min recovery.

The concentration of strong ions, with the exception of the venous [Cl^-], increased in the forearm artery and vein after maximal leg exercise. The increase in concentration was due to a decrease in plasma volume since the strong ions were taken up by the inactive forearm muscle. The concentration of strong ions was corrected for the decrease in plasma volume and the v-a concentration difference was used to estimate strong ion fluxes across the inactive forearm muscle.

Potassium exchange across the inactive muscle : The forearm arterial and venous [K^f] increased after maximal leg exercise (Table 12). Potassium was taken up by the inactive muscle immediately after exercise (5 umol/100 gm/min) (Fig. 11b). Potassium uptake by the inactive muscle was less than its release from the working muscle and caused the arterial [K^+] to increase immediately after exercise. After 2 min recovery the v-a [K^+] difference was almost zero. Sjøgaard (1985) reported that during one-legged knee extension potassium was released by the muscle but was taken up by the inactive resting leg. However the potassium content of the inactive triceps brachii muscle did not change during maximal leg cycling (Sjøgaard, 1983).

Sodium exchange across the inactive muscle : The plasma [Na^+] decreased below resting levels after correcting for the decrease in plasma volume (Table 12). Sodium was taken up by the inactive forearm during recovery (Table 11b); sodium uptake increased to 30-40 umol/100 gm/min during recovery. Sodium uptake by the inactive muscle was less than sodium release by the working muscle and yet the arterial [Na^+] decreased after exercise. This implies that sodium must be taken up by other tissues in addition to inactive muscle. The strong ion content of inactive tissue was not examined but sodium uptake by the muscle would be expected to increase the tissue sodium content. The sodium content of the inactive triceps brachii muscle did not change after following maximal leg cycling (Sjøgaard, 1983).

Chloride exchange across the inactive muscle : The arterial and venous [Cl^-] decreased after exercise as chloride was taken up by the inactive muscle (Fig. 11b); chloride uptake increased from approximately 10 umol/100 gm/min at rest to approximately 55 umol/100 gm/min during recovery. Chloride uptake by the nonworking tissue contributed to the increase in the plasma [SID] across the muscle.

After maximal cycle ergometer exercise there is an exchange of strong ions across the inactive muscle which effectively maintains a higher [SID] in the forearm vein than in the artery. Lactate, sodium, potassium and chloride were taken up by the nonworking muscle. Throughout the recovery the uptake of anions by the inactive muscle exceeded the uptake of cations, thereby increasing the [SID] across the muscle. When the CO_2-induced acidosis is eliminated in the lungs, the remaining strong ion-induced acid load will be less than what was present in the arterial plasma flowing into the nonworking muscle. Electrical neutrality is preserved in plasma and muscle as the dependent variables redistribute themselves according to physicochemical principles governing the system. At 30 s recovery, the forearm venous [cation] - [anion] difference was 8 mEq/l: [SID], 50.3 mEq/l; [H^+], 67.7 nEq/l; [HCO_3^-], 26.1 mEq/l; [A^-], 16.3 mEq/l; [OH^-], 0.65 uEq/l; [CO_2], 23 uEq/l.

4.6 CONTROL OF CO_2

CO_2 production increases in muscle during maximal exercise. The increase in intracellular PCO_2 that was assumed to occur during maximal exercise was responsible for approximately 25% of the intracellular acid load, the remaining 75% was due to the [SID]. CO_2 release from muscle reduces the intracelluar CO_2 load, thereby contributing to the recovery of the intracellular $[H^+]$.

4.6.1 CO_2 output across the quadriceps femoris muscle during recovery from maximal exercise

The CO_2 content of the plasma is a balance between the amount CO_2 added to it from tissue metabolism and the amount of CO_2 excreted from the body by the lungs. The fv-a plasma CO_2 content difference across the muscle increased after exercise as the fv-a PCO_2 difference (Fig. 6a) and fv-a $[HCO_3^-]$ difference (Fig. 10a) increased. The CO_2 output from the working muscle increased from 3 umol/100 gm/min (fv-a [total CO_2] x blood flow) at rest to 915 umol/100 gm/min at 30 s recovery. The CO_2 output from the muscle (3060 ml/min) corresponds to the excess $\dot{V}CO_2$ above rest (2965 ml/min) measured at the mouth after exercise. CO_2 output from the muscle decreased soon after exercise and was approximately 10 umol/100 gm/min at the end of the recovery.

In the first 30 s of recovery the acidosis in the femoral vein was primarily related to the PCO_2 since the [SID] was still at resting levels (Fig. 14). The PCO_2 had a greater effect on the $[H^+]$ in the plasma than in the intracellular fluid; an increase in PCO_2 raised the $[H^+]$ approximately 1 nmol/l/mm Hg in the plasma and 0.5 nmol/l/mm Hg in the intracellular fluid. The difference was due to the higher intracellular [ATOT]. The PCO_2 decreased during recovery and was less than resting levels at the end of recovery. The PCO_2 did not contribute to the elevated $[H^+]$ in the latter part of recovery; at this time the $[H^+]$ was maintained by a depressed [SID] and an elevated [ATOT] (Fig. 14). The arterial PCO_2 was less than resting levels throughout recovery; the elevated arterial $[H^+]$ was due to the depressed [SID] and an elevated [ATOT] (Fig. 15).

4.6.2 CO_2 output by the lung during recovery from maximal exercise

The CO_2 produced in active and inactive muscle diffuses into the blood and is transported to the lungs where it can be eliminated from the body. Ventilation ($\dot{V}E$) and whole body CO_2 output ($\dot{V}CO_2$) increase during heavy exercise; the $\dot{V}E$ and $\dot{V}CO_2$ both increased by approximately 12 fold during maximal exercise; the $\dot{V}CO_2$ increased to 3240 ml/min after exercise. The excess $\dot{V}CO_2$ measured at the end of exercise (2970 ml/min) was similar to the calculated CO_2 output from the working muscle (3060 ml/min) at the end of exercise (Section 4.6.1). The high $\dot{V}CO_2$ continued into the first part of recovery and

coincided with the continued output of CO_2 from the muscle; the t1/2 for recovery of $\dot{V}CO_2$ was approximately 105 s. In contrast, the $\dot{V}O_2$ decreased immediately after exercise (t1/2 approximately 45 s) and caused the respiratory exchange ratio (ie. the ratio $\dot{V}CO_2/\dot{V}O_2$) to increase. During recovery the fall in femoral venous PCO_2 and $\dot{V}CO_2$ followed a similar time course indicating that the two processes were related. Pulmonary gas exchange was effective in preventing a respiratory acidosis in the artery; the arterial PCO_2 was less than resting levels throughout recovery.

4.6.3 CO_2 output across the inactive forearm muscle during recovery from maximal exercise

CO_2 output by the inactive muscle produces an additional respiratory load in the body that must be handled by the lungs. The increased v-a PCO_2 difference (Fig. 6b) and v-a $[HCO_3^-]$ difference (Fig. 10b) contributed to an increase in the v-a plasma CO_2 content difference after exercise. CO_2 output across the inactive tissue increased from approximately 5 umol/100 gm/min at rest to approximately 50 umol/100 gm/min after maximal leg exercise. This represents an additional 190 ml/min (CO_2 output x 17 kg inactive muscle x 22.3 ml/mmol) that must be eliminated from the body. CO_2 output decreased to approximately 15 umol/100 gm/min after 10 min recovery. The increase in venous PCO_2 raised the venous $[H^+]$ above that found in the artery, even though the venous [SID] was higher

(Fig. 16). As demonstrated previously, the lung was effective in eliminating the excess CO_2 produced in the working and nonworking muscle. The fall in arterial PCO_2 that occurred after exercise prevented a large increase in the arterial $[H^+]$ (Fig. 15); the increase in arteral $[H^+]$ was caused by a decrease in the [SID].

4.7 THE CONCENTRATION OF WEAK ACIDS IN THE PLASMA DURING RECOVERY FROM MAXIMAL EXERCISE

The plasma [ATOT] consists of proteins and various organic weak acids including fatty acids, ketoacids, ammonia and phosphate. Plasma proteins make up more than 80% of the [ATOT]. The [ATOT] was not measured but the plasma [ATOT] was assumed to be 20 mEq/l (Stewart, 1981). Plasma proteins do not readily diffuse across the capillary membrane and any change in plasma volume will change the plasma protein concentration. The estimated decrease in plasma volume found in this study would be expected to increase [ATOT] to approximately 23 mEq/l (ie. a 10-13% increase). The concentration change of other weak acids are too small to significantly affect the [ATOT]. Fatty acids and ketoacids do not increase during short-term maximal exercise. We observed that after 30 s maximal exercise the plasma fatty acid concentration did not change significantly from resting levels (ie. 0.21 mmol/l) (McCartney et al., 1985). The concentration of ammonia increases during heavy exercise but its concentration is too low to significantly affect the [ATOT]; the

ammonia concentration increases from 30-70 umol/l at rest to 140-230 umol/l after maximal exercise (Dudley et al., 1983; Mutch and Banister, 1983; Buono, Clancy and Cook, 1984). The decrease in plasma volume appears to be the main factor contributing to the increase in [ATOT] after exercise. The [ATOT] increases the $[H^+]$ approximately 1-2 nmol/l/(mEq/l) increase in the [ATOT] but this effect is minor unless the [ATOT] increases dramatically. Thus the [ATOT] increases during exercise as fluid leaves the vascular space and contributes to the elevated plasma $[H^+]$. The [ATOT] returns to resting levels as the plasma volume is restored; the plasma volume was still 5-10% below resting levels after 10 min recovery (Tables 6 and 12).

4.8 HYDROGEN ION AND BICARBONATE CONCENTRATIONS AFTER MAXIMAL EXERCISE: COMPARISON OF MEASURED AND CALCULATED VALUES

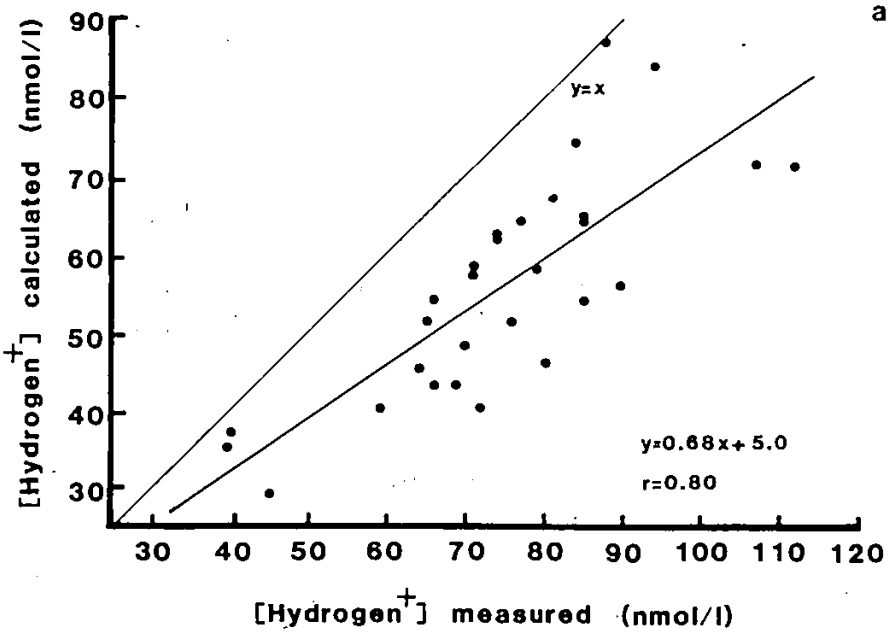
One of the aims of this thesis was to use the physicochemical principles outlined by Stewart (1981) to describe the factors contributing to the intra- and extracellular acidosis after 30 s maximal exercise. In using this approach I was faced with the problem encountered by many investigators of not being able to make all the necessary measurements, and thus having to assume certain values. In some instances the true value cannot be determined and only assumed values are reported in the literature. It is because of these unknown values that discrepancies were found between the two methods used to calculate the plasma $[H^+]$ and $[HCO_3^-]$.

The $[H^+]$ was calculated as a) the antilog (-pH) ("measured $[H^+]$") and b) according to the equations presented in Stewart (1981) ("calculated $[H^+]$"). The $[HCO_3^-]$ was calculated using a) the Henderson-Hasselbalch equation with the measured pH and PCO_2 ("measured $[HCO_3^-]$"), and b) according to the equations presented in Stewart (1981) ("calculated $[HCO_3^-]$"). The relationship between the measured and calculated $[H^+]$ and $[HCO_3^-]$ in the femoral vein, artery and forearm vein are presented in Figures 17, 18 and 19, respectively. In all cases the calculated $[H^+]$ and $[HCO_3^-]$ appear to qualitatively reflect the actual measured variables. However, the calculated $[H^+]$ consistently underestimated the measured $[H^+]$ and the calculated $[HCO_3^-]$ consistently overestimated the measured $[HCO_3^-]$. Some of the factors contributing to this discrepancy are discussed below.

The independent variable [ATOT] was not measured in this study. The [ATOT] represents the total concentration of weak acid in the intracellular or extracellular fluids. Stewart (1981) reported [ATOT] values of 200 mEq/l for intracellular fluid and 20 mEq/l for plasma. The protein concentration of plasma ranges between 6.5 and 8.5 gm/100 ml and contributes approximately 15.5-20.5 mEq/l (protein concentration (gm/100 ml) x 2.41) to the plasma [ATOT]. The actual value for [ATOT] may be difficult to measure but an effective [ATOT] may be assumed. Two curves have been plotted in each of Figures 14-16; the solid curve was plotted using the measured PCO_2 and [SID],

Figure 17 a. Relationship between measured and calculated plasma hydrogen ion concentration in the femoral vein. The hydrogen ion concentration was calculated using PCO_2, strong ion difference and the concentration of weak acid (Stewart, 1981). The PCO_2 and strong ion difference were measured in the femoral vein and the concentration of weak acid was assumed to be 20 mEq/l (Stewart, 1981) but corrected for the decrease in plasma volume.

b. Relationship between measured and calculated plasma bicarbonate concentration in the femoral vein. The bicarbonate concentration was calculated using PCO_2, strong ion difference and the concentration of weak acid (Stewart, 1981).


a
90
80
70
60
50
40
30
[Hydrogen+] calculated (nmol/l)
y=x
y=0.68x+5.0
r=0.80
30 40 50 60 70 80 90 100 110 120
[Hydrogen+] measured (nmol/l)

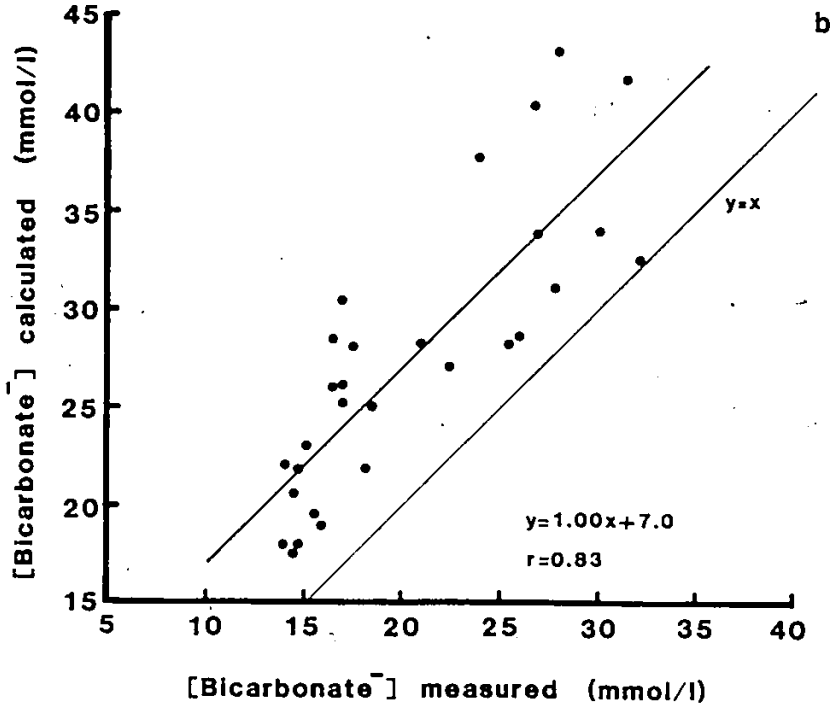
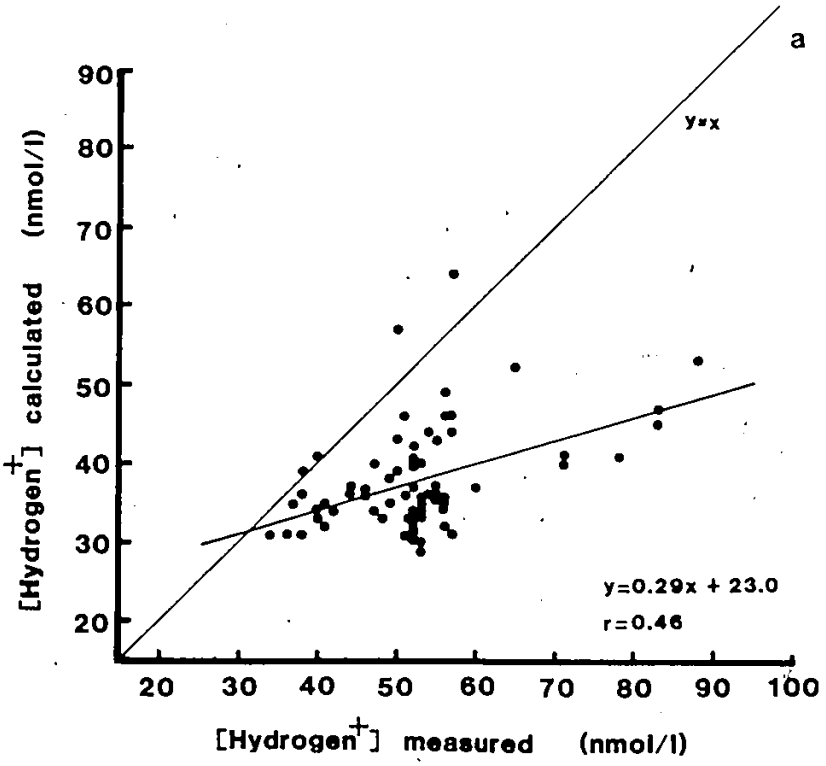

b
45
40
35
30
25
20
15
[Bicarbonate-] calculated (mmol/l)
y=x
y=1.00x+7.0
r=0.83
5 10 15 20 25 30 35 40
[Bicarbonate-] measured (mmol/l)

Figure 18 a. Relationship between measured and calculated plasma hydrogen ion concentration in the artery. The hydrogen ion concentration was calculated using PCO_2, strong ion difference and the concentration of weak acid (Stewart, 1981). The PCO_2 and strong ion difference were measured in the artery and the concentration of weak acid was assumed to be 20 mEq/l (Stewart, 1981) but corrected for the decrease in plasma volume.

b. Relationship between measured and calculated plasma bicarbonate concentration in the artery. The bicarbonate concentration was calculated using PCO_2, strong ion difference and the concentration of weak acid (Stewart, 1981).


a
y=x
y=0.29x + 23.0
r=0.46
[Hydrogen+] calculated (nmol/l)
[Hydrogen+] measured (nmol/l)
90
80
70
60
50
40
30
20
20 30 40 50 60 70 80 90 100

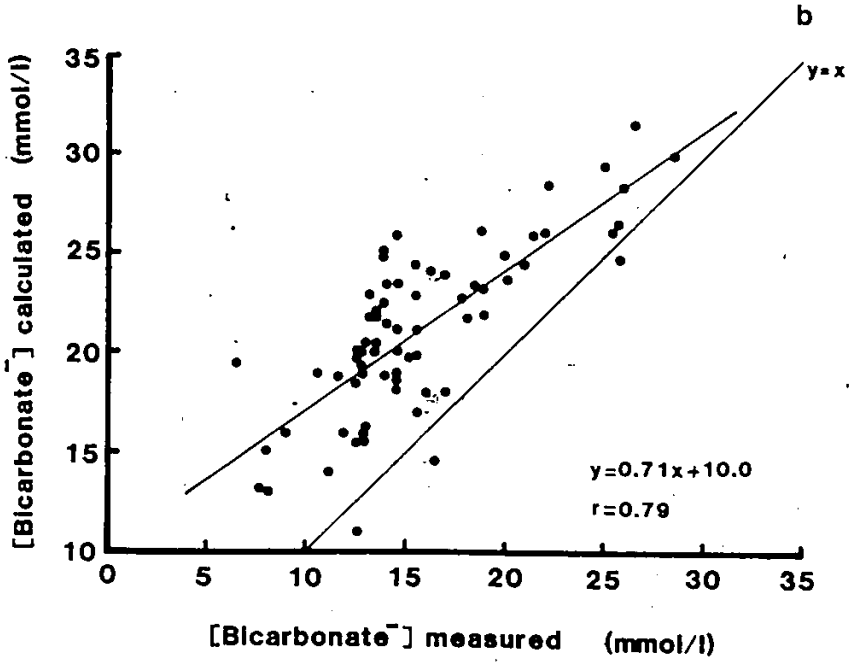
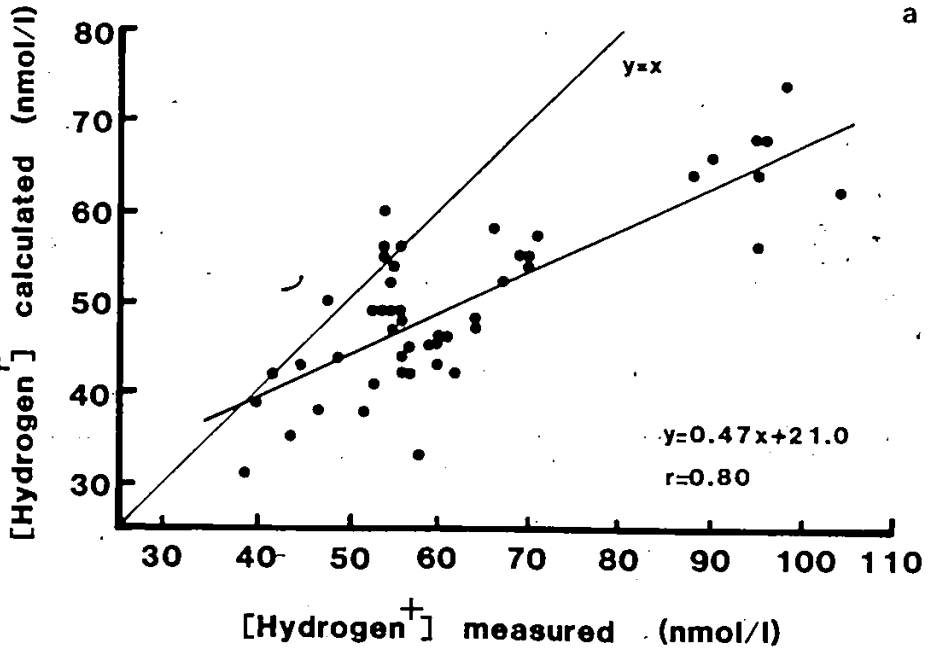
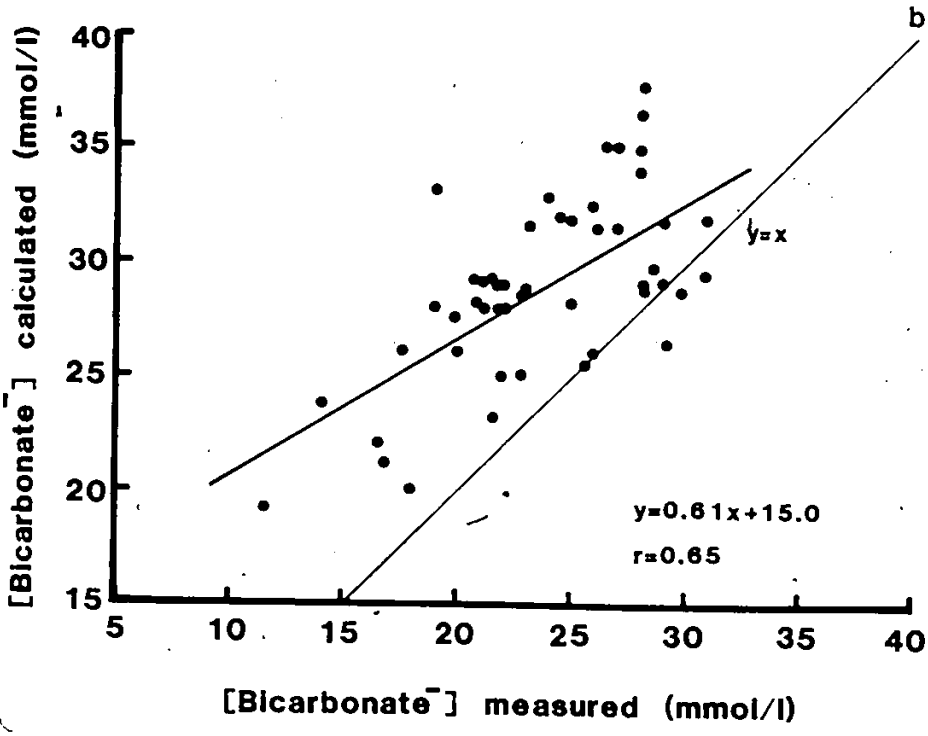

b
y=x
y=0.71x+10.0
r=0.79
[Bicarbonate-] calculated (mmol/l)
[Bicarbonate-] measured (mmol/l)
35
30
25
20
15
10
0 5 10 15 20 25 30 35

Figure 19 a. Relationship between measured and calculated plasma hydrogen ion concentration in the forearm vein. The hydrogen ion concentration was calculated using PCO_2, strong ion difference and the concentration of weak acid (Stewart, 1981). The PCO_2 and the strong ion difference were measured in the forearm vein and the concentration of weak acid was assumed to be 20 mEq/l (Stewart, 1981) but corrected for the decrease in plasma volume.

b. Relationship between measured and calculated plasma bicarbonate concentration in the forearm vein. The bicarbonate concentration was calculated using PCO_2, strong ion difference and the concentration of weak acid (Stewart, 1981).


a
y=x
y=0.47x+21.0
r=0.80
[Hydrogen+] calculated (nmol/l)
[Hydrogen+] measured (nmol/l)
80
70
60
50
40
30
30
40
50
60
70
80
90
100
110


b
y=x
y=0.61x+15.0
r=0.65
[Bicarbonate-] calculated (mmol/l)
[Bicarbonate-] measured (mmol/l)
40
35
30
25
20
15
5
10
15
20
25
30
35
40

and the open curve was plotted using the measured PCO_2 and $[H^+]$ (antilog -pH). In each figure the solid curve underestimated the $[H^+]$ as plotted on the open curve. This difference in $[H^+]$ plotted in this manner may be attributed to the difference between the actual [ATOT] and the value of 20 mEq/l assumed for the [ATOT]. Estimating the change in [ATOT] from plasma volume changes may underestimate the increase in [ATOT] that occurs after maximal exercise. Increasing the [ATOT] by 5-10 mEq/l will will improve the relationships presented in Figures 14-16.

The [ATOT] is comprised of proteins, phosphates, and other weak acids all having different dissociation constants (ie. Ka). For physiological purposes it is necessary to substitute a single representative value for the pKa that explains the behaviour of all the weak acids. An inappropriate choice for the dissociation constant will introduce an error into the calculations.

The values of the constants (K'w, Ka, Kc, K3) required in the $[H^+]$ equation were given in Stewart (1981, 1983). The pK of water (K'w), bicarbonate (Kc, K3), phosphate (Ka) and the imidazole group of histidine (Ka) decrease with increases in temperature (Rahn, Reeves and Howell, 1975). For the purposes of the anaylsis their values were assumed not to change after maximal exercise. Muscle and blood temperature increase and pH decreases during heavy exercise; during recovery the temperature and pH return to resting values. At 38 °C

the dpK/dT for carbonic acid and proteins were -0.0027 and -0.0159, respectively (Reeves, 1976). These factors may change the value of the constants after exercise and contribute to the difference between the measured and calculated values.

The $[H^+]$ of intracellular and extracellular fluid is related to the PCO_2, [SID] and [ATOT] of the respective fluid compartments. There were quantitative discrepancies between the $[H^+]$ measured in plasma and that calculated from the PCO_2, [SID] and [ATOT]. Part of the discrepancy may be attributed to the uncertainty for some of the assumed values such as the weak acid concentration and the various "K" values. The values used for these variables were those given by Stewart (1981, 1983) and have not been tested during steady-state or nonsteady-state exercise. However the qualitative relationship between the $[H^+]$ and the independent variables and the theoretically sound development of this approach justifies its continued use when attempting to understand acid-base disturbances.

5 GENERAL SUMMARY

5.1 INTRODUCTION

Maximal exercise of short duration produces a severe intracellular acidosis; as increases in intracellular hydrogen ion concentration have been implicated in excitation-contraction coupling failure and energy production impairment ultimately leading to fatigue or "exhaustion", mechanisms to control the acidosis are of great importance. The hydrogen ion concentration in muscle is regulated by the PCO_2, the concentration of strong ions ([SID]) and weak acids ([ATOT]). Thus several mechanisms are available to meet this acid-base homeostatic challenge. The work in this thesis used short-term, maximal exercise to generate the acidosis and examined the relative contributions of these mechanisms to the resolution of the acidosis.

5.2 A Summary of the Response to the Acidosis of Maximal Exercise

The changes in acid-base balance were examined after 30 s of maximal constant-velocity cycle ergometer exercise. A gross increase in acid production occurred in the quadriceps femoris muscle during exercise due to both increased CO_2 production and strong anion

production. The intracellular PCO_2 was presumed to increase to over 100 mm Hg and the intracellular lactate concentration increased to 45 mmol/l; the intracellular concentration of the other strong ions did not change. The total phosphate concentration did not change suggesting that the intracellular weak acid concentration did not change during maximal exercise. Thus the calculated intracellular hydrogen ion concentration increased from 70 nmol/l (pH 7.14) at rest to 170 nmol/l (pH 6.78) immediately after maximal exercise. Of the total change in hydrogen ion concentration, 25% was due to CO_2 and 75% was due to the lactate-induced fall in the strong ion difference.

Recovery from the acidosis involved the elimination of both CO_2 and lactate from within the muscle. Initially CO_2 release from the muscle was important in partially reducing the intracellular hydrogen ion concentration. CO_2 output from the muscle was elevated during the first part of recovery; the femoral venous PCO_2 increased to 105 mm Hg after exercise. CO_2 elimination from the body increased in response to the elevated CO_2 flux delivered to the lungs during and immediately following maximal exercise; the CO_2 output at the lungs increased to over 3000 ml/min by the end of exercise. Removal of excess CO_2 from the muscle was a relatively fast process and complete within the first 3 minutes of recovery. The lungs were effective in eliminating the CO_2-induced acidosis as the arterial PCO_2 was reduced below resting levels throughout recovery. Thus CO_2 elimination from the body is a fast, efficient mechanism for regulating the acid-base

balance of the body, but as the CO_2 contributes only partially to the acid load of the muscle, acid-base balance was not completely restored by this process.

Strong anion (ie. lactate) production was responsible for a greater portion of the intracellular acid load. Lactate disappearance from muscle is a relatively slow process and occurred at a rate of 2 mmol/kg/min. Immediately after exercise the intracellular-femoral venous lactate gradient was 40 mmol/l and favoured diffusion of lactate from the muscle into the circulation; the femoral venous lactate concentration rose to 15 mmol/l during recovery. The rate of diffusion from muscle decreased during recovery as the muscle-plasma lactate gradient decreased to approximately 2 mmol/l. Diffusion accounted for approximately 55-60% of the lactate disappearing from muscle. The remaining lactate was metabolised within the muscle and either converted to glycogen or oxidised. Once in the circulation the nonactive tissues of the body assume the role of removing lactate. The venous-arterial difference across inactive forearm muscle averaged 4.5 mmol/l. The arterial-venous O_2 content difference across the forearm indicated that only about 45% of the lactate taken up by the forearm could be oxidised, the remaining lactate underwent other metabolic fates. By reducing the lactate concentration of the body, the nonworking tissue contributed to the recovery of acid-base balance by effectively reducing the nonCO_2 acid load.

Strong ions in addition to lactate also contribute to the intra- and extracellular strong ion difference. Although the concentration of sodium, potassium, calcium, magnesium and chloride did not change in the working muscle during exercise or recovery, the venous-arterial concentration differences across the leg indicated that sodium and potassium were released and chloride was taken up by the muscle. The nonworking muscle appeared to take up sodium, potassium and chloride. While lactate was shown to significantly affect the acid-base status of the muscle, the exchange of other strong ions did not appear to contribute to the intracellular acidosis.

The weak acid concentration of the plasma was presumed to increase during maximal exercise as the plasma volume decreased. The plasma volume was slowly restored during recovery; the plasma volume (as determined by the change in hemoglobin concentration) was still reduced after 10 min recovery.

5.3 Questions for Future Research

The interpretation of these data in terms of the variables that determine the hydrogen ion concentration, depends on an understanding of the independent variables in muscle; the PCO_2, the strong ion difference and the concentration of weak acids. To

understand intracellular acid-base balance quantitatively requires accurate measurement of these variables. The intracellular PCO_2 probably cannot be measured, especially during exercise. The femoral venous PCO_2 was assumed to reflect the intracellular PCO_2. The strong ion difference in the cell cannot be measured accurately without knowing the intracellular and interstitial fluid volumes, and the interstitial strong ion concentration. The intracellular concentration of weak acids is not known and must be assumed. Thus all three independent variables responsible for the intracellular hydrogen ion concentration cannot at present be accurately measured. Research must be directed towards obtaining better estimates of these variables.

Muscle pH has not yet been accurately measured to provide a reliable reference with which to compare the calculated values for the intracellular hydrogen ion concentration. The acid-base changes should also be followed for a longer time in recovery to obtain the complete time course for recovery of acid-base balance after short-term, maximal exercise.

APPENDIX A

VALIDATION OF THE BREATH-BY-BREATH GAS ANALYSIS SYSTEM

Breath-by-breath analysis of ventilation ($\dot{V}E$), O_2 intake ($\dot{V}O_2$) and CO_2 output ($\dot{V}CO_2$) is useful when examining changes in gas exchange during nonsteady-state conditions as occurs during and following short-term, maximal exercise. The accuracy with which breath-by-breath measurements reflect what is actually occurring in the body can best be determined during steady-state conditions by comparing to an alternate referee system. In the present study the computer breath-by-breath gas analysis system was compared against mechanical and biological referee systems. In all cases, the computer breath-by-breath system was calibrated before use according to the protocol outlined in Chapter 2.

Mechanical validation : The accuracy with which the breath-by-breath system measured tidal volume was determined by passing measured volumes of air into the system and comparing them to the volumes detected by the computer system. A 9 l respirometer (Collins #718B) was connected in series to the pneumotachograph and transducer by a length of low-resistance, flexible tubing. Fixed volumes ranging from 0.2-6.5 l were introduced into the system by

raising and lowering the bell of the spirometer. The volume displacement was recorded on the spirometer and the actual volume was calculated by comparing the records to those obtained from a calibrated 2 l syringe. The relationship between the volumes generated by the spirometer and those detected by the computer system is presented in Figure 20a and Table 20.

A standard artificial lung (SAL) (Beckman Instruments) was used to deliver fixed volumes of air ranging from 0.4-1.6 l at frequencies ranging from 4-50 strokes/min. The SAL was connected in series to the pneumotachograph and transducer by a length of low-resistance tubing. The SAL was calibrated with respect to both frequency and volume before use. The relationship between the frequency generated by the SAL and that detected by the breath-by-breath system is presented in Figure 20b and Table 20. The relationship between the SAL-generated $\dot{V}E$ and the computer detected $\dot{V}E$ is shown in Figure 21 and Table 20. The error in $\dot{V}E$ amounted to 0.5 l/min or 1.6% at the mean $\dot{V}E$ (31.0 l/min).

Biological validation : Four subjects were studied at rest and during steady-state cycle ergometer exercise at 150, 300, 500, 900, 1200 and 1500 kpm/min. In addition, three subjects performed a progressive incremental exercise test where the power output was increased every 30 s. Ventilation, $\dot{V}O_2$ and $\dot{V}CO_2$ were measured breath-by-breath and comparisons were made with values obtained by one

Table 20. Regression analyses for comparisons between referee systems (Douglas Bag Collection, Beckman SAL and MMC) and computer breath-by-breath system.

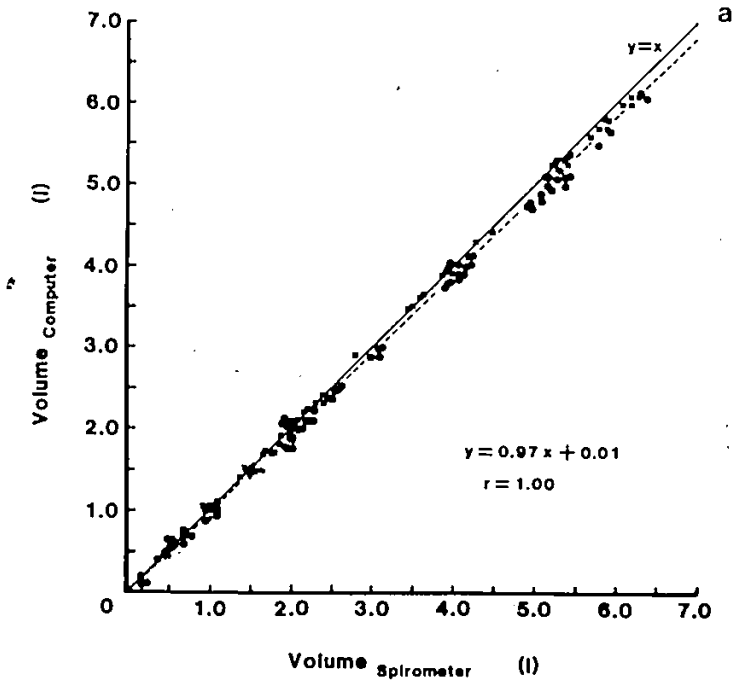
Variable	Range	Units	Slope	Intercept	r	n	Covar
V_T	0.2 - 6.5	l	0.97*	0.01	0.999	180	2.5
f	4 - 50	br/min	0.99	0.79**	0.999	49	2.0
$\dot{V}_E$SAL	3.5 - 77.5	l/min	0.99	0.76**	0.999	43	2.4
$\dot{V}_E$Bag	8 - 111	l/min	1.04*	-0.73	0.998	58	4.0
$\dot{V}O_2$Bag	290 - 3440	ml/min	1.01	9.51	0.997	55	5.0
$\dot{V}CO_2$Bag	215 - 3580	ml/min	1.02*	20.00	0.997	56	5.0
$\dot{V}_E$MMC	8 - 107	l/min	1.06*	-0.68	0.999	42	3.7
$\dot{V}O_2$MMC	270 - 3280	ml/min	1.04*	-9.53	0.997	42	4.7
$\dot{V}CO_2$MMC	210 - 3370	ml/min	1.03*	-1.37	0.998	42	4.2

* significantly different from slope = 1 ($p < 0.05$)

** significantly different from intercept = 0 ($p < 0.05$)

Figure 20 a. Volume detection with breath-by-breath gas analysis system.

b. Frequency detection with breath-by-breath gas analysis system.


a
y=x
y = 0.97 x + 0.01
r = 1.00
Volume Computer (l)
Volume Spirometer (l)
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0

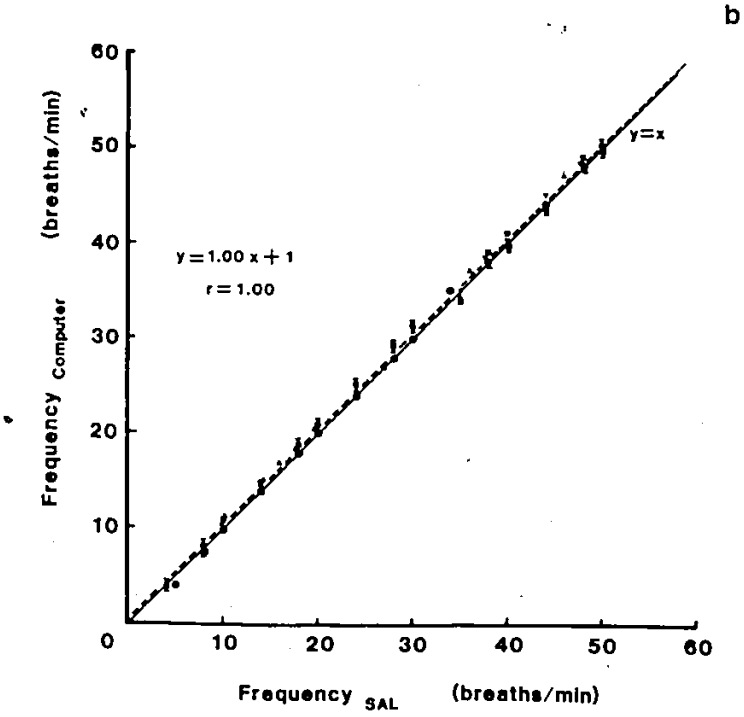
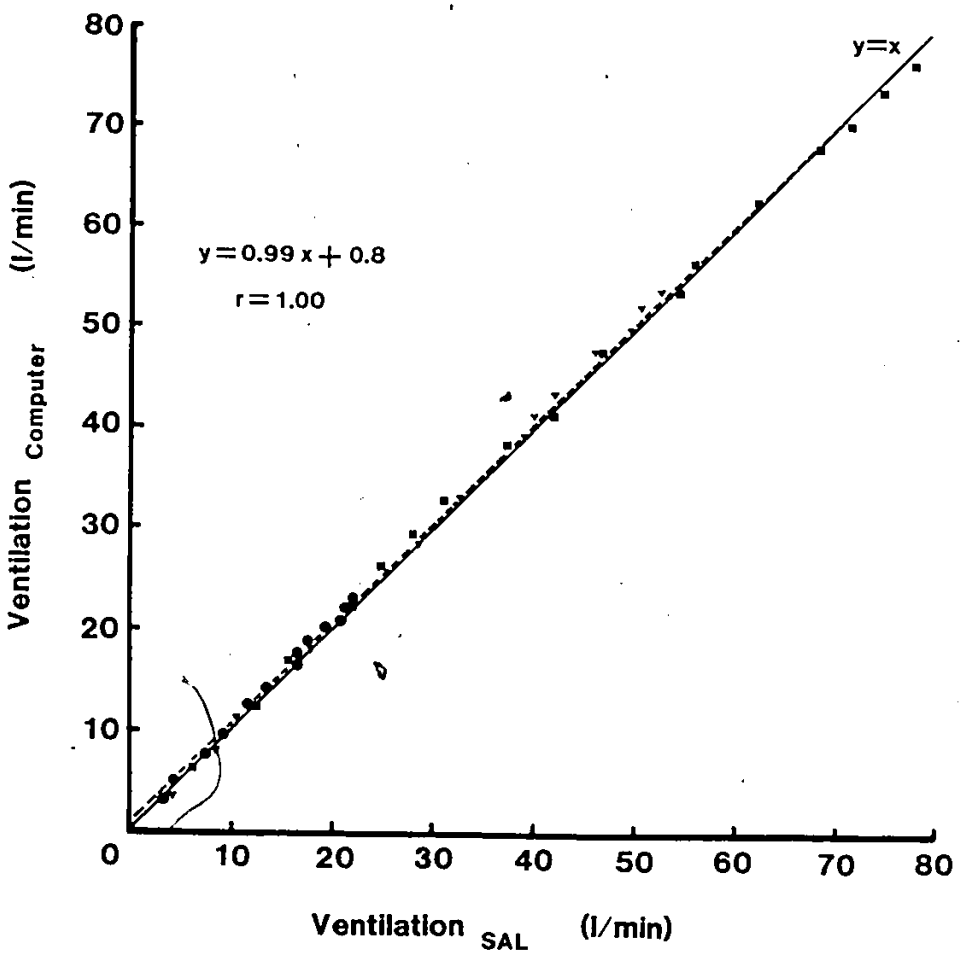

b
y=x
y = 1.00 x + 1
r = 1.00
Frequency Computer (breaths/min)
Frequency SAL (breaths/min)
0
10
20
30
40
50
60

Figure 21. Mean ventilation detected with breath-by-breath gas analysis system.


y=x
y=0.99 x + 0.8
r=1.00
Ventilation Computer (l/min)
Ventilation SAL (l/min)
0
10
20
30
40
50
60
70
80

of two referee systems: Douglas bag collection of expired gas or gas analysis using a Metabolic Measurement Cart (MMC) Horizon System (Beckman Instruments) (Norton, 1982; Jones, 1984).

The exercise tests were performed on an electrically braked cycle ergometer (Elema AM 370) in the seated position. Ventilation and gas exchange were measured with the computer breath-by-breath system for 10 min. During specific periods of the test expired gas was also collected in either meteorological balloons or the Beckman MMC. The balloons or MMC were connected in series and downstream from the pneumotachograph and transducer. The periods for the gas collections were timed using a stop watch so that later, the samples could be synchronized with the breath-by-breath output. The breath-by-breath output was averaged over the period corresponding to the time of gas collection by the referee system and compared to the calculated values obtained by that system.

Collection of expired gas using the Douglas bag method began and ended at the end of an expiration. The gas bags were capped and their contents well mixed. The concentration of O_2, CO_2 and N_2 in the mixed expired gas was determined by the mass spectrometer (Perkin Elmer, MGA 1100). The expired volume was recorded on the respirometer (Collins #718B). The $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated according to standard equations (Jones and Campbell, 1982).

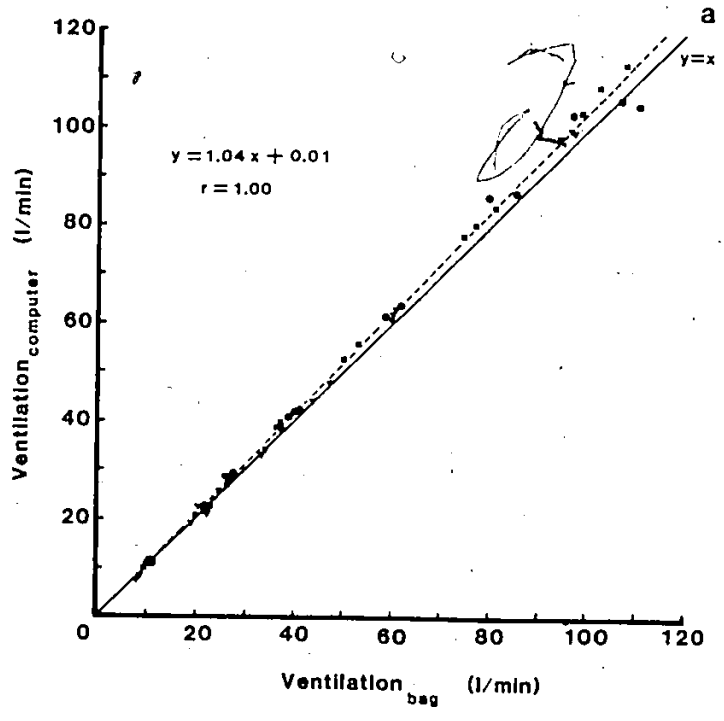
The Beckman MMC was connected to the breath-by-breath system 1-2 min before collecting gas so that the system dead space could be flushed. The collection period began and ended with an expiration. Ventilation and gas exchange were calculated as 15 s averages and comparisons were made with the breath-by-breath system by averaging the values during the collection period.

During incremental nonsteady-state exercise, comparisons were made only between the computer breath-by-breath system and the Beckman MMC. The exercise test consisted of pedalling at 0, 100 and 200 kpm/min for 1 min each, followed by a rapid increase in power output of 100 kpm/min every 30 s for the next 7 min (final power output 1600 kpm/min). Regression analysis of $\dot{V}E$, $\dot{V}O_2$ or $\dot{V}CO_2$ against time was performed by computer. Ventilation and $\dot{V}CO_2$ data were fit using a power function while the $\dot{V}O_2$ data were fit using linear regression.

The relationships between the steady-state $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ as determined by the Douglas bag and Beckman MMC referee systems, and the computer breath-by-breath system are presented in Figures 22-24 and Table 20. In general the relationships between the breath-by-breath system and the referee systems were very close as indicated by the slope (approximately 1.0), the intercept (approximately 0.0) and the high correlation (approxiamtely 1.0). The standard deviation expressed as a percent of the mean response (coefficient of variation) ranged between 3.7% and 5.0% (Table 20).

Figure 22 a. Ventilation: mean values for varying intensities of steady-state exercise - comparison between referee system (Douglas bag) and breath-by-breath gas analysis system.

b. Ventilation: mean values for varying intensities of steady-state exercise - comparison between referee system (Beckman MMC) and breath-by-breath gas analysis system.


a
y=x
y = 1.04 x + 0.01
r = 1.00
Ventilation computer (l/min)
Ventilation bag (l/min)
0
20
40
60
80
100
120

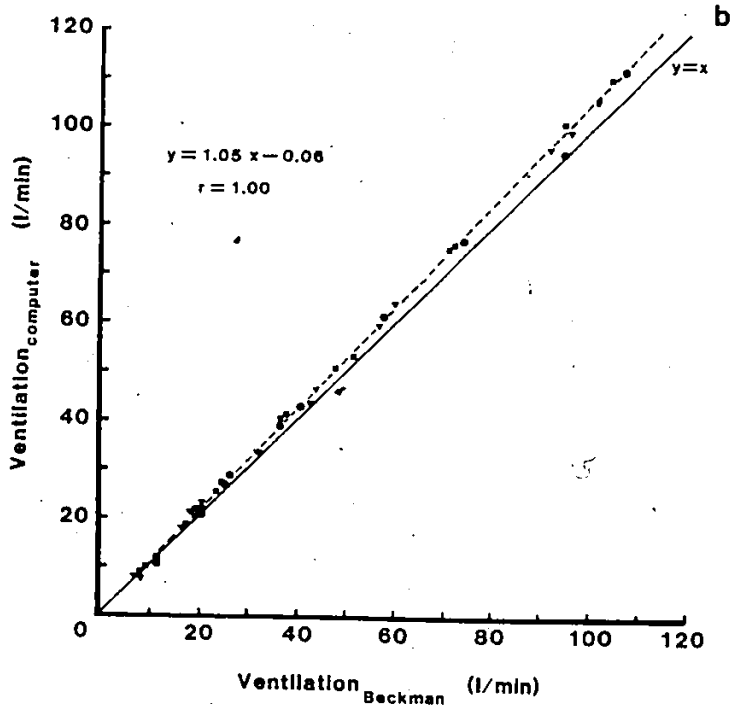
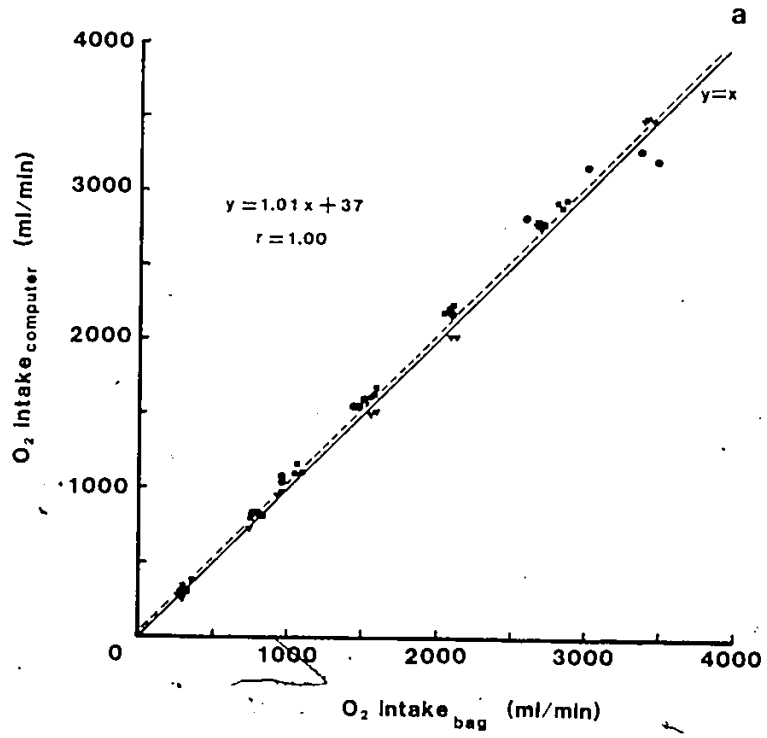

b
y=x
y = 1.05 x − 0.06
r = 1.00
Ventilation computer (l/min)
Ventilation Beckman (l/min)
0
20
40
60
80
100
120

Figure 23 a. O_2 Intake: mean values for varying intensities of steady-state exercise - comparison between referee system (Douglas bag) and breath-by-breath gas analysis system.

b. O_2 Intake: mean values for varying intensities of steady-state exercise - comparison between referee system (Beckman MMC) and breath-by-breath gas analysis system.


a
4000
3000
2000
1000
0
y=x
y = 1.01 x + 37
r = 1.00
O2 intake computer (ml/min)
1000
2000
3000
4000
O2 intake bag (ml/min)

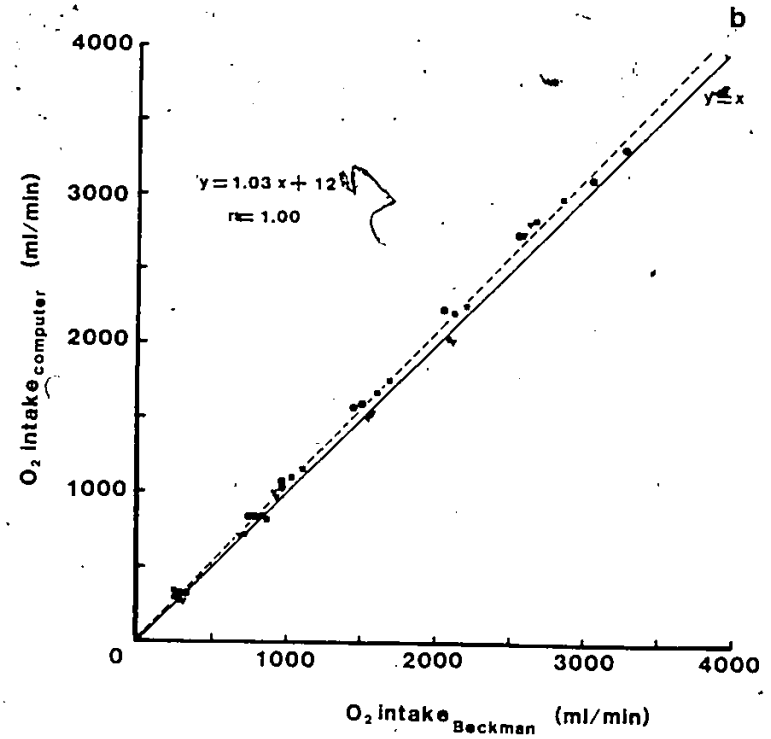
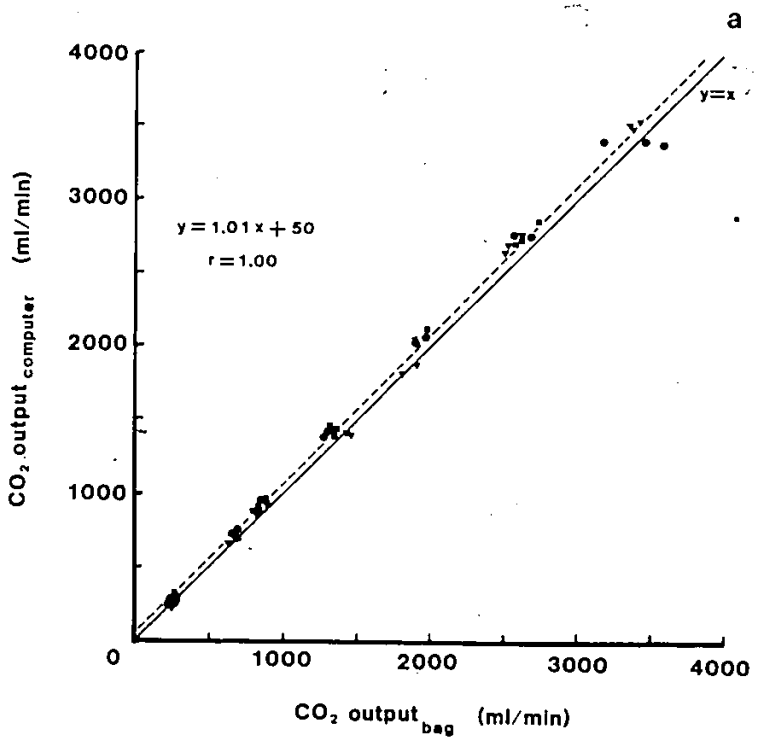
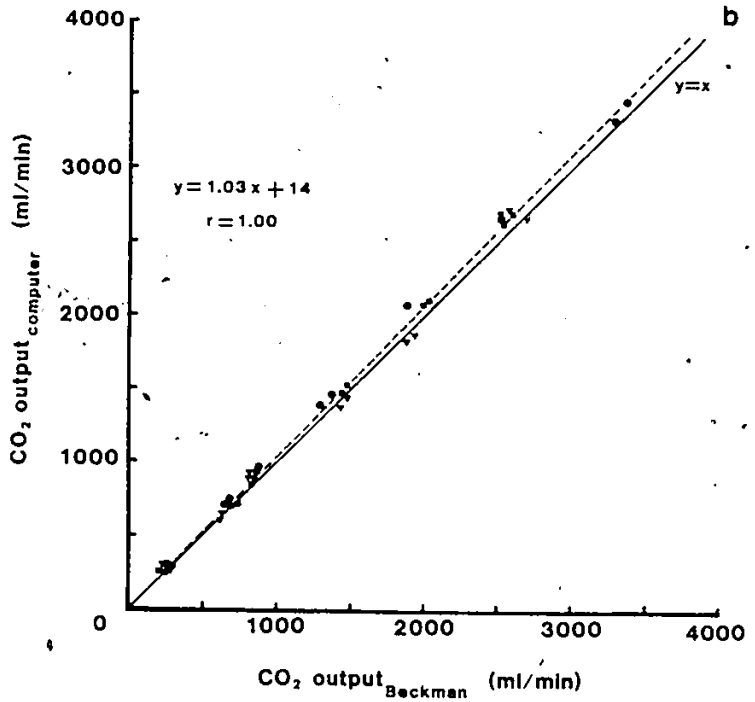

b
4000
3000
2000
1000
0
y=x
y = 1.03 x + 12
r = 1.00
O2 intake computer (ml/min)
1000
2000
3000
4000
O2 intake Beckman (ml/min)

Figure 24 a. CO_2 Output: mean values for varying intensities of steady-state exercise - comparison between referee system (Douglas bag) and breath-by-breath gas analysis system.

b. CO_2 Output: mean values for varying intensities of steady-state exercise - comparison between referee system (Beckman MMC) and breath-by-breath gas analysis system.


a
4000
3000
2000
1000
0
y = 1.01 x + 50
r = 1.00
y=x
1000
2000
3000
4000
CO2 output computer (ml/min)
CO2 output bag (ml/min)


b
4000
3000
2000
1000
0
y = 1.03 x + 14
r = 1.00
y=x
1000
2000
3000
4000
CO2 output computer (ml/min)
CO2 output Beckman (ml/min)

The relationship between the nonsteady-state $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ as determined by the Beckman MMC referee system and the breath-by-breath system are presented in Table 21 and Figure 25. The slopes and intercepts were similar between the computer breath-by-breath system and the Beckman MMC, the only difference being a higher ($p<0.05$) intercept for $\dot{V}O_2$ determined by the breath-by-breath system.

In general, the measurement of ventilation and gas exchange by the breath-by-breath system compares favourably with simultaneous measurements made by two independent referee systems. The computer breath-by-breath gas analysis system can be used to measure gas exchange during the nonsteady-state of short-term maximal exercise, and during recovery from that exercise.

Table 21. Regression analyses for comparisons between referee system (Beckman MMC) and computer breath-by-breath system during progressive exercise.

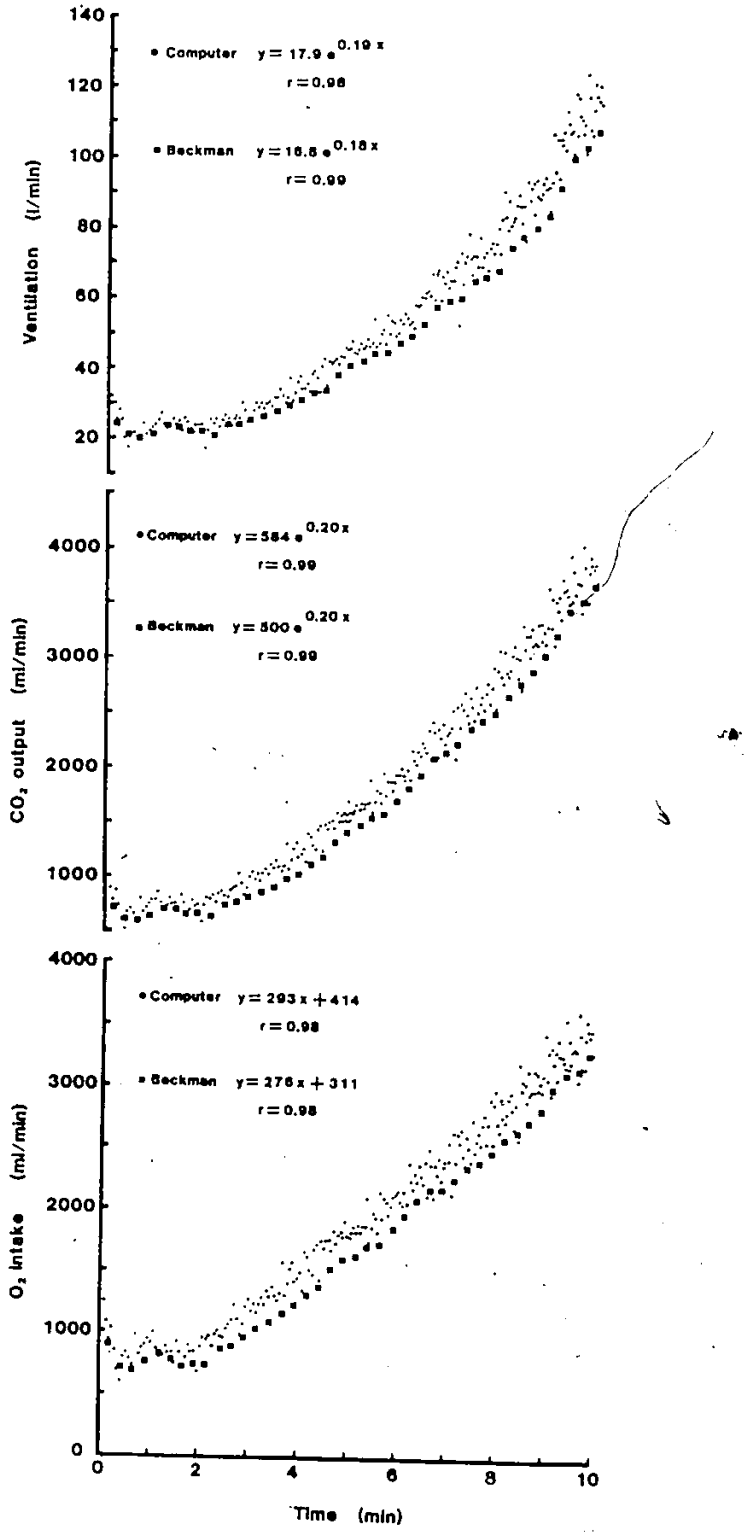
Variable	Slope	Intercept	r
$\dot{V}_E$Beck	0.18 ± 0.00	14.6 ± 2.0	0.987
$\dot{V}_E$Comp	0.19 ± 0.01	15.5 ± 2.4	0.976
$\dot{V}O_2$Beck	280 ± 12	277 ± 43*	0.983
$\dot{V}O_2$Comp	289 ± 6	350 ± 61*	0.976
$\dot{V}CO_2$Beck	0.21 ± 0.00	500 ± 26	0.990
$\dot{V}CO_2$Comp	0.20 ± 0.00	552 ± 36	0.981

* Beckman vs Computer ($p < 0.05$)

$\dot{V}_E$ and $\dot{V}CO_2$: $y = be^{mx}$

$\dot{V}O_2$: $y = mx + b$

Figure 25. Ventilation, CO_2 output and O_2 intake during nonsteady-state progressive exercise of a single subject (6). Comparison between referee system (Beckman MMC) and breath-by-breath gas analysis system.


Ventilation (l/min)
• Computer y = 17.9 e^0.19 x
r = 0.98
• Beckman y = 16.8 e^0.18 x
r = 0.99
CO2 output (ml/min)
• Computer y = 584 e^0.20 x
r = 0.99
• Beckman y = 500 e^0.20 x
r = 0.99
O2 intake (ml/min)
• Computer y = 293 x + 414
r = 0.98
• Beckman y = 276 x + 311
r = 0.98
Time (min)

APPENDIX B

CALCULATIONS

B1. Calculation of $[H^+]$ and $[HCO_3^-]$ from blood measurements

$$[H^+] = 10^{-pH}$$

$$[HCO_3^-] = 0.031 \times PCO_2 \times 10^{pH-6.1}$$

B2. Calculation of $[H^+]$ and $[HCO_3^-]$ according to Stewart (1981).

Water dissociation equilibrium:

$$[H^+] \times [OH^-] = K'w$$

Weak acid dissociation equilibrium:

$$[H^+] \times [A^-] = Ka \times [HA]$$

Conservation of mass for "A":

$$[HA] + [A^-] = [ATOT]$$

Bicarbonate ion formation equilibrium:

$$[H^+] \times [HCO_3^-] = Kc \times PCO_2$$

Carbonate ion formation equilibrium:

$$[H^+] \times [CO_3^{2-}] = K3 \times [HCO_3^-]$$

Electrical neutrality:

$$[SID] + [H^+] - [HCO_3^-] - [A^-] - [CO_3^{2-}] - [OH^-] = 0$$

where [HA] and [A^-] are the concentration of the undissociated weak acid and its conjugate base, [ATOT] is the total concentration of weak acid, [HCO_3^-] is the bicarbonate concentration, [H^+] is the hydrogen ion concentration, [CO_3^{2-}] is the carbonate ion concentration, [OH^-] is the hydroxyl ion concentration, and [SID] is the strong ion difference. The constants "K" represent the following: K'w is the ion product of water, Ka is the weak acid dissociation constant, Kc is the dissociation for bicarbonate into dissolved CO_2 and hydroxyl ions, and K3 is the dissociation constant for bicarbonate into hydrogen ions and carbonate ions.

The value for the [H^+] was determined according to the following equation (Stewart, 1981):

$$[H^+]^4 + (([SID] + Ka) \times [H^+]^3) + ((Ka \times ([SID] - [ATOT]) - K'w - Kc \times PCO_2) \times [H^+]^2) - ((Ka \times (K'w + Kc \times PCO_2) + K3 \times Kc \times PCO_2) \times [H^+]) - Ka \times K3 \times Kc \times PCO_2 = 0.$$

The "K" values given for plasma extracellular fluid are (Stewart, 1981, 1983): K'w, 4.4×10^{-14} $(Eq/l)^2$; Ka, 3.0×10^{-7} Eq/l; Kc, 2.46×10^{-11} $(Eq/l)^2$ /mm Hg; K3, 6.0×10^{-11} Eq/l. The "K" values given for intracellular fluid are (Stewart, 1981): K'w, 4.4×10^{-14} $(Eq/l)^2$; Ka, 1.5×10^{-7} Eq/l; Kc, 2.40×10^{-11} $(Eq/l)^2$/mm Hg; K3, 6.0×10^{-11} Eq/l.

B3. Calculation of the intracellular strong ion concentration

Neutron activation analysis (NAA) was used to measure the tissue strong ion content. The calculations and assumptions used to determine the intracellular strong ion concentration are as follows (a sample calculation for the resting potassium concentration is presented):

interstitial fluid volume (assumed) = 0.39 ml/gm d.w.

plasma $[K^+]$ (measured) = 4.2 mmol/l (or 4.2 umol/ml)

valence = 1

tissue potassium content (measured from NAA) = 13717.5 ug/gm d.w.

molecular weight of potassium = 39.102 ug/umol

wet weight/dry weight ratio (measured) = 3.97 gm w.w./gm d.w.

interstitial fluid potassium content = Donnan ratio x interstitial fluid volume x plasma concentration x valence

= 0.96 x 0.39 ml/gm d.w. x 4.2 umol/ml x 1
= 1.57 uEq/gm d.w.

total muscle potassium content = tissue potassium content x 1/molecular weight x valence

= 13717.5 ug/gm d.w. x 1/(39.102 umol/ug) x 1
= 350.81 uEq/gm d.w.

intracellular potassium content = (total muscle content) - (interstitial fluid content)

= 350.81 uEq/gm d.w. - 1.57 uEq/gm d.w.
= 349.24 uEq/gm d.w.

conversion from dry weight to wet weight = (intracellular content /d.w.) x 1/(w.w/d.w. ratio)

= 349.24 uEq/gm d.w. x 1/(3.97 gm w.w./gm d.w.)
= 88.0 uEq/gm w.w.

total muscle water content = ((w.w./d.w. ratio) - 1) x 1/(w.w./d.w. ratio)

= (3.97 - 1)/3.97
= 0.75 ml/gm w.w.

intracellular water content = (total muscle water content) - ((interstitial water content)/(w.w./d.w. ratio)

= 0.75 ml/gm w.w. - ((0.39 ml/gm d.w.)/(3.97 gm w.w./gm d.w.))
= 0.65 ml/gm w.w.

intracellular $[K^+]$ = (intracellular content /gm w.w.) x 1/(intracellular water content) x 1/valence

= 88.0 uEq/gm w.w. x 1/(0.65 ml/gm w.w.) x 1
= 135.34 umol/ml (or 135.34 mmol/l)

B4. Estimation of plasma volume change from hemoglobin concentration

During heavy exercise fluid moves from the vascular compartment into the muscle. The % change in plasma volume was estimated from the plasma hemoglobin concentration according to the following equation:

$$([Hb]r/[Hb]t) - 1) \times 100$$

where [Hb]r is the resting hemoglobin concentration and [Hb]t is the hemoglobin concentration measured at time "t".

B5. Correction of strong ion concentration for changes in plasma volume

Strong ions move between the intra- and extracellular fluid compartments during heavy exercise. However the accompanying change in plasma volume can disguise the direction of movement for the strong ions by artificially elevating the existing concentrations. Therefore, the concentration of strong ions that were measured in the plasma were corrected for the estimated change in the plasma volume according to the following equation:

$$[X]t \,/\, (1 - (([Hb]r/[Hb]t) - 1))$$

where [X]t is the strong ion concentration measured at time "t", [Hb]r is the resting hemoglobin concentration and [Hb]t is the hemoglobin concentration measured at time "t".

B6. Calculation of muscle blood flow

The blood flow in the active muscle at the end of exercise was calculated according to the following equations:

$$\text{blood flow} = (\dot{V}O_2)\text{muscle} / \text{a-v } O_2 \text{ content difference}$$

where $(\dot{V}O_2)$muscle is the O_2 intake in the active muscle and assumed to be

the difference between the resting whole body $\dot{V}O_2$ and the $\dot{V}O_2$ measured at the end of exercise. The O_2 content difference is:

$$O_2 \text{ content difference} = (1.34 \times [Hb] \times \% O_2 \text{ saturation}/100) + (PO_2 \times 0.003)$$

where [Hb] is the hemoglobin concentration measured in the femoral vein or artery.

APPENDIX C

INDIVIDUAL DATA

The individual and mean data are presented in the following tables. Performance data and femoral venous and arterial data for the 3 subjects participating in Part A of this study are presented in Tables 22-35. Performance data and foream venous and arterial data for the 6 subjects participating in Part B of this study are presented in Tables 36-49. Performance data, arterial and muscle data for the 6 subjects participating in Part C of this study are presented in Tables 50-69.

Table 22. Performance variables during 30 s of maximal exercise (Part A).

Subject	PT N.m	AT N.m	PP W	AP W	MW J	TW J	Fatigue Index PT %	Fatigue Index PP %	Fatigue Index AP %
1	114	78	1196	821	492	19006	41.0	41.0	42.2
2	148	90	1571	955	567	19482	51.9	52.4	54.5
6	117	73	1224	760	457	17231	42.3	43.2	47.2
$\bar{x}$	127	80	1330	845	505	18573	45.1	45.5	48.0
±SD	20	9	209	100	56	1186	6.0	6.1	6.2

PT = peak torque
AT = average torque
PP = peak power
AP = average power
MW = maximal work
TW = total work

Table 23. Individual arterial and femoral venous hydrogen ion concentrations (nmol/l) at rest and during recovery from 30 s of maximal exercise (Part A).

		Time Post-Exercise, min								
	Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery: 1	36.4	41.4	45.8	46.6	48.4	52.4	52.5	53.3	52.2	51.5
2	40.2	41.3	50.5	52.2	52.7	56.8	56.1	56.0	56.0	54.6
6	36.9	37.7	44.2	47.0	49.8	51.6	52.8	53.8	53.3	54.8
$\bar{x}$	37.8	40.1	46.8	48.6	50.3	53.6	53.8	54.4	53.8	53.6
±SD	2.1	2.1	3.3	3.1	2.2	2.8	2.0	1.4	2.0	1.9
Femoral Vein: 1	38.8	66.4	84.3	85.1	78.7	75.9	70.1	68.5	64.3	59.4
2	45.2	85.1	107.2	112.2	103.0	90.2	84.9	79.6	71.6	66.1
6	39.5	87.9	94.4	80.5	74.1	71.0	74.1	76.7	71.0	65.0
$\bar{x}$	41.2	79.8	95.3	92.6	85.3	79.0	76.4	74.9	69.0	63.5
±SD	3.5	11.7	11.5	17.1	15.5	10.0	7.8	5.8	4.1	3.6

Table 24. Individual arterial and femoral venous pH values at rest and during recovery from 30 s of maximal exercise (Part A).

		Rest	Time Post-Exercise, min								
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	7.439	7.383	7.339	7.332	7.315	7.281	7.280	7.273	7.282	7.288
	2	7.396	7.384	7.297	7.282	7.278	7.246	7.251	7.252	7.252	7.263
	6	7.433	7.424	7.355	7.328	7.303	7.287	7.277	7.269	7.273	7.261
	$\bar{x}$	7.423	7.397	7.330	7.314	7.299	7.271	7.269	7.265	7.269	7.271
	±SD	0.023	0.023	0.030	0.028	0.019	0.022	0.016	0.011	0.015	0.015
Femoral Vein:	1	7.411	7.178	7.074	7.070	7.104	7.120	7.154	7.164	7.192	7.226
	2	7.345	7.070	6.970	6.950	6.987	7.045	7.071	7.099	7.145	7.180
	6	7.403	7.056	7.025	7.094	7.130	7.149	7.130	7.115	7.149	7.187
	$\bar{x}$	7.386	7.101	7.023	7.038	7.074	7.105	7.118	7.126	7.162	7.198
	±SD	0.036	0.067	0.052	0.077	0.076	0.054	0.043	0.034	0.036	0.025

Table 25. Individual arterial and femoral venous PCO_2 values (mm Hg) at rest and during recovery from 30 s of maximal exercise (Part A).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	39.2	35.1	34.6	29.5	28.4	28.0	28.4	27.4	26.8	28.2
	2	40.8	37.5	38.6	35.9	33.0	31.8	31.1	33.4	33.5	34.6
	6	42.9	33.3	36.2	34.8	33.4	32.1	30.1	26.9	29.2	31.8
	$\bar{x}$	41.0	35.3	36.5	33.4	31.6	30.6	29.9	29.2	29.8	31.5
	±SD	1.9	2.1	2.0	3.4	2.8	2.3	1.4	3.6	3.4	3.2
Femoral Vein:	1	43.9	74.4	85.4	73.1	58.7	52.3	42.2	38.7	37.4	36.9
	2	51.3	109.3	116.3	109.7	88.8	64.3	57.1	53.6	49.9	45.5
	6	42.3	115.0	115.8	74.0	54.9	45.1	43.8	49.3	42.3	37.1
	$\bar{x}$	45.8	99.6	105.8	85.6	67.5	53.9	47.7	47.2	43.2	39.8
	±SD	4.8	22.0	17.7	20.9	18.6	9.7	8.2	7.7	6.3	4.9

Table 26. Individual arterial and femoral venous strong ion difference values (mEq/l) at rest and during recovery from 30 s of maximal exercise (Part A).

		Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	49.8	44.1	43.1	40.8	40.8	41.6	42.7	39.4	39.5	41.4
	2	47.5	48.3	45.9	43.3	44.2	44.7	41.5	43.5	45.2	42.1
	6	48.0	46.6	43.7	41.4	33.2	39.4	41.2	38.1	40.0	37.4
	$\bar{x}$	48,3	46.3	44.2	41.8	39.4	41.9	41.8	40.3	41.6	40.3
	±SD	1.2	2.1	1.5	1.3	5.6	2.7	0.8	2.8	3.2	2.5
Femoral Vein:	1	49.0	53.1	46.0	46.9	43.0	44.3	41.0	40.7	39.2	42.4
	2	61.5	59.8	57.2	54.2	-	45.6	43.5	44.8	48.3	42.1
	6	46.4	50.7	52.2	45.6	40.4	38.6	36.3	37.5	36.7	36.3
	$\bar{x}$	52.3	54.5	51.8	48.9	41.7	42.8	40.3	41.0	41.4	40.3
	±SD	8.1	4.7	5.6	4.6	1.8	3.7	3.7	3.7	6.1	3.4

Table 27. Individual arterial and femoral venous lactate concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part A).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	0.4	8.9	11.2	13.5	14.0	14.7	15.4	14.7	12.8	12.0
	2	1.3	3.1	8.4	11.3	12.3	13.5	13.7	12.5	11.8	11.9
	6	1.0	7.0	9.2	11.8	14.2	13.4	12.4	14.2	12.4	13.1
	$\bar{x}$	0.9	6.3	9.6	12.2	13.5	13.9	13.8	13.8	12.3	12.3
	±SD	0.5	3.0	1.4	1.2	1.0	0.7	1.5	1.2	0.5	0.7
Femoral Vein:	1	0.5	9.2	12.2	13.4	15.4	17.1	16.3	15.6	15.4	14.1
	2	1.4	9.7	14.1	16.2	17.3	18.1	20.1	16.7	15.2	13.4
	6	1.2	10.2	13.1	14.8	14.4	15.5	16.3	14.2	15.0	15.0
	$\bar{x}$	1.0	9.7	13.1	14.8	15.7	16.9	17.6	15.5	15.2	14.2
	±SD	0.5	0.5	1.0	1.4	1.5	1.3	2.2	1.3	0.2	0.8

Table 28. Individual arterial and femoral venous sodium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part A).

		Rest	Time Post-Exercise, min								
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	137	146	145	144	143	141	141	139	139	139
	2	138	142	145	144	143	142	141	140	139	138
	6	136	145	144	143	142	141	140	139	137	137
	$\bar{x}$	137	144	145	144	143	141	141	139	138	138
	±SD	1	2	1	1	1	1	1	1	1	1
Femoral Vein:	1	138	148	147	146	145	142	141	139	138	139
	2	140	154	151	149	-	145	142	140	139	138
	6	136	149	149	146	144	142	141	139	138	137
	$\bar{x}$	138	150	149	147	144	143	141	139	138	138
	±SD	2	3	2	2	1	2	1	1	1	1

Table 29. Individual arterial and femoral venous potassium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part A).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	4.0	6.4	5.7	4.9	4.4	3.9	3.7	3.7	3.9	4.0
	2	4.6	6.2	5.9	5.4	5.3	5.0	5.0	4.8	4.8	4.8
	6	4.8	8.0	7.3	6.6	6.0	5.4	5.2	4.9	5.0	5.1
	$\bar{x}$	4.5	6.9	6.3	5.6	5.2	4.8	4.6	4.5	4.6	4.6
	±SD	0.4	1.0	0.9	0.9	0.8	0.8	0.8	0.7	0.6	0.6
Femoral Vein:	1	4.3	6.7	5.6	4.7	4.0	4.0	3.9	3.9	4.2	4.2
	2	5.5	7.7	6.7	5.8	-	5.3	5.2	5.3	5.3	5.3
	6	6.4	9.1	8.5	7.8	7.4	6.7	6.4	6.3	6.3	6.1
	$\bar{x}$	5.4	7.8	6.9	6.1	5.7	5.3	5.2	5.2	5.3	5.2
	±SD	1.1	1.2	1.5	1.6	2.4	1.4	1.3	1.3	1.1	1.0

Table 30. Individual arterial and femoral venous calcium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part A).

		Time Post-Exercise, min									
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	1.13	1.25	1.25	1.22	1.22	1.17	1.19	1.17	1.17	1.15
	2	1.06	1.12	1.15	1.11	1.10	1.12	1.13	1.10	1.08	1.10
	6	1.14	1.27	1.27	1.25	1.20	1.19	1.20	1.18	1.17	1.17
	$\bar{x}$	1.11	1.21	1.22	1.19	1.17	1.16	1.17	1.15	1.14	1.14
	±SD	0.04	0.08	0.06	0.07	0.06	0.04	0.04	0.04	0.05	0.04
Femoral Vein:	1	1.09	1.33	1.33	1.30	1.20	1.23	1.21	1.19	1.21	1.17
	2	1.15	1.38	1.30	1.26	-	1.21	1.17	1.11	1.14	1.11
	6	1.09	1.35	1.35	1.28	1.24	1.19	1.11	1.17	1.16	1.10
	$\bar{x}$	1.11	1.35	1.33	1.28	1.22	1.21	1.16	1.16	1.17	1.13
	±SD	0.04	0.03	0.03	0.02	0.03	0.02	0.05	0.04	0.04	0.04

Table 31. Individual arterial and femoral venous chloride concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part A).

		Rest	Time Post-Exercise, min							
			0	.0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	93	102	99	97	95	91	89	91	93	92
	2	96	99	99	97	94	91	93	91	89	91
	6	94	102	101	99	103	96	94	93	92	94
	$\bar{x}$	94	101	100	98	97	93	92	92	91	92
	±SD	2	2	1	1	5	3	3	1	2	2
Femoral Vein:	1	95	95	97	93	93	87	90	89	90	89
	2	85	95	89	87	86	89	86	86	83	90
	6	97	100	95	96	99	97	97	96	95	94
	$\bar{x}$	92	97	94	92	93	91	91	90	89	91
	±SD	6	3	4	5	7	5	6	5	6	3

Table 32. Individual arterial and femoral venous bicarbonate concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part A).

		Rest	Time Post-Exercise, min								
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	26.5	20.9	18.6	15.6	14.5	13.2	13.3	12.6	12.7	13.5
	2	25.0	22.3	18.8	16.9	15.4	13.8	13.6	14.7	14.7	15.6
	6	28.4	21.9	20.2	18.2	16.5	15.3	14.0	12.4	13.4	14.3
	$\bar{x}$	26.6	21.7	19.2	16.9	15.5	14.1	13.6	13.2	13.6	14.5
	±SD	1.7	0.7	0.9	1.3	1.0	1.1	0.4	1.3	1.0	1.1
Femoral Vein:	1	27.8	27.0	25.6	21.1	18.4	17.0	14.8	13.9	14.4	15.2
	2	27.9	31.4	26.7	24.1	21.2	17.6	16.6	16.5	17.1	17.0
	6	26.3	32.2	30.2	22.6	18.3	15.6	14.4	15.8	14.7	14.1
	$\bar{x}$	27.3	30.2	27.5	22.6	19.3	16.7	15.3	15.4	15.4	15.4
	±SD	0.9	2.8	2.4	1.5	1.6	1.0	1.2	1.3	1.5	1.5

Table 33. Individual arterial and femoral venous PO_2 values (mm Hg) at rest and during recovery from 30 s of maximal exercise (Part A).

		Rest	Time Post-Exercise, min 0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	96.5	101.4	110.4	123.3	122.7	119.8	117.0	119.3	113.5	110.4
	2	91.8	103.3	113.2	129.1	137.5	136.0	131.6	127.1	125.3	117.4
	6	88.0	118.3	123.9	122.1	125.6	123.6	122.8	122.5	121.3	114.4
	$\bar{x}$	92.1	107.7	115.8	124.8	128.6	126.5	123.8	123.0	120.0	114.1
	±SD	4.3	9.3	7.2	3.7	7.8	8.5	7.4	3.9	6.0	3.5
Femoral Vein:	1	28.4	19.3	34.4	47.6	57.0	57.5	59.6	51.9	50.0	46.3
	2	19.0	12.6	34.4	59.0	64.3	65.1	59.6	54.8	58.4	56.4
	6	40.9	24.2	42.2	60.0	62.1	61.5	61.8	62.6	63.6	66.0
	$\bar{x}$	29.4	18.7	37.0	55.5	61.1	61.4	60.3	56.4	57.3	56.2

Table 34. Individual arterial and femoral venous hemoglobin concentrations (gm/100 ml) at rest and during recovery from 30 s of maximal exercise (Part A).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	11.4	12.9	12.5	12.3	12.6	12.6	12.6	12.8	12.4	12.2
	2	14.6	15.7	16.0	15.9	15.9	16.1	16.0	15.7	15.4	15.6
	6	12.7	14.2	14.3	14.0	13.9	13.6	13.5	14.3	13.5	14.2
	$\bar{x}$	12.9	14.3	14.3	14.1	14.1	14.1	14.0	14.3	13.8	14.0
	±SD	1.6	1.4	1.8	1.8	1.7	1.8	1.8	1.5	1.5	1.7
Femoral Vein:	1	11.5	13.4	12.8	13.3	12.3	12.9	12.9	12.3	12.5	12.8
	2	17.0	18.8	17.4	17.2	17.7	17.6	17.4	16.1	17.3	15.5
	6	12.1	14.7	14.3	14.1	13.8	13.9	13.9	13.7	13.6	13.1
	$\bar{x}$	13.5	15.6	14.8	14.9	14.6	14.8	14.7	14.0	14.5	13.8
	±SD	3.0	2.8	2.3	2.1	2.8	2.5	2.4	1.9	2.5	1.5

Table 35. Individual arterial and femoral venous oxygen saturation values (%) at rest and during recovery from 30 s of maximal exercise (Part A).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	100	99	100	99	100	100	99	99	100	99
	2	95	94	95	94	95	95	95	95	95	95
	6	100	101	99	99	99	104	103	104	102	103
	$\bar{x}$	98	98	98	97	98	100	99	99	99	99
	±SD	3	4	3	3	3	5	4	5	4	4
Femoral Vein:	1	54	19	43	66	79	81	83	77	76	72
	2	26	9	37	67	73	75	72	72	75	76
	6	79	23	55	82	86	87	86	85	88	90
	$\bar{x}$	53	17	45	72	79	81	80	78	80	79
	±SD	26	7	9	9	7	6	8	7	7	10

Table 36. Performance variables during 30 s of maximal exercise (Part B).

Subject	PT N.m	AT N.m	PP W	AP W	MW J	TW J	Fatigue Index PT %	Fatigue Index PP %	Fatigue Index AP %
1	107	70	1121	732	439	16274	46.5	46.5	47.0
2	154	90	1640	955	564	20479	55.3	56.1	48.9
3	149	93	1571	980	585	22954	35.7	36.0	35.3
4	183	104	1962	1116	654	21880	61.8	63.3	58.7
5	176	120	1900	1301	755	28653	52.1	54.2	54.9
6	121	69	1253	718	433	15687	44.3	44.3	47.6
$\bar{x}$	148	91	1575	967	572	20988	49.2	50.1	48.7
±SD	30	20	338	224	124	4775	9.2	9.7	8.0

PT = peak torque
AT = average torque
PP = peak power
AP = average power
MW = maximal work
TW = total work

Table 37. Individual arterial and deep forearm venous hydrogen ion concentrations (nmol/l) at rest and during recovery form 30 s of maximal exercise (Part B).

		Rest	Time Post-Exercise, min								
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	39.0	44.4	52.4	51.9	52.4	57.4	57.1	60.0	56.6	56.1
	2	37.5	40.0	50.4	50.7	51.8	51.3	52.6	52.5	53.3	52.2
	3	33.8	41.9	45.7	48.1	49.4	52.6	54.6	55.3	55.6	54.0
	4	39.1	48.5	53.6	57.8	61.0	62.5	65.5	61.9	64.6	62.1
	5	39.6	50.2	55.6	64.9	70.6	71.3	77.8	83.2	83.1	87.5
	6	36.9	37.7	44.2	47.0	49.8	51.6	52.8	53.8	53.3	54.8
	$\bar{x}$	37.7	43.8	50.3	53.4	55.8	57.8	60.1	61.1	61.1	61.1
	±SD	2.2	4.9	4.5	6.8	8.4	7.9	9.9	11.5	11.6	13.4
Forearm Vein:	1	46.6	60.4	69.5	70.1	70.8	67.2	69.3	66.1	63.5	61.7
	2	42.4	48.5	51.6	53.0	55.6	55.3	55.5	55.7	54.1	53.3
	3	38.7	55.5	-	56.6	57.1	59.3	60.8	59.8	59.5	58.1
	4	43.3	69.7	81.7	82.0	81.5	85.7	88.3	75.3	72.8	66.8
	5	44.0	64.0	87.5	90.2	95.5	94.8	95.1	98.4	95.3	104.2
	6	39.9	44.7	48.1	54.1	54.0	54.8	54.3	55.0	55.3	56.2
	$\bar{x}$	42.5	57.1	67.7	67.7	69.1	69.5	70.6	68.4	66.8	66.7
	±SD	2.9	9.5	17.6	15.8	16.8	16.9	17.3	16.5	15.5	18.9

Table 38. Individual arterial and deep forearm venous pH values at rest and during recovery from 30 s of maximal exercise (Part B).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	7.409	7.353	7.281	7.285	7.281	7.241	7.243	7.222	7.247	7.251
	2	7.426	7.398	7.298	7.295	7.286	7.290	7.279	7.282	7.273	7.282
	3	7.471	7.378	7.340	7.318	7.306	7.279	7.263	7.257	7.255	7.268
	4	7.408	7.314	7.271	7.238	7.215	7.204	7.184	7.204	7.190	7.207
	5	7.402	7.299	7.255	7.188	7.151	7.147	7.109	7.080	7.081	7.058
	6	7.433	7.424	7.355	7.328	7.303	7.287	7.277	7.269	7.273	7.261
	$\bar{x}$	7.425	7.361	7.300	7.275	7.257	7.241	7.226	7.220	7.220	7.221
	±SD	0.026	0.048	0.040	0.053	0.061	0.057	0.067	0.074	0.075	0.084
Forearm Vein:	1	7.332	7.219	7.158	7.154	7.150	7.173	7.159	7.180	7.197	7.210
	2	7.373	7.314	7.287	7.276	7.255	7.257	7.256	7.254	7.267	7.273
	3	7.413	7.256	-	7.247	7.243	7.227	7.216	7.223	7.226	7.236
	4	7.363	7.157	7.088	7.086	7.089	7.067	7.054	7.123	7.138	7.175
	5	7.357	7.194	7.058	7.045	7.020	7.023	7.022	7.007	7.021	6.982
	6	7.399	7.349	7.318	7.267	7.268	7.261	7.265	7.260	7.257	7.250
	$\bar{x}$	7.373	7.248	7.182	7.179	7.171	7.168	7.162	7.175	7.184	7.188
	±SD	0.029	0.073	0.117	0.099	0.102	0.101	0.104	0.097	0.093	0.106

Table 39. Individual arterial and deep forearm venous PCO_2 values (mm Hg) at rest and during recovery from 30 s of maximal exercise (Part B).

		Rest	Time Post-Exercise, min							
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	40.8	34.1	33.8	30.7	27.1	28.2	26.2	28.2	28.6	28.7
	2	38.9	35.0	36.5	31.7	29.7	28.6	29.8	27.7	30.4	30.5
	3	35.6	34.2	31.5	30.3	28.9	28.6	28.0	28.2	28.5	28.8
	4	40.0	36.5	33.1	34.7	33.2	30.3	31.4	28.6	32.8	30.6
	5	41.3	38.4	35.9	33.6	30.6	26.3	25.2	21.9	25.7	28.1
	6	42.9	33.3	36.2	34.8	33.4	32.1	30.1	26.9	29.2	31.8
	$\bar{x}$	39.9	25.3	34.5	32.6	30.5	29.0	28.5	26.9	29.2	29.8
	±SD	2.5	1.9	2.0	2.0	2.5	2.0	2.4	2.5	2.3	1.4
Forearm Vein:	1	52.7	65.4	72.7	69.7	65.4	59.3	63.8	58.7	56.7	47.9
	2	49.7	57.2	58.6	60.4	60.9	59.4	57.7	56.8	50.1	46.3
	3	44.0	60.7	-	57.8	53.8	52.3	52.4	51.6	48.5	45.0
	4	49.5	76.7	84.4	82.0	78.2	77.5	76.9	59.2	62.4	50.3
	5	41.1	62.7	73.7	78.8	80.7	75.7	68.2	66.6	53.7	48.5
	6	45.8	56.1	60.1	64.3	65.1	63.4	62.4	57.5	40.3	38.5
	$\bar{x}$	47.1	63.1	69.9	68.8	67.4	64.6	63.4	58.4	52.0	46.1
	±SD	4.3	7.5	10.7	9.9	10.3	10.0	8.5	4.8	7.6	4.1

Table 40. Individual arterial and deep forearm venous strong ion difference values (mEq/l) at rest and during recovery from 30 s of maximal exercise (Part B).

		Rest	Time Post-Exercise, min								
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	44.4	43.0	44.0	38.2	34.8	34.7	32.6	38.4	28.5	34.4
	2	44.2	46.1	42.5	36.2	43.6	42.8	45.1	40.3	38.0	38.0
	3	46.7	45.2	41.7	39.6	40.1	40.2	38.5	38.9	39.8	35.2
	4	-	-	-	-	-	-	-	-	-	-
	5	42.5	41.7	37.1	34.8	38.7	35.4	34.2	31.0	32.1	31.7
	6	48.1	46.5	43.6	41.3	33.2	39.4	41.2	38.1	39.9	37.3
	$\bar{x}$	45.2	44.5	41.8	38.0	38.1	38.5	38.3	37.3	35.7	35.3
	±SD	2.2	2.1	2.8	2.6	4.2	3.4	5.1	3.6	5.1	2.5
Forearm Vein:	1	51.8	54.8	52.0	51.2	47.5	47.2	47.2	44.0	48.3	47.0
	2	46.8	51.4	58.3	56.5	51.0	51.0	44.6	47.7	44.3	42.1
	3	53.1	55.1	-	51.5	51.3	48.6	47.8	49.0	45.3	52.9
	4	-	-	-	-	-	-	-	-	-	-
	5	46.6	52.2	47.2	47.8	47.8	45.9	44.9	40.2	42.5	37.4
	6	46.4	51.6	49.1	45.3	47.8	49.0	47.2	45.3	39.3	40.3
	$\bar{x}$	48.9	53.0	51.7	50.5	49.1	48.3	46.3	45.2	43.9	43.9
	±SD	3.2	1.8	4.9	4.2	1.9	1.9	1.5	3.4	3.3	6.1

Table 41. Individual arterial and deep forearm venous lactate concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part B).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	0.7	8.9	10.7	13.9	13.7	14.1	14.0	13.5	14.3	12.7
	2	1.1	5.3	13.1	13.0	13.5	12.8	13.6	13.2	12.5	11.7
	3	1.0	8.6	11.3	12.8	13.7	13.8	14.3	14.0	13.2	12.7
	4	0.8	10.2	12.3	14.4	14.8	15.5	15.5	15.7	15.0	14.0
	5	1.0	9.9	12.5	15.4	16.9	17.5	17.5	17.8	17.7	17.9
	6	1.0	7.0	9.2	11.8	14.2	13.4	12.4	14.2	12.4	13.1
	$\bar{x}$	0.9	8.3	11.5	13.6	14.5	14.5	14.6	14.7	14.2	13.7
	±SD	0.2	1.9	1.4	1.3	1.3	1.7	1.8	1.7	2.0	2.2
Forearm Vein:	1	1.4	5.2	8.8	9.0	9.5	9.4	10.1	9.0	8.9	9.3
	2	1.1	3.2	4.5	5.1	5.6	6.1	5.4	6.6	7.6	7.4
	3	1.2	5.8	-	7.8	8.1	9.2	9.0	8.8	8.6	9.2
	4	0.8	8.9	11.4	11.6	11.5	11.9	13.2	11.8	10.0	10.8
	5	1.1	4.7	11.1	10.9	12.4	12.1	12.8	13.4	13.5	15.8
	6	0.9	2.2	3.6	5.2	5.3	5.8	5.5	5.9	10.0	10.7
	$\bar{x}$	1.1	5.0	7.9	8.3	8.7	9.1	9.3	9.3	9.8	10.5
	±SD	0.2	2.3	3.7	2.8	3.0	2.7	3.4	2.9	2.0	2.9

Table 42. Individual arterial and deep forearm venous sodium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part B).

		Rest	Time Post-Exercise, min								
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	138	147	145	144	143	142	141	140	138	138
	2	139	145	145	143	143	142	141	140	139	139
	3	139	147	145	144	144	142	141	140	140	139
	4	-	-	-	-	-	-	-	-	-	-
	5	135	148	148	146	145	144	143	140	139	138
	6	136	145	144	143	142	141	140	138	137	137
	$\bar{x}$	137	146	145	144	143	142	141	140	139	138
	±SD	2	1	2	1	1	1	1	1	1	1
Forearm Vein:	1	138	141	143	144	143	142	142	141	141	140
	2	138	141	141	141	141	141	140	141	140	140
	3	140	143	-	142	143	142	142	141	140	140
	4	-	-	-	-	-	-	-	-	-	-
	5	135	140	143	142	142	141	142	-	139	139
	6	137	139	140	140	141	139	140	139	139	138
	$\bar{x}$	138	141	142	142	142	141	141	141	140	139
	±SD	2	1	2	1	1	1	1	1	1	1

Table 43. Individual arterial and deep forearm venous potassium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part B).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	3.9	6.5	5.4	4.8	4.3	3.7	3.5	3.6	3.6	3.8
	2	4.2	6.2	5.3	5.0	4.9	4.5	4.6	4.4	4.4	4.6
	3	4.4	7.4	6.5	5.9	5.3	4.6	4.4	4.5	4.6	4.5
	4	-	-	-	-	-	-	-	-	-	-
	5	4.2	7.9	6.8	5.5	4.9	4.3	4.2	4.4	4.4	4.2
	6	4.8	8.0	7.3	6.6	6.0	5.4	5.2	4.9	5.0	5.1
	$\bar{x}$	4.3	7.2	6.3	5.6	5.1	4.5	4.4	4.4	4.4	4.4
	±SD	0.3	0.8	0.9	0.7	0.6	0.6	0.6	0.5	0.5	0.5
Forearm Vein:	1	4.1	5.6	5.2	4.8	4.6	4.3	4.0	3.8	3.9	4.0
	2	4.9	6.4	5.6	5.4	5.4	4.9	4.9	5.1	4.8	4.5
	3	4.1	5.6	-	5.0	5.0	4.5	4.4	4.5	4.6	4.7
	4	-	-	-	-	-	-	-	-	-	-
	5	4.4	6.2	5.6	5.0	4.6	4.4	4.1	3.9	4.6	4.8
	6	4.1	5.5	5.3	5.1	4.8	4.5	4.4	4.1	4.2	4.0
	$\bar{x}$	4.3	5.9	5.4	5.1	4.9	4.5	4.4	4.3	4.4	4.4
	±SD	0.3	0.4	0.2	0.2	0.3	0.2	0.4	0.5	0.4	0.4

Table 44. Individual arterial and deep forearm venous calcium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part B).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	1.10	1.17	1.13	1.14	1.12	1.09	1.09	1.14	1.10	1.13
	2	1.03	1.10	1.13	1.12	1.07	1.05	1.05	1.05	1.03	1.05
	3	1.13	1.22	1.25	1.26	1.27	1.20	1.19	1.18	1.20	1.18
	4	1.11	1.19	1.17	1.20	1.30	1.21	1.11	1.09	1.09	1.09
	5	1.17	1.37	1.39	1.36	1.34	1.31	1.26	1.20	1.19	1.21
	6	1.14	1.27	1.27	1.25	1.20	1.19	1.20	1.18	1.17	1.17
	$\bar{x}$	1.11	1.22	1.22	1.22	1.22	1.18	1.15	1.14	1.13	1.14
	±SD	0.05	0.09	0.10	0.09	0.11	0.09	0.08	0.06	0.07	0.06
Forearm:	1	1.09	1.22	1.32	1.21	1.19	1.14	1.16	1.12	1.14	1.14
	2	1.00	1.11	1.08	1.11	1.11	1.09	1.05	1.12	1.05	1.02
	3	1.10	1.18	-	1.13	1.19	1.16	1.20	1.17	1.15	1.19
	4	1.08	1.27	1.25	1.26	1.25	1.23	1.21	1.16	1.15	1.14
	5	1.14	1.33	1.37	1.33	1.32	1.28	1.32	1.36	1.21	1.22
	6	1.08	1.14	1.18	1.19	1.16	1.13	1.15	1.06	1.07	1.01
	$\bar{x}$	1.08	1.20	1.24	1.21	1.20	1.17	1.18	1.17	1.13	1.12
	±SD	0.05	0.08	0.12	0.08	0.07	0.07	0.09	0.10	0.06	0.09

Table 45. Individual arterial and deep forearm venous chloride concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part B).

		Time Post-Exercise, min									
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	99	104	98	99	101	99	100	94	101	97
	2	100	102	97	101	93	93	89	93	95	96
	3	98	103	101	100	98	95	95	94	94	98
	4	93	96	95	95	91	94	88	92	96	95
	5	98	107	108	104	97	98	98	98	96	95
	6	94	102	101	99	103	96	94	93	92	94
	$\bar{x}$	97	102	100	100	97	96	94	94	96	96
	±SD	3	4	5	3	5	2	5	2	3	2
Forearm Vein:	1	91	89	90	91	93	92	91	94	90	90
	2	97	95	86	87	92	91	97	94	95	97
	3	92	90	-	90	91	91	92	90	93	85
	4	88	85	88	84	85	86	87	88	85	84
	5	94	92	93	91	89	90	91	93	90	93
	6	96	93	95	97	95	91	94	94	96	93
	$\bar{x}$	93	91	90	90	91	90	92	92	92	90
	±SD	3	4	4	4	3	2	3	3	4	5

Table 46. Individual arterial and deep forearm venous bicarbonate concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part B).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	25.7	18.9	16.3	14.6	12.8	12.1	11.3	11.6	12.4	12.6
	2	25.5	21.6	17.8	15.4	14.1	13.8	13.8	13.1	14.0	14.4
	3	25.9	20.1	16.9	15.5	14.4	13.4	12.7	12.5	12.6	13.1
	4	25.2	18.5	15.2	14.8	13.4	11.9	11.8	11.4	12.5	12.2
	5	25.8	18.9	15.9	12.8	10.6	9.1	8.0	6.5	7.7	8.0
	6	28.4	21.9	20.2	18.2	16.5	15.3	14.0	12.4	13.4	14.3
	$\bar{x}$	26.1	20.0	17.1	15.2	13.6	12.6	11.9	11.3	12.1	12.4
	±SD	1.2	1.5	1.8	1.8	1.9	2.1	2.2	2.4	2.2	2.3
Forearm Vein:	1	27.9	26.6	25.8	24.5	22.8	21.7	22.3	21.9	21.9	19.1
	2	28.9	29.1	27.9	28.1	27.0	26.2	25.6	25.1	22.8	21.4
	3	28.0	26.9	-	24.9	23.2	21.7	21.2	21.3	20.1	19.1
	4	28.1	27.1	25.4	24.7	23.6	22.3	21.5	19.4	21.1	18.5
	5	23.0	24.0	20.8	21.5	20.8	19.7	17.6	16.6	13.9	11.5
	6	28.2	30.9	30.8	29.3	29.7	28.5	28.3	25.8	17.9	16.8
	$\bar{x}$	27.4	27.4	26.1	25.5	24.5	23.4	22.8	21.7	19.6	17.7
	±SD	2.2	2.4	3.7	2.8	3.2	3.3	3.7	3.5	3.3	3.4

Table 47. Individual arterial and deep forearm venous PO_2 values (mm Hg) at rest and during recovery from 30 s of maximal exercise (Part B).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	100.1	105.3	113.3	128.2	129.6	133.2	122.4	118.3	116.0	119.0
	2	90.5	120.8	130.5	137.4	138.0	136.2	129.7	128.8	124.3	124.4
	3	98.6	114.6	124.0	127.6	122.0	121.3	120.2	124.8	116.3	114.6
	4	96.9	108.8	126.7	126.0	124.5	131.2	124.5	120.9	118.6	118.9
	5	85.0	113.9	116.0	120.1	123.3	128.9	125.4	127.1	123.3	127.1
	6	88.0	118.3	123.9	122.1	125.6	129.6	122.8	122.5	121.3	114.4
	$\bar{x}$	93.2	113.6	122.4	126.9	127.2	129.1	124.2	123.7	120.0	118.7
	±SD	6.2	5.8	6.5	6.0	5.9	5.7	3.3	3.9	3.5	5.1
Forearm Vein:	1	27.9	16.4	18.2	29.8	28.1	24.9	20.6	23.5	25.6	37.3
	2	21.6	9.3	11.0	12.8	13.1	15.2	17.2	21.1	29.4	28.7
	3	33.7	19.6	-	23.5	27.9	26.7	26.7	27.9	27.0	35.9
	4	32.8	7.3	16.6	20.9	17.3	25.5	27.5	32.7	20.4	34.5
	5	34.9	24.5	14.3	12.5	16.0	20.6	24.0	27.3	31.7	52.8
	6	35.2	16.5	13.9	10.9	15.2	17.8	18.2	21.4	42.5	42.0
	$\bar{x}$	31.0	15.6	14.8	18.4	19.6	21.8	22.4	25.7	29.4	38.5
	±SD	5.3	6.4	2.8	7.5	6.6	4.7	4.4	4.5	7.5	8.2

Table 48. Individual arterial and deep forearm venous hemoglobin concentration (gm/100 ml) at rest and during recovery from 30 s of maximal exercise (Part B).

			Time Post-Exercise, min								
		Rest	0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	12.3	13.9	14.2	14.1	14.3	14.2	14.0	14.1	13.8	14.0
	2	13.5	15.4	15.3	15.3	15.4	15.3	15.2	15.2	15.3	15.4
	3	12.6	14.4	14.3	14.4	14.3	14.2	14.1	13.9	14.1	14.2
	4	13.3	15.2	14.7	14.5	15.3	14.9	14.4	14.2	14.0	14.2
	5	13.7	15.1	14.9	15.5	14.4	15.2	14.3	14.9	14.3	14.6
	6	12.7	14.2	14.3	14.0	13.9	13.6	13.5	14.3	13.5	14.2
	$\bar{x}$	13.0	14.7	14.6	14.6	14.6	14.6	14.3	14.4	14.2	14.3
	±SD	0.6	0.6	0.4	0.6	0.6	0.7	0.6	0.5	0.6	0.7
Forearm Vein:	1	12.8	15.2	15.2	14.8	14.7	15.0	15.1	15.1	15.1	14.5
	2	13.6	15.4	15.6	15.4	15.6	15.4	16.9	16.7	16.2	15.9
	3	12.4	14.3	-	13.9	14.2	14.5	14.2	14.3	14.0	14.0
	4	13.9	15.9	16.0	15.7	15.5	15.4	15.4	14.8	15.3	14.6
	5	13.2	15.4	16.0	15.7	15.7	15.5	14.9	15.9	14.2	14.6
	6	12.6	14.0	14.3	14.8	14.8	14.6	14.9	14.0	13.7	12.6
	$\bar{x}$	13.1	15.0	15.4	15.1	15.1	15.1	15.2	15.1	14.8	14.4
	±SD	0.6	0.7	0.7	0.7	0.6	0.4	0.9	1.0	0.9	1.1

Table 49. Individual arterial and deep forearm venous oxygen saturation values (%) at rest and during recovery from 30 s of maximal exercise (Part B).

		Rest	Time Post-Exercise, min								
			0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Artery:	1	100	100	100	101	100	99	99	100	99	98
	2	97	98	98	99	99	99	98	98	98	98
	3	104	104	104	104	104	104	104	104	104	103
	4	100	99	101	100	100	99	100	99	100	100
	5	102	103	102	102	102	102	103	102	102	101
	6	100	101	99	99	99	104	103	104	102	103
	$\bar{x}$	100	101	101	101	101	101	101	101	101	100
	±SD	2	2	2	2	2	3	2	2	2	2
Forearm Vein:	1	50	17	18	40	37	33	23	28	34	58
	2	34	7	9	12	12	15	19	27	46	46
	3	64	21	-	29	38	37	33	37	39	53
	4	65	7	16	22	17	30	32	47	23	54
	5	70	35	10	9	12	20	23	32	43	75
	6	72	24	17	12	17	22	23	31	75	74
	$\bar{x}$	59	19	14	21	22	26	26	34	43	60
	±SD	15	11	4	12	12	8	6	7	17	12

Table 50. Performance variables during 30 s of maximal exercise (Part C).

Subject	PT N.m	AT N.m	PP W	AP W	MW J	TW J	Fatigue Index PT %	Fatigue Index PP %	Fatigue Index AP %
7	159	104	1663	1087	652	25809	52.1	52.9	51.0
8	168	115	1799	1235	720	28618	45.5	46.7	47.2
9	193	113	2099	1234	712	26678	54.1	58.6	61.6
10	189	116	2133	1313	727	26556	-	-	-
11	136	85	1493	932	534	23305	36.4	37.2	34.5
12	154	101	1605	1057	637	24185	50.5	51.1	46.9
$\bar{x}$	166	106	1799	1143*	664	25859	40.6	42.6	50.6
±SD	22	12	265	142	74	1903	16.1	15.8	10.5

PT = peak torque
AT = average torque
PP = peak power
AP = average power
MW = maximal work
TW = total work

Table 51. Individual arterial hydrogen ion concentration (nmol/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	35.6	57.1	76.9	76.2
9	35.1	57.3	70.5	71.4
10	36.5	55.3	65.9	67.9
11	36.9	47.6	53.2	51.8
12	-	-	-	-
$\bar{x}$	36.0	54.3	66.6	66.8
±SD	0.8	4.6	10.0	10.6

Table 52. Individual arterial pH values at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	7.449	7.243	7.114	7.118
9	7.455	7.242	7.152	7.146
10	7.438	7.257	7.181	7.168
11	7.433	7.322	7.274	7.286
12	-	-	-	-
$\bar{x}$	7.444	7.266	7.180	7.180
±SD	0.010	0.038	0.068	0.074

Table 53. Individual arterial PCO_2 values (mm Hg) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	39.1	36.3	31.1	30.8
9	38.5	36.1	26.9	27.4
10	36.8	35.9	28.8	26.4
11	41.3	40.3	32.3	32.9
12	-	-	-	-
$\bar{x}$	38.9	37.2	29.8	29.4
±SD	1.9	2.1	2.4	3.0

Table 54. Individual arterial strong ion difference values (mEq/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	51.0	44.7	38.0	38.4
9	49.1	42.1	38.5	38.2
10	52.4	44.7	40.9	39.2
11	50.0	46.9	43.3	39.4
12	-	-	-	-
$\bar{x}$	50.6	44.6	40.2	38.8
±SD	1.4	2.0	2.4	0.6

Table 55. Individual arterial lactate concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	0.6	15.8	19.3	16.9
9	0.8	16.0	18.9	18.4
10	0.6	14.2	18.1	17.8
11	1.0	10.8	13.6	12.6
12	-	-	-	-
$\bar{x}$	0.8	14.2	17.5	16.4
±SD	0.2	2.4	2.6	2.6

Table 56. Individual arterial sodium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	138	148	143	140
9	136	148	143	140
10	138	150	145	141
11	139	147	142	139
12	-	-	-	-
$\bar{x}$	138	148	143	140
±SD	1	1	1	1

Table 57. Individual arterial potassium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	4.4	6.9	3.8	3.9
9	4.6	6.5	3.9	4.1
10	3.9	6.4	3.7	3.8
11	3.8	6.2	3.6	3.7
12	-	-	-	-
$\bar{x}$	4.2	6.5	3.8	3.9
±SD	0.4	0.3	0.1	0.2

Table 58. Individual arterial calcium concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	1.12	1.28	1.25	1.18
9	1.14	1.30	1.24	1.25
10	1.07	1.24	1.14	1.12
11	1.13	1.23	1.17	1.16
12	-	-	-	-
$\bar{X}$	1.12	1.26	1.20	1.18
±SD	-.03	0.03	0.05	0.05

Table 59. Individual arterial chloride concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	93	97	92	91
9	93	99	92	90
10	91	100	92	90
11	94	98	91	93
12	-	-	-	-
$\bar{x}$	93	99	92	91
±SD	1	1	1	1

Table 60. Individual arterial bicarbonate concentrations (mmol/l) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	27.1	15.6	10.0	10.0
9	27.0	15.5	9.4	9.4
10	24.8	16.0	10.8	9.6
11	27.5	20.8	14.9	15.7
12	-	-	-	-
$\bar{x}$	26.6	17.0	11.3	11.2
±SD	1.2	2.6	2.5	3.0

Table 61. Individual arterial PO_2 values (mm Hg) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	99.2	118.0	127.5	120.6
9	105.7	116.0	127.6	117.5
10	92.2	109.9	121.4	113.5
11	91.8	99.2	115.7	104.9
12	-	-	-	-
$\bar{X}$	97.2	110.8	123.1	114.1
±SD	6.6	8.5	5.7	6.8

Table 62. Individual arterial hemoglobin concentrations (gm/100 ml) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	14.8	16.1	15.7	15.2
9	13.0	14.2	13.9	13.5
10	14.6	16.3	16.2	15.7
11	14.7	16.0	15.8	15.4
12	-	-	-	-
$\bar{x}$	14.3	15.7	15.4	15.0
±SD	0.9	1.0	1.0	1.0

Table 63. Individual arterial oxygen saturation values (%) at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	-	-	-	-
8	99	98	98	98
9	100	99	99	98
10	94	94	94	93
11	94	93	94	93
12	-	-	-	-
$\bar{x}$	97	96	96	96
±SD	3	3	3	3

Table 64. Wet weight/dry weight ratio of muscle samples taken at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
7	3.97	4.31	4.13	4.24
8	-	4.07	4.38	4.38
9	4.29	4.29	4.62	4.60
10	4.45	4.67	4.85	4.64
11	-	4.29	4.34	4.21
12	4.07	4.18	4.25	-
$\bar{x}$	4.20	4.30	4.43	4.41
±SD	0.22	0.20	0.26	0.20

Table 65. Intracellular concentation (mmol/l) and content (μmol/gm d.w.) of sodium at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
Concentration, mmol/l				
7	-	-	28	0
8	-	-	-	-
9	-	-	16	-
10	7	13	6	5
11	-	-	-	1
12	11	19	-	-
$\bar{x}$	9	16	17	2
±SD	3	4	11	3
Content, μmol/gm d.w.				
7	-	-	72	1
8	-	-	-	-
9	-	-	50	-
10	22	40	19	16
11	-	-	-	0
12	30	49	-	-
$\bar{x}$	26	44	47	6
±SD	5	6	27	8

Table 66. Intracellular concentration (mmol/l) and content (μmol/gm d.w.) of potassium at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
Concentration, mmol/l				
7	135	128	108	107
8	-	153	124	126
9	113	127	147	98
10	138	118	121	136
11	-	142	114	123
12	107	90	123	-
$\bar{x}$	124	126	123	118
±SD	16	22	13	15
Content, μmol/gm d.w.				
7	349	349	281	292
8	-	382	353	363
9	328	344	453	305
10	424	364	402	428
11	-	384	320	335
12	287	233	334	-
$\bar{x}$	347	342	357	344
±SD	57	56	62	54

Table 67. Intracellular concentration (mmol/l) and content (µmol/gm d.w.) of ionised calcium at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
Concentration, mmol/l				
7	2	1	1	1
8	-	1	1	1
9	1	1	2	1
10	1	1	1	2
11	-	1	2	1
12	1	2	2	-
$\bar{x}$	1	1	1	1
±SD	0	0	1	0
Content, µmol/gm d.w.				
7	8	5	5	5
8	-	5	4	6
9	5	6	13	5
10	7	7	7	7
11	-	4	9	6
12	5	9	9	-
$\bar{x}$	6	6	8	6
±SD	2	2	3	1

Table 68. Intracellular concentration (mmol/l) and content (μmol/gm d.w.) of magnesium at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
Concentration, mmol/l				
7	15	15	9	16
8	-	14	14	14
9	14	16	18	13
10	15	13	13	14
11	-	15	14	12
12	13	11	14	-
$\bar{x}$	14	14	14	14
±SD	1	2	3	2
Content, μmol/gm d.w.				
7	79	79	46	89
8	-	69	79	81
9	84	84	113	81
10	89	81	86	89
11	-	79	79	66
12	69	55	75	-
$\bar{x}$	80	75	80	81
±SD	9	11	21	9

Table 69. Intracellular concentration (mmol/l) and content (µmol/gm d.w.) of chloride at rest and during recovery from 30 s of maximal exercise (Part C).

Subject	Rest	Time Post-Exercise, min		
		0	3.5	9.5
Concentration, mmol/l				
7	4	1	52	7
8	-	7	3	8
9	6	-	31	0
10	13	35	18	12
11	-	5	12	10
12	16	35	3	-
$\bar{x}$	10	17	20	7
±SD	6	17	19	4
Content, µmol/gm d.w.				
7	11	-	135	19
8	-	17	8	23
9	18	-	95	1
10	41	110	58	37
11	-	13	33	27
12	43	92	7	-
$\bar{x}$	28	58	56	21
±SD	16	50	51	13

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