Effects of in Vivo and in Vitro Production of Lactic Acid on Ionized, Protein-Bound, and Complex-Bound Calcium in Blood

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We have studied, both in vitro and in vivo, the quantitative effects of lactic acid production on concentrations of ionized calcium, bound calcium, pH, bicarbonate, and albumin. To do so, we examined the effects of addition of aqueous solutions of either hydrochloric acid, lactic acid, or lithium lactate to blood; we studied in vitro accumulation by storing blood sealed in tubes at room temperature for 5 h, then exposing the blood to air; and we induced in vivo production of lactic acid in healthy individuals who climbed stairs for 10 min. Lactic acid evidently affects the ionized, protein-bound, and complex-bound calcium concentrations in the following ways: (a) hydrogen ions from lactic acid bind to protein, which decreases protein-bound calcium; (b) lactate chelates calcium ions from free ionized calcium and protein-bound calcium about equally; and (c) the loss of a millimole of bicarbonate, either by exposure of blood to air or by respiratory alkalosis, results in the release of about 7 μmol of calcium ions, which re-equilibrate with both the protein-bound and ionized calcium. Because lactate apparently removes calcium ions directly from albumin, our study indicates that protein-bound calcium readily provides calcium ions that buffer changes in the concentration of ionized calcium.

Additional Keyphrases: acidosis • hypoxia • effects of exercise

In several reports it was noted that lactic acid produced in vitro in whole blood by cellular metabolism increases the concentration of free ionized calcium (1–4), but this increase is less than that expected from the observed decrease in pH (1–3). One mechanism proposed (2) to explain this observation is that hydrogen ions and lactate anions have the following effects on the free ionized calcium concentration, which offset each other: (a) by binding to albumin, hydrogen ions from lactic acid release calcium ions from protein, and (b) lactate anions chelate free calcium ions to form calcium–lactate complexes.

Because this mechanism apparently has not been confirmed by measurements of the ionized, protein-bound, and complex-bound calcium, we studied, both in vitro and in vivo, the accumulation of lactic acid under both anaerobic and combined anaerobic/ aerobic conditions to determine how these conditions affect the ionized, complex-bound, and protein-bound calcium concentrations. This was done by (a) adding solutions of either hydrochloric acid, lactic acid, or lithium lactate to blood as it was collected; (b) storing blood in sealed syringes at room temperature for 5 h, then exposing the blood to air for 15 min; and (c) sampling blood from volunteers before and after they climbed stairs for 10 min.

These studies led us to conclude that lactic acid has somewhat more complicated effects on the free and bound portions of calcium than proposed before. Lactate apparently removes calcium ions from protein, which indicates that the protein-bound calcium must readily provide free calcium ions, even to a relatively weak chelator such as lactate. Patients who are critically ill frequently display lactic acidosis as a result of hypoxia. This study may help explain how ionized calcium changes in these patients.

Materials and Methods

Throughout our study, the same six volunteers, all apparently healthy, donated blood during each experiment in the three phases of our study.

In the first phase of our study, keeping the solution:blood ratio (by volume) equal to 1:100, we added solutions of either 160 mmol/L NaCl ("saline"), 0.3 mol/L HCl, 0.4 mol/L lithium lactate, or 0.5 mol/L lactic acid to blood when collected. These concentrations of hydrochloric acid and lactic acid each decreased the pH of blood by about 0.1 unit.

Next, we collected heparinized blood in syringes, then stored the blood at room temperature (23–25 °C) for 5 h in sealed syringes. After removing aliquots of this stored blood for analysis, we exposed the remaining blood for 15 min to air in an open cup, which was swirled occasionally. The blood was then aspirated back into the syringe for further analyses.

Finally, we collected blood without anticoagulants from individuals before and after they walked up and down stairs for 10 min. The paired t-test was used to determine the P values shown in the tables.

We measured ionized calcium with ion-selective electrodes (Radiometer ICA-1; Radiometer America, Inc., Westlake, OH 44145), total calcium by atomic absorption spectrophotometry, and ultratitratable calcium (5) by using disposable centrifugal ultratilters ("Centrifree"; Amicon Corp, Danvers, MA 01923). Because these ultratilters do not prevent loss of CO2 during the 15 min of centrifugation, we applied a 1- to 2-mm layer of mineral oil to the serum, using a 3.75-cm needle attached to a syringe containing the oil. Apparently because oil prevents an increase in sample pH, the results for ultratitratable calcium were now about 0.06 mmol/L higher. After centrifugation, the ultratilters were analyzed for calcium content by atomic absorption. Protein-bound calcium (Ca-PB) was calculated as the difference between total and ultrafiltrable calcium. Complex-bound calcium (Ca-CPX) was calculated as the difference between ultrafiltrable and ionized calcium.

Both pH and bicarbonate (calculated from pCO2) were measured with conventional blood-gas analyzers. Lactate was measured in the cox (DuPont Co, Wilmington, DE). Albumin and total protein were measured in an Ektachem 100 (Eastman Kodak Co, Clinical Products Division, Rochester, NY). We used a relative molecular mass of 66 000 for albumin in converting results from grams per deciliter to

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millimoles per liter.

We calculated association constants for calcium albumin \(K_{CaAlb}\) by using an equation derived elsewhere (6, 7):

\[
K_{CaAlb} = \frac{[Ca_{pp}]}{[Ca^2+](12[Alb] - [Ca_{pp}])}
\]

Briefly described, \(Ca_{pp}\) is the concentration of protein-bound calcium used to approximate the average number of calcium ions bound per mole of albumin. \(Ca^2+\) is the measured concentration of ionized calcium. The term \(12[Alb] - [Ca_{pp}]\) represents the unoccupied calcium-binding sites on albumin.

**Results**

Table 1 shows the effects of added hydrochloric acid, lactic acid, and lithium lactate on the measured concentrations of ionized, complex-bound, and protein-bound calcium in blood. These data demonstrate that: (a) for similar decreases in pH, lactic acid increases ionized calcium less than half as much as does hydrochloric acid; and (b) independent of pH, lactate anions (from the lithium lactate solution) not only bind free calcium ions but also lower protein-bound calcium, and to an even greater extent.

Because pH, lactate, albumin, and bicarbonate all affect calcium binding, we determined if the changes in these could account for the changes that we measured in ionized, complex-bound, and protein-bound calcium shown in Tables 1, 3, and 4. Table 2 lists the factors we used to calculate the quantitative effects of pH, lactate, albumin, and bicarbonate on the ionized and bound calcium. For example, from the data in Table 2, one can see that each millimole per liter increase in lactate should decrease ionized calcium by 6 \(\mu\)mol/L, increase complex-bound calcium by 14 \(\mu\)mol/L, and decrease protein-bound calcium by 12 \(\mu\)mol/L. Hemoconcentration caused by fluid movement will increase the albumin concentration, so protein-bound calcium should increase in proportion to albumin. Finally, by using the association constant for calcium bicarbonate, 5.4 L/mol (8), each millimole of bicarbonate lost per liter should decrease complex-bound calcium by 7 \(\mu\)mol/L. We assume the calcium ions released from bicarbonate are distributed about equally between the ionized and protein-bound calcium.

When we used these factors to calculate the changes in calcium components caused by adding lactic acid to blood (Table 1), the calculated changes agreed closely with the measured changes (Table 5). This confirms that the effects of hydrogen ions or lactate ions, added separately, can be used to predict the effects of hydrogen ions and lactate ions added together in the form of lactic acid.

Table 3 shows the changes in calcium components that occur during storage of heparinized blood in sealed syringes kept at room temperature for 5 h, then poured into an open container and left for 15 min. Lactate increased relatively little, 2.8 mmol/L, for a pH decrease of 0.07. Apparently, acids other than lactic are produced during storage. As bicarbonate decreased from loss of carbon dioxide during exposure to air, both pH and ionized calcium returned to nearly their initial values. After either storage of blood or exposure to air (Table 5), the calculated changes generally agreed with the measured changes. The exception was complex-bound calcium after exposure to air, where the loss of 4 mmol of bicarbonate per liter coupled with the gain of 2.3 mmol of lactate per liter calculated to a change in complex-bound calcium of nearly zero, whereas the measured change was +0.04 mmol/L. This indicates that some other anions, such as pyruvate, are binding calcium ions.

The individuals who exercised for 10 min showed marked changes in pH, lactate, albumin, and bicarbonate (Table 4). Lactate, albumin, and bicarbonate changed consistently among individuals, but pH changes ranged from −0.06 to +0.04. This is because pH reflects the dynamic balance between lactic acid production and CO₂ lost by ventilation, both of which are changing rapidly by the end of the exercise period. Changes in protein-bound calcium ranged from −0.07 to 0.15 mmol/L. This variation is probably the result of the changes in pH, lactate, bicarbonate, and albumin—all of which influence the concentration of protein-bound calcium. Despite the variability of these changes, the calculated and measured changes in ionized and bound calcium agreed closely, as shown in Table 5.

### Table 1. Effects of Adding Solutions to Blood

<table>
<thead>
<tr>
<th>Component</th>
<th>Mean changes (± SD) after adding</th>
<th>Li lactate</th>
<th>Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca^2+</td>
<td>1.25 ± 0.03</td>
<td>+0.07 ± 0.01^a</td>
<td>−0.02 ± 0.01^a</td>
</tr>
<tr>
<td>Ca-CPX</td>
<td>0.15 ± 0.03</td>
<td>−0.01 ± 0.02^c</td>
<td>+0.06 ± 0.003^d</td>
</tr>
<tr>
<td>Ca-PB</td>
<td>0.95 ± 0.06</td>
<td>−0.04 ± 0.02^a</td>
<td>−0.05 ± 0.002^d</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.01</td>
<td>−0.10 ± 0.01^a</td>
<td>0.0 ± 0.01^c</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.62 ± 0.02</td>
<td>+0.02 ± 0.01 (0.01)</td>
<td>+0.01 ± 0.06^c</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.4 ± 0.2</td>
<td>+0.01 ± 0.08^d</td>
<td>+4.1 ± 0.02^d</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>28 ± 1</td>
<td>−0.08 ± 1.2^a</td>
<td>0.0 ± 0.1^a</td>
</tr>
<tr>
<td>K_{CaAlb} L/mol</td>
<td>117 ± 12</td>
<td>−16 ± 3.9^d</td>
<td>−8.7 ± 1.6^a</td>
</tr>
</tbody>
</table>

Concentrations are in mmol/L, N = 6 different subjects. P values of the differences, shown in parentheses:^a P < 0.0005. ^b P < 0.001. ^c P > 0.10 (NS). ^d P < 0.0001. ^e P < 0.005.
Ca2⁺, Albumin, Ca-PB, Ca-CPX

This indicates that the changes in pH, lactate, albumin, and bicarbonate during exercise account for most of the changes in ionized and bound calcium. Also note that the mean change in protein-bound calcium of 15 μmol/L is much less than the increase of 60 μmol/L predicted solely from the changes in albumin and pH, which also suggests that the increase in lactate decreases the concentration of protein-bound calcium.

In most of our experiments the association constant calculated for calcium albumin decreased, independent of pH, whenever lactate was present, suggesting that lactate somehow alters the affinity of albumin for calcium. Lactate could possibly bind to albumin, but when we compared measurements of total and ultrafiltrable lactate, we found no evidence of it (data not shown).

Discussion

By measuring ultrafiltrable calcium in addition to ionized and total calcium in our study of the mechanism by which lactic acid affects ionized calcium, we have shown that the offsetting effect of lactic acid on the ionized calcium concentration in blood involves several events. These are: (a) hydrogen ions release calcium ions from protein; (b) lactate chelates free calcium ions; (c) calcium ions dissociate from albumin to maintain equilibrium; (d) lactate removes some calcium ions directly from protein; and (e) the loss of bicarbonate on exposure of blood to air also results in release of calcium ions, which redistribute with both the ionized and protein-bound calcium. This mechanism is illustrated in Figure 1.

The finding that lactate removes calcium ions from protein is consistent with an earlier study of platelet donors who were receiving citrate: concentrations of both ionized calcium (25% decrease) and protein-bound calcium (35% decrease) markedly decreased (7). In retrospect, the decrease in the association constants on addition of citrate in that study is consistent with citrate chelating protein-bound calcium—which is not surprising, because citrate has a large association constant for calcium ions (1300 L/

Table 3. Changes during Storage of Blood and after Exposure to Air

<table>
<thead>
<tr>
<th>Ca²⁺</th>
<th>Mean changes (± SD) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 h storage</td>
</tr>
<tr>
<td></td>
<td>+0.031 ± 0.01³</td>
</tr>
<tr>
<td></td>
<td>+0.033 ± 0.02 (0.01)</td>
</tr>
<tr>
<td></td>
<td>+0.042 ± 0.04 (0.02)</td>
</tr>
<tr>
<td></td>
<td>+0.070 ± 0.01³</td>
</tr>
<tr>
<td></td>
<td>+0.005 ± 0.01³</td>
</tr>
<tr>
<td></td>
<td>+2.7 ± 0.5³</td>
</tr>
<tr>
<td></td>
<td>-1.5 ± 1.0 (0.01)</td>
</tr>
</tbody>
</table>

Concentrations are in mmol/L. n = 6 different subjects. * P < 0.0005. b P = 0.10 (NS). c P < 0.0001. d P < 0.005. e P < 0.001.

Table 4. Effects of 10-Min Stair Walking

<table>
<thead>
<tr>
<th>Ca²⁺</th>
<th>Mean ± SD</th>
<th>Changes after walking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Changes after walking</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>1.21 ± 0.05</td>
<td>+0.020 ± 0.02 (0.05)</td>
</tr>
<tr>
<td>Ca-CPX</td>
<td>0.20 ± 0.07</td>
<td>+0.043 ± 0.04 (0.05)</td>
</tr>
<tr>
<td>Ca-PB</td>
<td>0.97 ± 0.07</td>
<td>+0.015 ± 0.09³</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 ± 0.01</td>
<td>-0.03 ± 0.04³</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.65 ± 0.04</td>
<td>+0.06 ± 0.03³</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.5 ± 0.4</td>
<td>+5.2 ± 2.6³</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>27 ± 3</td>
<td>-5.5 ± 2.8</td>
</tr>
</tbody>
</table>

Concentrations are in mmol/L. n = 6 different subjects. * P < 0.10 (NS). b P < 0.0005.

Table 5. Calculated and Measured Changes in Ionized and Bound Calcium Attributable to Lactic Acid

<table>
<thead>
<tr>
<th>Measured mean changes</th>
<th>Calculated changes in</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺</td>
<td>Ca-CPX</td>
</tr>
<tr>
<td>Addition of lactic acid (from Table 1)</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>-0.10</td>
</tr>
<tr>
<td>Lactate</td>
<td>+4.9</td>
</tr>
<tr>
<td>Albumin</td>
<td>+0.03</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>—</td>
</tr>
</tbody>
</table>

Sum of calculated changes: +0.041 +0.069 -0.081

Measured changes (Table 1): +0.03 +0.07 -0.09

Storage of blood (from Table 3)

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Changes after walking</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.07</td>
</tr>
<tr>
<td>Lactate</td>
<td>+2.8</td>
</tr>
<tr>
<td>Albumin</td>
<td>0</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>-1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sum of calculated changes: +0.037 +0.032 -0.079

Measured changes (Table 3): +0.03 +0.04 -0.05

After exposure to air (from Table 3)

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Changes after walking</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.01</td>
</tr>
<tr>
<td>Lactate</td>
<td>+2.3</td>
</tr>
<tr>
<td>Albumin</td>
<td>+0.02</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>-4</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Sum of calculated changes: +0.009 +0.004 +0.013

Measured changes (Table 3): 0 +0.04 +0.02

After 10 min of exercise (from Table 4)

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Changes after walking</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-0.03</td>
</tr>
<tr>
<td>Lactate</td>
<td>+5.2</td>
</tr>
<tr>
<td>Albumin</td>
<td>+0.06</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>-6</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sum of calculated changes: +0.014 +0.031 +0.037

Measured changes (Table 4): +0.02 +0.04 +0.01
hypocalcemia.

changes

redistributed

bonate

938

with

bone;

reasons:

is

calcium

ions,

or

lactate

fractions

Hydrogen

Lactic

added

CLINICAL

changes

so

in

the

Ions

changes

there

mobilization

in

support

is

the

patients

more

important

finding

is

lactate

apparently

removes

calcium

ions

protein,

which

emphasizes

that

albumin-bound

calcium

readily

dissociates

to

buffer

changes

in

the

concentration

of

increased

calcium

and

to

developing

symptoms

of

hypocalcemia.

Studies

are

needed

on

patients

with

low

albumin

and

total-protein

concentrations

in

their

serum,

both

to

prove

that

such

patients

are

more

susceptible

to

hypocalcemia

and

that

correction

of

the

hypoproteinemia

prevents

hypocalcemia.

Both

the

present

study

of

lactate

and

a

previous

study

of

citrate

infusions

during

plateletapheresis

(7)

demonstrate

that,

in

addition

to

measurement

of

the

ions

and

calcium,

measurement

of

the

ultrafiltrable

calcium

is

also

essential

if

one

is

to

monitor

adequately

the

changes

in

protein-bound

calcium.

Citrate

and

lactate

decrease

both

protein-bound

calcium

and

ionized

calcium

concentrations

by

approximately

the

same

amounts,

despite

the

100-fold

differences

in

association

constants

for

calcium

citrate

and

calcium

lactate.

The

apparent

decrease

in

association

constant

for

calcium

albumin

suggests

that

lactate

somehow

lowers

the

affinity

of

albumin

for

calcium.

Lactate

apparently

does

not

bind

to

albumin,

so

we

cannot

yet

explain

how

lactate

has

this

effect.

In

patients

with

good

ventilatory

capacity

and

sufficient

albumin

and

bicarbonate

concentrations,

lactic

acidosis

should

have

little

effect

on

the

ionized

calcium

concentration.

This

would

be

partly

similar

to

the

conditions

shown

in

Table

3.

However,

in

critically

ill

patients

with

poor

pulmonary

function

and

low

concentrations

of

albumin

and

bicarbonate,

the

effects

of

lactic

acidosis

on

ionized

calcium

are

more

difficult

to

predict.

Schaer

and

Bachmann

(1)

point

out

that

an

increased

concentration

of

ionized

calcium

may

improve

cardiac

contractility

in

the

presence

of

acidosis.

Thus,

in

hypercapnic

(respiratory)

acidosis

the

increased

ionized

calcium

would

be

beneficial,

whereas

in

lactic

acidosis

the

small

increase

in

ionized

calcium

would

not

overcome

the

myocardial

depression

from

acidosis.

We

believe

that

accurate

measurements

of

protein-bound,

complex-bound,

and

ionized

calcium

related

to

variables

of

cardiac

function

in

patients

with

acidosis

may

determine

how

pH,

calcium,

and

albumin

affect

cardiac

contractility.

We
	hank

all

the

dedicated

workers

at

the

Blood

Gas

Laboratory

at

Duke

Medical

Center,

who

did

many

of

these

anayses.

and

graciously

volunteered

to

donate

blood.

We

are

especially

indebted

to

Paul

Williams,

Susan

Jones,

Darrell

Sandiford,

Tammy

Mathes,

Alan

Culley,

and

Faye

Watson.

References