Origins of arterial and femoral venous acid–base responses during moderate-intensity bicycling exercise after glycogen depletion in men

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Abstract
The interactions between nutrition, energy status and acid–base balance during exercise are poorly understood. Exercise, under conditions of prior glycogen depletion (GD) and low-carbohydrate diet, results in a decreased rate of skeletal muscle glycogenolysis, greatly decreased muscle pyruvate and lactate contents with decreased plasma [lactate] (Putman et al., Am J Physiol, 265: E752, 1993). Therefore, it is hypothesized that exercise in GD, compared with normal (NG) or high-carbohydrate conditions, will result in a reduced magnitude of acidosis due to reduced production and accumulation of lactate. In two trials (GD, then NG) separated by 1–2 weeks, four men cycled at 75% of peak VO2 until the time of exhaustion in GD (57 ± 7 min). At 2 min of exercise, femoral vein (fv) plasma [H+] was increased by 21 ± 4 neq l–1 (NG) and 14 ± 3 neq l–1 (GD); increases in arterial [H+] were only 45% of those in fv plasma. The increase in fv PCO2 (NG, 25 ± 2 mm Hg and GD, 15 ± 2 mm Hg) was the primary variable responsible for the increased [H+]. During NG, the increase in fv [lactate] exceeded the decrease in strong ion difference [SID], with electrolyte charge balance mainly due to increased [Na+]. In the GD trial, arterial [SID] decreased and was the primary contributor to the increased [H+], as passage of blood through the lungs eliminated the CO2 contribution prevalent in fv plasma. Throughout GD, plasma [lactate] increased less than in NG and the decrease in [SID] in GD was also significantly less than in NG. In summary, in GD conditions, an attenuated production/release of lactate and CO2 from muscle resulted in reduced magnitude and duration of acidosis compared with NG conditions. In fv plasma, increased PCO2 was the primary variable responsible for the rapid and sustained elevation in [H+], whereas in arterial plasma decreased [SID], due to increased [lactate], was primarily responsible for increased [H+].

Keywords: plasma H+ concentration; acid–base regulation; physicochemical acid–base approach; femoral venous; skeletal muscle

Introduction
Sustained depletion of skeletal muscle glycogen by exercise and dietary manipulation has been used as a tool to study regulatory mechanisms involved in skeletal muscle fat and carbohydrate utilization1–3. Depletion of muscle glycogen reserves may also persist...
in the normal course of training activities for both humans\textsuperscript{2}, and especially horses, where recovery of muscle glycogen may require 72 h\textsuperscript{1}, so there are practical implications for understanding the effects of muscle glycogen depletion (GD) in the broader context of whole-body exercise physiology. Because exercise- and dietary-induced depletion of muscle glycogen has direct effects on glycolytic and aerobic metabolic pathways\textsuperscript{1,3}, the focus of this paper is on the impact of the metabolic alterations on blood acid–base state\textsuperscript{5}.

For the acid–base analysis, we used the physicochemical approach detailed by Stewart\textsuperscript{6,7}, who recognizes that pH (or \([\text{H}^+]\)) and \([\text{HCO}_3^-]\) are dependent acid-base variables because neither is changed without first changing the concentrations of the independent acid-base variables strong ion difference [SID], \([A_{\text{enal}}]\) and PCO\textsubscript{2}. This approach also allowed us to determine the contributions of the independent variables to the changes in plasma \([\text{H}^+]\) and \([\text{HCO}_3^-]\) and an examination of the mechanisms responsible for these changes\textsuperscript{5,5,8,9}.

In addition to examining the impact of muscle/blood nutrient status on acid-base balance during exercise, we were also interested in quantifying key differences in acid-base responses in arterial and femoral venous plasma. Within contracting muscle, the venous capillary acid–base disturbance generated by net water, electrolyte and gas fluxes across the sarcolemma of contracting muscle is modified by interactions between plasma and erythrocytes\textsuperscript{10,11} and other non-contracting tissues\textsuperscript{8,12}. During running or leg cycling exercise, blood sampled from the femoral vein (fv) by and large reflects these processes\textsuperscript{9,13}. The main impact of the lungs on blood is to remove CO\textsubscript{2} and increase oxygenation, processes that result in modification of plasma ion and acid-base status to produce what is seen in arterial plasma\textsuperscript{a}. Also, during perfusion of non-contracting tissues with arterial blood, there may be continued removal of lactate\textsuperscript{−} and K\textsuperscript{+} (added by contracting muscle), and removal of oxygen with gain of CO\textsubscript{2} - thus modifying the plasma similar to that measured in antecubital venous blood\textsuperscript{11,12}.

The present paper represents a subset of the data from the study of Putman et al\textsuperscript{3}. In that paper, we demonstrated that moderate-intensity exercise, under conditions of prior GD and low-carbohydrate diet, results in a decreased rate of skeletal muscle glycogenolysis, greatly decreased muscle pyruvate and lactate contents with decreased plasma [lactate]. Based on these results, we hypothesized that exercise in GD, compared with normal muscle glycogen (NG) or high-carbohydrate conditions, should result in a reduced magnitude of acidosis due to reduced production and accumulation of lactate. It is further hypothesized that the fv acid–base disturbance will be characterized primarily by decreased [SID] and increased PCO\textsubscript{2}, while the arterial acidosis will be characterized mainly by decreased [SID] and increased \([A_{\text{enal}}]\) resulting from increased plasma [protein].

The purposes of this paper are to compare the origins of the fv and arterial plasma acid-base responses with prolonged, submaximal exercise under conditions of no prior muscle GD (NG) and with prior muscle GD. The rapid changes occurring early in the rest-to-exercise transition are emphasized.

**Methods**

**Subjects**

Datasets of four subjects from the study described by Putman et al\textsuperscript{3} were used. Characteristics of these subjects include body mass of 79.6 ± 10.8 kg, height of 183 ± 3 cm, age of 23.4 ± 2.4 years and peak VO\textsubscript{2} of 4.92 ± 0.281 min\textsuperscript{−1}. Subjects abstained from caffeine and alcohol consumption for at least 24 h before reporting to the lab on four occasions. The procedures and risks were fully explained to the subjects both verbally and in writing, and written informed consent was obtained. The study was approved by McMaster University’s Research Ethics Committee.

**Experimental protocol**

Each subject’s peak VO\textsubscript{2} was determined using a progressive exercise test on a Monark cycle ergometer. This was immediately followed by continuous bicycling at high and low intensities until exhaustion (60–80 min) to deplete liver and muscle glycogen stores. Subjects then consumed a low-carbohydrate diet (3% carbohydrate, 51% fat and 46% protein) for 2.5 days and reported to the lab for the GD trial the morning of the third day. At rest, just prior to starting exercise, muscle glycogen was 185 ± 19 mmol glucosyl units/kg dry muscle\textsuperscript{3}. The NG trial was performed 7–14 days after GD. This ordering was necessary to provide timing of muscle biopsy samples\textsuperscript{3}. Three days prior to NG, the subjects repeated the exhaustive exercise procedure to deplete liver and muscle glycogen stores, then consumed a high-carbohydrate diet (86% carbohydrate, 4% fat and 10% protein), also for 2.5 days. At rest, prior to starting the NG, muscle glycogen was 655 ± 71 mmol/glucosyl units/kg dry muscle\textsuperscript{3}.

Prior to conducting the GD and NG trials, subjects reported to the lab in the morning. Catheters were inserted into a brachial artery and fv, and one leg was prepared for needle biopsy of the \textit{vastus lateralis}. After 15 min of quiet sitting, the resting blood samples and muscle biopsy were obtained. The subjects then cycled (intensity 75% of peak VO\textsubscript{2}) on an electronically braked cycle ergometer to exhaustion in GD and to the GD exhaustion time in the NG trial.
Blood
Blood samples were simultaneously drawn from the artery and fv in 10 ml lithium-heparinized plastic syringes at rest, 0.5, 1, 2, 5, 10, 15, 30 and 45 min of exercise and at exhaustion. Blood was immediately analysed for plasma pH, PCO2 and PO2 using a Corning 178 pH/blood gas analyzer corrected to 37°C. Plasma was immediately separated from a 3 ml blood sample by centrifugation for 2 min at 15 000 × g. A 200 μl aliquot of plasma was deproteinized in 400 μl of 6% perchloric acid and the supernatant analysed for [lactate]14. Plasma was analysed for Na+, K+ and Cl− using ion-selective electrodes (AVL 983-S electrolyte analyzer) and for total protein using a clinical refractometer (Atago model 331, Atago, Japan). Haemoglobin ([Hb]) was measured using a haemoximeter (Radiometer model OS3, Copenhagen, Denmark). Haematocrit was determined in duplicate using heparinized capillary tubes centrifuged for 5 min at 15 000 × g. The mean differences between duplicate measures were 0.006 ± 0.003 for pH, 0.8 ± 0.5 mm Hg for PCO2, 1.1 ± 0.7 mm Hg for O2, 0.3 ± 0.1 meq l−1 for [Na+], 0.6 ± 0.2 meq l−1 for [Cl−], 0.02 ± 0.02 meq l−1 for [K+], 0.2 ± 0.2 meq l−1 for [lactate−], 0.4 ± 0.3% for haematocrit and 0.2 ± 0.2 g dl−1 for [Hb].

Calculations
The change in plasma volume was calculated using Eq. 1 of Gillen et al.15 from rest and exercise measures of [Hb] and Hct. Hct (%) was multiplied by 0.96 and 0.91, giving H, to correct for trapped plasma and peripheral sampling, respectively.

\[ \%dPV = 100 \times \left( \frac{[Hb]}{Hb_e} \times \frac{100 - H_e}{100 - H_c} - 1 \right) \]

The physicochemical approach to understanding acid–base physiology treats physiological solutions as systems possessing three independent variables: the SID, PCO2 and the total weak acid concentration ([A_{tot}]) that determine the dependent variables \( [H^+] \) and \( [\text{HCO}_3^-] \); the approach has been analytically evaluated and validated16,17.

Plasma \([A_{tot}]\) was calculated by multiplying plasma protein concentration (IPP) in g–dl−1 by 2.45, which ignores an insignificant and constant contribution from inorganic phosphate9,17,18. Plasma [SID] was calculated as the sum of the strong cation concentrations minus the sum of the strong anion concentrations6,7:

\[ [\text{SID}] \text{meq l}^{-1} = ([\text{Na}^+] + [K^+]) - ([\text{Cl}^-] + [\text{Lac}^-]) \]

Other strong ions (Ca2+, Mg2+ and SO42−) were ignored because their concentrations are low and their charges cancel each other.

Predicted plasma \([H^+]\) was calculated as6,7:

\[ [H^+]_t = (K_a + [\text{SID}]) \times [H^+]_s + \frac{([\text{SID}] - [A_{tot}])}{K_a} \]

\[ + (K_C \times \text{PCO}_2 + K'_W) \times [H^+]_s \]

\[ - ([K_C \times \text{PCO}_2 + K'_W] \times K_C \times K_C \times \text{PCO}_2) \times [H^+]_s - K_a \times K_3 \times K_C \times \text{PCO}_2 = 0 \]

where \( K_a = 3 \times 10^{-7} \) eq. l−1, \( K_C = 2.45 \times 10^{-11} \) eq. l−1, \( K_3 = 6.0 \times 10^{-11} \) eq. l−1 and \( K_W = 4.4 \times 10^{-11} \) eq. l−1.

Changes in [SID], [A_{tot}] and PCO2, independently of each other, contribute to changes in the dependent variables \([H^+], [\text{HCO}_3^-]\) and \([A^-]\). The contribution of each independent variable to each dependent variable was assessed by solving this equation with each variable being changed only singly or in combination5,9.

Statistics
Results are expressed as means ± SE. Data were analysed using two-way repeated measures ANOVA with respect to time and treatment (NG versus GD). When a significant F ratio was obtained, the Holm–Sidak post hoc test was used to compare means. Significance was accepted at \( P < 0.05 \) and power > 0.8.

Results
The time to exhaustion in GD was 57 ± 7 min (range 48–60 min). In NG, subjects exercised for the same duration as in GD.

The exercise-induced decrease in PV (Fig. 1) was rapid and similar in both trials during the first 2 min of exercise. Thereafter, the decrease in PV was significantly less in the GD than in the NG trial, in both arterial and fv blood. Since the decrease in PV represents a greater proportionate loss of water than of Na+, Cl− and protein, this fluid shift has direct effects on plasma acid–base balance. A primary effect is the increase in PP, manifest as the increase in plasma \([A_{tot}]\) (see below).

Dependent variables
In NG, fv plasma \([H^+]\) increased by 21 ± 4 neq l−1 compared (\( P < 0.05 \)) with a peak increase of 16 ± 3 neq l−1 at 2 min in the GD trial, and NG \([H^+]\) remained elevated (\( P < 0.05 \)) over that of the GD trial (Fig. 2). In NG, arterial \([H^+]\) increased by 10 ± 2 neq l−1 had a broad peak between 5 and 15 min and averaged 14 neq l−1 lower than fv \([H^+]\). In GD, arterial \([H^+]\) increased by only 8 ± 2 neq l−1
at 5 min, and thereafter significantly decreased to rest values by 30 min.

In NG, \(\text{fv } \text{HCO}_3^-\) was elevated for the first 5 min of exercise while there was no significant change in the GD trial (Fig. 3). At rest and during the first 5–10 min of exercise, \(\text{fv } \text{plasma } \text{HCO}_3^-\) were significantly lower in GD than in NG. In NG, arterial \(\text{HCO}_3^-\) decreased by 5.9 ± 1.6 \(\text{meq l}^{-1}\) in the first 15 min of exercise, compared with a 2.8 ± 0.9 \(\text{meq l}^{-1}\) decrease that occurred entirely in the first 5 min of exercise in GD. In NG, there was no recovery of arterial \(\text{HCO}_3^-\).

**Independent variables**

Femoral venous plasma \(\text{PCO}_2\) was 7.8 ± 1.7 mm Hg higher \((P < 0.05)\) at rest in NG than in GD and remained higher throughout exercise (Fig. 4). With the onset of exercise, the rate of increase in \(\text{fv } \text{PCO}_2\) was greater in NG than in GD, such that in NG \(\text{fv } \text{PCO}_2\) peaked at 2 min compared with 5 min in the GD trial. In NG, \(\text{fv } \text{PCO}_2\) decreased from its peak between 5 and 15 min and remained at c. 65 mm Hg for the remainder of the trial. Arterial \(\text{PCO}_2\) changes were small compared with those seen in \(\text{fv}\) plasma. In NG, there was a significant lowering of arterial \(\text{PCO}_2\) during the final 30 min of exercise. In GD, arterial \(\text{PCO}_2\) was decreased at 0.5 and 15 min of exercise.

There was a pronounced \(\text{fv-a plasma } \text{PCO}_2\) difference of c. 24 mm Hg by 5 min of exercise in both trials with the increase in \(\text{fv } \text{PCO}_2\) faster in the NG trial (Fig. 5). In the NG trial, \(\text{fv-a } \text{PCO}_2\) remained constant throughout exercise and, in contrast, decreased \((P < 0.05)\) during the last 30 min of exercise in GD compared with NG.

At rest, both \(\text{fv}\) and arterial \([\text{A}_{\text{tot}}]\) tended to be higher \((P < 0.05)\) in NG than in GD and remained elevated throughout exercise. In both trials, \(\text{fv } [\text{A}_{\text{tot}}]\) peaked at 2–5 min of exercise and remained elevated throughout exercise, with no difference between trials. In both trials, arterial \([\text{A}_{\text{tot}}]\) peaked at 5 min and remained elevated throughout exercise, with no difference between trials. The increases in arterial \([\text{A}_{\text{tot}}]\) were significantly less than in \(\text{fv}\) plasma.

The exercise-induced increases in \([\text{A}_{\text{tot}}]\) are primarily due to the increases in plasma \([\text{protein}]\), reflecting the net gain of fluid by contracting muscles. In both trials, there was an initial rapid increase in \(\text{fv-a } \text{plasma } \text{protein}\) and, in NG, this was sustained throughout the period of exercise (Fig. 5). In GD, after the initial
rapid increase, fv-a [protein] decreased to resting levels within 10 min of exercise such that fv-a plasma [protein] was negative in the GD trial compared with positive in the NG trial.

The plasma ion concentrations used for the calculation of [SID] are provided in Table 1. There were significant increases in fv [Na\(^+\)] in both trials, with the increase in the GD trials being less than in the NG trial. Arterial [Na\(^+\)] increased in the NG but not in the GD trial. Arterial and fv [K\(^+\)] increased in both trials, with no difference between trials. Arterial [Cl\(^-\)] increased similarly in both trials, but decreased in fv plasma. Arterial and fv [lactate\(^-\)] increased to a greater extent in the NG compared with the GD trial. The increase in fv plasma [lactate\(^-\)] in GD was c. 2/3 that of NG and, at the end of exercise, was only 5 meq l\(^{-1}\) compared with 10 meq l\(^{-1}\) in NG (P < 0.05).

The time course of change in plasma [SID] (Fig. 7) and [lactate\(^-\)] were similar, and [lactate\(^-\)] was quantitatively the largest contributor to the decreases in plasma [SID] in both trials. Exercise resulted in decreases in fv and arterial [SID] in both trials (Fig. 7). In fv plasma, the decrease in plasma [SID] tended (P = 0.07) to be slower in the GD compared with the NG trial, and remained significantly higher in the GD trial throughout exercise. The same trends were observed in arterial plasma [SID] and, importantly, the decreases in arterial plasma [SID] were greater than for fv [SID]. In NG, the increase in plasma [lactate\(^-\)] (from 2.1 ± 0.3 meq l\(^{-1}\) at rest to 13.4 ± 1.5 meq l\(^{-1}\) at 5 min) was c. 50% greater than the decrease in [SID]. The discrepancy was made up by a 4–5 meq l\(^{-1}\) rise in [Na\(^+\)] and a 1.4 meq l\(^{-1}\) rise in [K\(^+\)], exceeding the c. 3 meq l\(^{-1}\) decrease in [Cl\(^-\)].

In GD, the increase in arterial plasma [lactate\(^-\)] (from 1.4 ± 0.4 meq l\(^{-1}\) at rest to 9.4 ± 2.5 meq l\(^{-1}\) at 5 min) nearly matched the decrease in [SID]. As predicted, the increase in fv-a [lactate\(^-\)] was greater (nearly twofold) in the NG compared with the GD trial (Fig. 5). The fv-a plasma [lactate\(^-\)] difference increased to a peak of 2.4 ± 0.7 meq l\(^{-1}\) at the first minute of the NG trial and remained elevated for the first 2 min of exercise, whereas in the GD trial the peak increase of 1.7 ± 0.9 meq l\(^{-1}\) occurred at 0.5 min and was not different from baseline at 2 min.
Origins of changes in $[\text{H}^+]$ and $[\text{HCO}_3^-]$  

The contributions from each of the independent, physicochemical acid–base variables $\text{PCO}_2$, $[A_{\text{tot}}]$ and $[\text{SID}]$ to the calculated $[\text{H}^+]$ responses are shown in Figs 8 (NG) and 9 (GD). In these figures, the abscissa is plotted in a log scale so as to clearly show the changes during the first 5 min of exercise. In NG, the increase in $\text{PCO}_2$ and decrease in $[\text{SID}]$ contributed nearly equally to the rapid (first 2 min) and sustained increase in $f_v [\text{H}^+]$ (Fig. 8); the increase in $[A_{\text{tot}}]$ made only a minor contribution to the increase in $f_v [\text{H}^+]$. In GD, the increase in $f_v [\text{H}^+]$ was completely due to the increase in $\text{PCO}_2$ (Fig. 9). The increase in $[A_{\text{tot}}]$ had a minor acidifying effect while $[\text{SID}]$, which changed little, had no effect.

In arterial plasma, the decrease in $[\text{SID}]$ was the primary contributor to the increase in $[\text{H}^+]$ in both the NG (Fig. 8) and GD trials (Fig. 9). In both trials, the elevation in $[A_{\text{tot}}]$ made a minor contribution to the increased $[\text{H}^+]$, while the decreased $\text{PCO}_2$ contributed to a reduction in $[\text{H}^+]$ (alkalinizing effect).

The contribution from each of the independent, physicochemical acid–base variables $\text{PCO}_2$, $[A_{\text{tot}}]$ and $[\text{SID}]$ to the calculated $[\text{HCO}_3^-]$ response is shown in Figs 10 (NG) and 11 (GD). In these figures, the abscissa is plotted in a log scale so as to clearly show the changes during the first 5 min of exercise. In both trials, in both $f_v$ and arterial plasma, the decreases in $[\text{SID}]$ were the main contributors to the decreases in $[\text{HCO}_3^-]$. The increases in $\text{PCO}_2$ contributed to small increases in $[\text{HCO}_3^-]$, while the increased $[A_{\text{tot}}]$ had a minor acidifying effect.

Discussion

The muscle GD and low-CHO diet aspects of this study were designed to force contracting skeletal muscle away from carbohydrate metabolism (NG trial) and towards fat metabolism (GD trial)\(^1\,^3\). We demonstrated that the predominant pathways of energy provision\(^3\) markedly affect arterial and femoral venous blood acid–base balance during moderate-intensity exercise.
Importantly, these effects are manifest within the first 0.5–2 min of exercise, suggestive of altered metabolic control occurring prior to or within the rest-to-exercise transition. The greatest effects of GD on plasma acid–base balance during exercise were a decreased rise in fv PCO₂ and [lactate²⁻], with lower rates of CO₂ and [lactate²⁻] production/release ¹,³. The present study also extends the recent results of Putman et al. ¹⁹ that examined the acid–base responses at 15 min of exercise in subjects that exercised at 75% of peak VO₂, as well as those of Miller et al. ¹⁸, by providing a detailed time course showing the rapidity and magnitude of acid–base responses early in the exercise period.

Comparison of origins of acid–base changes in the artery and femoral vein

Very little disturbance to acid–base state occurred during the first 30 s of exercise. This observation is consistent with the initial intracellular alkalization that occurs upon phosphocreatine hydrolysis within contracting muscle and the time dependency required to achieve peak rates of glycolytic ATP and lactate²⁻ production²⁰ with lactate²⁻ efflux from muscle. After the initial 30 s of the rest-to-exercise transition, very rapid changes occurred, generating differing acid–base responses during the next 30 s, with peak acid–base effects occurring as early as 2 min after onset of exercise. It is noteworthy that, when expressed in traditional terms (pH, PCO₂, ½HCO₃⁻/C1₃8), the acid–base disturbance persisted in NG until the end of exercise, in contrast to GD where a traditional acid–base disturbance was not evident after the first 15 min of exercise.

The physicochemical approach to analysing acid–base disturbances allowed us to identify the origins of arterial and fv plasma acid–base disturbances. Using a traditional acid–base approach, the fv plasma acid–base disturbance would be characterized as a mixed respiratory/metabolic acidosis, identifying increased CO₂ and ‘metabolic’ acid as contributing variables. The traditional approach fails to identify and quantify the contribution of changes in plasma proteins (weak acids) and strong ions, and indeed of individual strong ions, to the acidosis of exercise⁹,¹⁹,²¹.

| Arterial [Na⁺] | 142.5 145.0* 145.9* 147.8* 146.7* 147.2* 147.4* 146.8* 146.1* 147.2* |
| fv [Na⁺] | 0.4 0.3 0.7 0.4 0.7 0.5 0.4 0.9 1.2 0.8 |
| Arterial [K⁺] | 4.18 5.04* 5.50* 5.64* 5.31* 5.23* 5.23* 5.23* 5.43* 5.43* |
| fv [K⁺] | 0.08 0.11 0.14 0.12 0.11 0.08 0.15 0.11 0.16 0.19 |
| Arterial [Cl₂⁻] | 106.4 108.3* 109.1* 109.1 109.0* 108.3* 108.4* 108.9* 107.5* 108.8* |
| fv [Cl₂⁻] | 0.7 0.4 0.4 0.8 0.8 0.6 0.6 0.6 0.2 0.5 |
| Arterial [lactate⁻] | 2.1 3.4* 6.8* 11.1* 13.4* 12.7* 13.3* 13.0* 10.4* 12.8* |
| fv [lactate⁻] | 0.7 0.7 0.6 0.5 0.6 0.4 0.7 0.7 0.2 0.5 |

Table 1 Arterial and femoral venous (fv) plasma ion concentrations in the control and the glycogen-depleted (GD) trials

<table>
<thead>
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<th>Exercise time (min)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>57</th>
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<td></td>
</tr>
<tr>
<td>Arterial [Na⁺]</td>
<td>142.5</td>
<td>145.0</td>
<td>145.9</td>
<td>147.8</td>
<td>146.7</td>
<td>147.2</td>
<td>147.4</td>
<td>146.8</td>
<td>146.1</td>
<td>147.2</td>
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<tr>
<td>fv [Na⁺]</td>
<td>0.4</td>
<td>0.3</td>
<td>0.7</td>
<td>0.4</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
<td>0.9</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Arterial [K⁺]</td>
<td>4.18</td>
<td>5.04</td>
<td>5.50</td>
<td>5.64</td>
<td>5.31</td>
<td>5.23</td>
<td>5.23</td>
<td>5.23</td>
<td>5.43</td>
<td>5.43</td>
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<tr>
<td>fv [K⁺]</td>
<td>0.08</td>
<td>0.11</td>
<td>0.14</td>
<td>0.12</td>
<td>0.11</td>
<td>0.08</td>
<td>0.15</td>
<td>0.11</td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td>Arterial [Cl₂⁻]</td>
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<td>109.1</td>
<td>109.1</td>
<td>109.0</td>
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<td>108.4</td>
<td>108.9</td>
<td>107.5</td>
<td>108.8</td>
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<tr>
<td>fv [Cl₂⁻]</td>
<td>0.7</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
<td>0.8</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
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<td>0.5</td>
</tr>
</tbody>
</table>

| GD trial            |   |   |   |   |   |   |   |   |   |   |
| Arterial [Na⁺]      | 141.2 | 142.7 | 143.6 | 144.2 | 146.0 | 145.3 | 145.5 | 145.7 | 144.9 | 145.2 |
| fv [Na⁺] ² | 0.5 | 0.5 | 1.3 | 1.5 | 0.8 | 1.0 | 1.4 | 0.8 | 0.8 | 1.6 |
| Arterial [K⁺]       | 4.34 | 5.02 | 5.49 | 5.60 | 5.39 | 5.32 | 5.42 | 5.42 | 5.61 | 5.70 |
| fv [K⁺]             | 0.08 | 0.09 | 0.08 | 0.14 | 0.14 | 0.13 | 0.14 | 0.16 | 0.14 | 0.13 |
| Arterial [Cl₂⁻]     | 104.8 | 106.9 | 107.5 | 107.3 | 108.1 | 107.6 | 107.0 | 108.4 | 107.5 | 107.7 |
| fv [Cl₂⁻]           | 0.10 | 0.13 | 0.13 | 0.14 | 0.26 | 0.16 | 0.21 | 0.13 | 0.18 | 0.07 |
| Arterial [lactate⁻] | 1.4 | 1.6 | 4.3 | 8.5 | 9.4 | 8.0 | 7.5 | 6.4 | 4.2 | 3.8 |
| fv [lactate⁻] ² | 0.4 | 0.3 | 0.5 | 1.5 | 2.5 | 1.6 | 1.9 | 2.1 | 2.1 | 1.4 |

Data are mean ± SE, n = 4 subjects. * indicates significantly different (P < 0.05) from time 0. ² indicates significantly different (P ≤ 0.05) from control trial.

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Conclusion

The results of the present study suggest that glycogen depletion can alter the acid–base balance during exercise, with the greatest effects occurring within the first 0.5–2 min of exercise. This is consistent with previous research indicating that changes in plasma acid–base balance during exercise are associated with increased CO₂ and lactate production/release. The present study also extends previous research by providing a detailed time course showing the rapidity and magnitude of acid–base responses early in the exercise period.

Comparison of origins of acid–base changes in the artery and femoral vein

Table 1 presents the arterial and femoral venous plasma ion concentrations in the control and glycogen-depleted (GD) trials. The data show significant differences in plasma ion concentrations between the control and GD trials. For example, in the control trial, arterial [Na⁺] was significantly higher than in the GD trial after 2 min of exercise. Similarly, arterial [K⁺] was significantly lower in the GD trial compared to the control trial. These differences suggest that glycogen depletion alters the acid–base balance during exercise, with the greatest effects occurring early in the exercise period.

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Examination of femoral venous (fv) and arterial plasma reveals very different pictures of acid–base disturbance, and the differences provide mechanistic insight into the origins of the acid–base disturbance. Femoral venous blood draining from contracting leg muscles provides a representation of events occurring within contracting skeletal muscle cells. The greatest differences between arterial and fv plasma concerned the two independent variables PCO2 and [SID], with [A\text{tot}] playing a minor role. The influence of erythrocytes on modification of plasma composition has been considered previously\(^9,10,11\) and will not be discussed here.

It was a concern that the differences between measured and calculated values for [H\text{\textsuperscript{+}}] and \(\frac{\text{HCO}_3^-}{\text{C}}\) are largely due to the following: calculated values are based on measured values for [Na\textsuperscript{+}], [Cl\textsuperscript{−}], [K\textsuperscript{+}], [lactate\textsuperscript{−}], [protein] and PCO\textsubscript{2} - each of these measured values has its inherent errors. Also, the calculations are performed with the assumption that the KA for [A\text{tot}] is the same for each subject and is constant during the period of exercise – this is unlikely. The point of performing the calculation is to allow one to determine the contributions of each of the independent variables to the acid–base disturbance.

The agreement between measured and calculated [H\text{\textsuperscript{+}}] (within 10 nmol\textsuperscript{l\textsuperscript{-1}}) is reasonable, and the interpretation arising from the analysis of calculated values is warranted. In the case of \(\frac{\text{HCO}_3^-}{\text{C}}\), the differences between absolute values are a concern, indicating that calculated \(\frac{\text{HCO}_3^-}{\text{C}}\) is more sensitive to errors, but note that the magnitude and direction of change is consistent, hence providing a basis for the interpretation based on calculated values.

\textbf{PCO\textsubscript{2}}

In both trials, the increase in PCO\textsubscript{2} (NG, 27 ± 2 mm Hg and GD, 11 ± 2 mm Hg) was the primary variable responsible for the increased [H\text{\textsuperscript{+}}]. While PCO\textsubscript{2} is an independent variable in the “open” CO\textsubscript{2} : HCO\textsubscript{3}\textsuperscript{−} system, contributing to physicochemical equilibrium in arterial plasma and under the control of ventilation, venous plasma represents a closed system in which a change in venous CO\textsubscript{2} content is the main initiating event. In both trials, rapid and pronounced increases in fv plasma PCO\textsubscript{2} were the primary contributor to the initial acidosis of exercise.
The CO₂ produced within, and released from, contracting muscle has both glycolytic and β-oxidation contributions. The decarboxylation of pyruvate to acetyl CoA by pyruvate dehydrogenase (PDH) results in the production of 1 mol of CO₂ for each mole of pyruvate entering the TCA cycle. There was, however, an important difference in the magnitude of the fV PCO₂ response between the two trials. The metabolic changes that occur in muscle during exercise in a glycerogen-depleted state lead to a reduced CO₂ production compared with NG, evident as a lower fV PCO₂ as leg blood flow was similar during both trials. The reduced contribution of the glycolytic pathway to the increased ATP demand in GD results in a reduction in CO₂ production at the level of PDH. For a given rate of ATP formation, a shift from aerobic glycogenolysis to lipolysis had the potential to reduce CO₂ production by up to 28%. The reduced CO₂ release follows from decreases in PCO₂ within muscle, resulting in the diminished VCO₂ and RER in GD (0.83 ± 0.03 and 0.80 ± 0.03 at 5 and 30 min, respectively) compared with NG (0.95 ± 0.03 and 0.90 ± 0.02, respectively).

This reduced exercise RER in GD represents a 55% reduction in muscular carbohydrate utilization compared with NG, indicative of an increased reliance on fat as a fuel for ion transport and contractile ATPases within muscles.

The rapidity and magnitude of increase in PCO₂ seen in fV plasma were largely absent in arterial plasma, demonstrating the rapidity and efficacy of CO₂ elimination by the lungs. Indeed, arterial PCO₂ did not change significantly during exercise, except for a mild hypocapnia during the final 30 min of exercise in the NG trial. Thus, in contrast to the acidifying effects of raised PCO₂ seen in fV plasma, small decreases in arterial PCO₂ contributed to an alkalinizing effect that partially offset the acidifying effects of decreased [SID] and increased [A_{tot}].

[SID]
In both trials, changes in fV plasma [SID] were small, and the contributions of the initial decrease in [SID] to acidosis was minor, despite the initially rapid and large lactate release from contracting muscle.
This is in marked contrast to the rapid and pronounced decrease in arterial [SID] seen in both trials.

Fv plasma [SID] directly reflects the net flux of strong ions across the sarcolemma. In the present study, the strong anion lactate \(2^-\) (pK\(_0\) of 3.86) was the primary contributor to the decrease in [SID], which had an acidifying effect on plasma. The attenuated [SID] response in fv plasma, compared with arterial, was associated with an increase in plasma \([\text{Na}^+]\). The increase in plasma \([\text{K}^+]\) agrees with a net loss of \([\text{K}^+]\) from muscle through \([\text{K}^+]\) channels and inadequate rate of Na, K-ATPase activity to maintain intracellular \([\text{K}^+]\)\(^22\). The modest increases in fv \([\text{Na}^+]\) and \([\text{K}^+]\) serve to counter, in part, the effect of increased \([\text{lactate}^-\) in lowering [SID] and thus contributed to an alkalizing effect. The reduction in muscle lactate\(^-\) release in GD directly accounted for the attenuated decrease infv [SID], compared with NG; this, coupled with the lack of any effect of GD on fv concentrations of other strong ions, thus accounts for the reduced acidification seen in fv plasma during GD.

The magnitude of change in [SID] was more pronounced in arterial than in fv plasma. This may, in part, be attributed to the rapidity of gas and ion exchange processes within the femoral venous plasma\(^10\) and in part to the occurrence of these processes during transit through the lungs\(^8\). A key difference between arterial and fv plasma was the significantly greater increase in arterial \([\text{Cl}^-]\) which, with minor removal of lactate\(^-,\) contributed to negating the effects of increased \([\text{Na}^+]\) and \([\text{K}^+]\) seen in fv plasma, and hence accounted for the more pronounced decrease in fv than arterial [SID]. The rapid \([\text{Cl}^-]\) shift is attributed to erythrocyte CO\(_2\) and O\(_2\) handling\(^24\). Within fv plasma, the increase in [SID] appears to drive the net production of HCO\(_3^-\) in both plasma and erythrocytes, resulting in exchange of erythrocytic HCO\(_3^-\) for plasma Cl\(^-\).\(^{24,25}\) Thus, fv plasma [Cl\(^-\)] is lower than arterial plasma. At the lungs, the process of erythrocyte Cl\(^-\)/HCO\(_3^-\) exchange is reversed, favouring production of CO\(_2\) from HCO\(_3^-\) and diffusion of CO\(_2\) from erythrocytes and plasma into alveoli. The net effect is a decrease in PCO\(_2\), accompanied by an increase in plasma [Cl\(^-\)].

\([A_{\text{tot}}]\)

Plasma \([A_{\text{tot}}]\) is an independent acid–base variable that is affected by the rapid net flux of water between vascular and non-vascular compartments. Within contracting skeletal muscle, the transition from rest to exercise is accompanied by the rapid hydrolysis of phosphocreatine and increasing rates of glycolysis. These two metabolic events result in a rapid increase in intracellular osmolality that drives a net flux of fluid from the vascular compartment into contracting muscle\(^22\), an effect that is more pronounced with high-intensity exercise\(^9\). This net shift of fluid from the vascular compartment is evident as an increase in fv-a plasma [protein] and increased fv and arterial plasma \([A_{\text{tot}}]\), which contributed to an acidifying effect in plasma early in the exercise period. With submaximal exercise, a partial recovery of vascular water (coincident with partial recovery of intramuscular phosphocreatine and decreasing concentrations of lactate\(^-\) and \([\text{K}^+]\) may nearly restore plasma [protein] (and hence \([A_{\text{tot}}]\)) to pre-exercise levels\(^22\), so that its contribution to acid–base balance may be negligible after the first 15–20 min.

### Summary and conclusions

The present study has highlighted an effect of nutritional status on the early time course of acid–base responses to moderate-intensity exercise in normal glycogen and glycogen-depleted conditions in arterial and fv plasma. Profound differences between the NG and GD conditions in femoral venous plasma reflected the metabolic switch from primarily carbohydrate metabolism (NG condition) to fat metabolism (GD conditions). Notably, GD was associated with a decreased...
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date and magnitude of lactate and CO₂ appearance in plasma that altered the description of acid-base status in these conditions compared with NG. There were marked differences between the magnitude and types of acidosis present in arterial and plasma. In particular, in plasma increased PCO₂ was the primary variable responsible for the rapid and sustained elevation in [H⁺], whereas in arterial plasma decreased [SID] due to increased [lactate⁻] was primarily responsible for increased [H⁺].

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