Effect of HCO_3^- on Glutamine and Glucose Metabolism in Lymphocytes

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Lymphocytes play a quantitatively important role in glutamine utilization in the body. We hypothesized that in metabolic acidosis characterized by decreased extracellular HCO_3^- concentration ($[HCO_3^-]$), glutamine utilization by lymphocytes may decrease to compensate partially for the increased uptake of glutamine by the kidneys for ammoniagenesis. This study was therefore designed to quantify the effect of extracellular $[HCO_3^-]$ on glutamine metabolism in lymphocytes relative to glucose utilization. Mesenteric lymph node lymphocytes were incubated at 37°C for 1 hour in Krebs-Henseleit buffer containing 0, 12.5, and 25 mmol/L HCO_3^- at a constant pH of 7.4 or 15.7 and 25 mmol/L HCO_3^- at a constant CO_2 concentration of 1.25 mmol/L. Reducing extracellular $[HCO_3^-]$ from 25 to 12.5 mmol/L at constant pH or from 25 to 15.7 mmol/L at constant CO_2 concentration decreased glutamine utilization and the production of glutamate and ammonia. A reduction in $[HCO_3^-]$ from 12.5 to 0 mmol/L further decreased glutamine utilization, as well as the production of all measured glutamine metabolites. Interestingly, decreasing $[HCO_3^-]$ from 25 to 0 mmol/L had no significant effect on glucose metabolism, although the production of pyruvate (a minor product of glucose in lymphocytes) was decreased in the absence of medium HCO_3^-. The contribution of glutamine but not of glucose to lymphocyte adenosine triphosphate (ATP) production was decreased with reduced extracellular $[HCO_3^-]$. Thus, glucose was a more important fuel for lymphocytes than was glutamine at low $[HCO_3^-]$. Our results demonstrate for the first time that a decrease in extracellular $[HCO_3^-]$ reduces glutamine utilization and metabolism by lymphocytes, which may help to increase the availability of glutamine for renal ammoniagenesis under conditions of metabolic acidosis.

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GLUTAMINE is the most abundant free amino acid in the blood and is an essential precursor for the synthesis of purine and pyrimidine nucleotides in all animal cells.[1] Glutamine also plays an important role in interorgan metabolism of nitrogen and carbon. It serves as an important fuel for the small intestine[2] and lymphocytes.[3] Also, glutamine is the major source of ammonia in the kidneys for the regulation of acid/base balance in the body.[4] Accordingly, rates of glutamine utilization by these competing metabolic pathways must be precisely regulated in response to physiological demands. However, the mediator(s) involved has not yet been fully identified. During metabolic acidosis, uptake of glutamine by the kidneys markedly increases, which is compensated for by decreased glutamine utilization by the gut, increased glutamine release from skeletal muscle, and a shift in the balance of glutamine from net uptake to net release by the liver.[5] This illustrates exquisite interorgan cooperations in the regulation of mammalian pH homeostasis.

It is now well documented that glutamine is rapidly metabolized in rat[3,6] and human[7] lymphocytes with a high utilization rate, as previously reported for rat enterocytes.[8] Given the estimated level of 1.5 kg lymphocytes in an adult man,[9] which is equivalent to the liver mass, utilization of glutamine by lymphocytes may play a quantitatively important role in regulating the availability of glutamine for its utilization by nonlymphoid cells in response to altered physiological demands. In metabolic acidosis, which is characterized by a decreased extracellular HCO_3^- concentration ($[HCO_3^-]$) in a variety of nutritional, physiological, and pathological states,[10-13] the rate of glutamine metabolism in lymphocytes may decrease to spare glutamine partially for renal ammoniagenesis. The present study was therefore designed to test this hypothesis, in light of previous observations that HCO_3^- itself regulates mammalian glutamine metabolism in a tissue-dependent manner.[14] To investigate if $[HCO_3^-]$ affects the utilization of substrates other than glutamine, glucose metabolism, which occurs in lymphocytes at a high rate,[3,6,7] was also determined in this study.

MATERIALS AND METHODS

Chemicals

L-[U-^{14}C]glutamine and D-[U-^{14}C]glucose were obtained from Amersham (Arlington Heights, IL). L-[U-^{14}C]glutamine was routinely purified on the day of the experiment using Dowex AG-1-X8 (200-400 mesh, acetate form; Bio-Rad Laboratories, Richmond, CA) as previously described.[3] L-Glutamine, D-glucose, L-lactate, pyruvate, Hyamine hydroxide, bovine serum albumin ([BSA] fraction V, essential fatty acid–free), and HEPES were purchased from Sigma Chemical (St Louis, MO). L-Glutamate dehydrogenase, α-ketoglutarate dehydrogenase, α-ketoglutaric acid, L-lactate dehydrogenase, β-NADH, β-NAD^+, adenosine diphosphate, and EDTA (disodium salt) were obtained from Boehringer Mannheim (Indianapolis, IN).

Animals

Male Sprague-Dawley rats weighing 150 to 200 g were obtained from Harlan (Indianapolis, IN) and housed in a light (12-hour light/dark cycle)- and climate-controlled facility. The animals were fed ad libitum a laboratory rat chow containing 24% (wt/wt) crude protein (Hardland-Teklad, Bartonville, IL) and had free access to water. Rats weighing 220 to 260 g were used to prepare lymphocytes for metabolic studies. The experiments were performed in

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accordance with guidelines of the US National Research Council for the care and use of laboratory animals.

Preparation of Mesenteric Lymph Node Lymphocytes

Rat mesenteric lymph node lymphocytes were prepared at room temperature essentially as previously described.[3] Briefly, after rats were anesthetized with CO_2, they were killed by cervical dislocation. Mesenteric lymph nodes were passed through a 50-mesh stainless steel grid in oxygenated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate buffer, pH 7.4 (119 mmol/L NaCl, 4.8 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, and 25 mmol/L $NaHCO_3$) supplemented with 0.5% BSA and 20 mmol/L HEPES. After centrifugation at 200 × *g* for 5 minutes, red blood cells were lysed for 3 minutes in 1 mL of a solution containing 155 mmol/L NH_4Cl, 0.1 mmol/L disodium EDTA, and 10 mmol/L $KHCO_3$. Lymphocytes were washed three times with fresh Krebs-Henseleit buffer, pH 7.4 (119 mmol/L NaCl, 4.8 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_4$, and 1.2 mmol/L KH_2PO_4) containing no HCO_3^- but supplemented with 0.5% BSA and 20 mmol/L HEPES. Cells were then suspended in fresh Krebs buffer containing 0, 12.5, 15.7, or 25 mmol/L HCO_3^-, with appropriate amounts of NaCl to keep medium sodium concentration and osmolarity constant. Cell viability was greater than 96%, as determined by Trypan blue exclusion. Results are expressed per 10^6 viable cells.

Incubation of Lymphocytes

Incubations were performed at 37°C in 25-mL siliconized Erlenmeyer flasks placed in a shaking water bath (70 cycles/min). Lymphocytes (5 to 40 × 10^6/mL) were incubated for 1 hour in 2 mL Krebs buffer containing 0.5% BSA, 20 mmol/L HEPES, and 0.5 to 2.0 mmol/L glutamine. The medium contained 0, 12.5, and 25 mmol/L HCO_3^-, which was gassed with 100% O_2, 97.5% O_2/2.5% CO_2, and 95% O_2/5% CO_2, respectively, to obtain a constant pH of 7.4.[15] In some experiments, the medium contained 25 and 15.7 mmol/L HCO_3^-, which was gassed with 95%, O_2/5% CO_2 to obtain a constant CO_2 level of 1.25 mmol/L.[15] Appropriate amounts of NaCl were added to keep medium sodium concentration and osmolarity constant.[15] To study the oxidation of glutamine or glucose, the medium contained either 1 mmol/L [U-^{14}C]glutamine (250 dpm/nmol) plus 5 mmol/L glucose or 5 mmol/L [U-^{14}C]glucose (300 dpm/nmol) plus 1 mmol/L glutamine, respectively. The incubations were initiated by addition of cells. Flasks containing 0, 12.5, and 25 mmol/L HCO_3^- were filled with 100% O_2, 97.5% O_2/2.5% CO_2, and 95% O_2/5% CO_2, respectively, before sealing with rubber stoppers, to maintain medium pH at 7.4.[15] In some experiments, flasks containing 12.5 and 25 mmol/L HCO_3^- were filled with 95% O_2/5% CO_2, before sealing with rubber stoppers, to maintain medium CO_2 level at 1.25 mmol/L.[15] The incubations were terminated by addition of 0.2 mL 1.5-mol/L $HClO_4$ to the medium through the stopper. $^{14}CO_2$ was trapped, by a 1-hour further incubation, in 0.2 mL Hyamine hydroxide, which was added through the stopper into a suspended centerwell. After $^{14}CO_2$ collection, incubation media were immediately neutralized with 0.1 mL 2-mol/L K_2CO_3 to minimize hydrolysis of glutamine. Radioactivity was measured by a Beckman 3800 liquid scintillation counter (Beckman Instruments, Fullerton, CA), using a modified Bray's solution.[3] Parallel incubations, in which incubation media were acidified with 100 μL 1.5-mol/L $HClO_4$ before addition of cells, were run as blanks. Blanks were subtracted from sample values in all assays. It was established in preliminary experiments that rates of glutamine metabolism were linear for 1 hour. In this study, we found that there were no significant differences in glutamine or glucose metabolism in lymphocytes incubated in the presence of 1.4 or 2.5 mmol/L $CaCl_2$.

To measure intracellular glutamine concentration in lymphocytes, cells were incubated as described earlier. At the end of a 1-hour incubation period, cells were separated from the medium using a mixture of oil (bromododecane/dodecane) as described by Wu and Flynn.[16] Glutamine content in lysed lymphocytes was determined by a fluorimetric high-performance liquid chromatography (HPLC) method involving precolumn derivatization with *o*-phthaldialdehyde.[17]

Measurements of Ammonia, [^{14}C]Glutamate, [^{14}C]Aspartate, Lactate, and Pyruvate

Ammonia levels in neutralized samples were measured by an enzymatic method involving glutamate dehydrogenase as previously described,[3] except that the change in NADH was determined using a Perkin-Elmer LS-5 Fluorescence Spectrophotometer (Oak Brook, IL).[18] [^{14}C]glutamate and [^{14}C]aspartate were separated by Dowex AG-1-X8 chromatography, and their radioactivities were separately measured by a Beckman 3800 liquid scintillation counter.[3] Lactate and pyruvate were determined by a fluorimetric enzymatic method using L-lactate dehydrogenase as previously described.[18] Glutamate, aspartate, and alanine in cell extracts plus incubation medium were determined using a sensitive fluorimetric HPLC method.[17]

Potential Production of Adenosine Triphosphate From Glutamine and Glucose

The potential production of adenosine triphosphate (ATP) from glutamine and glucose was calculated on the basis of their measured end products.[3,19] It is assumed that formation of 1 mol lactate and 6 mol CO_2 from glucose yields 1 and 38 mol ATP, respectively. Production of 1 mol aspartate and 5 mol CO_2 from glutamine via complete oxidation of glutamine yields 9 and 24 mol ATP, respectively. The amount of CO_2 produced via complete oxidation of glutamine is equal to total CO_2 minus aspartate production, because 1 mol CO_2 is stoichiometrically produced for 1 mol aspartate formed.

Statistical Analysis

Data were analyzed by paired T test or by one-way ANOVA with the Student-Neuman-Keuls multiple-range test.[20] Probability values less than .05 were taken to indicate statistical significance.

RESULTS

Effects of [HCO_3^-] on Glutamine Metabolism

The effects of extracellular [HCO_3^-] at a constant pH of 7.4 on glutamine metabolism in lymphocytes are presented in Table 1. The major end products of glutamine in lymphocytes were ammonia, glutamate, aspartate, and CO_2. No measurable amount of lactate or a small amount of pyruvate (0.19 ± 0.04 nmol/h per 10^6 cells, mean ± SEM, n = 8) was produced from glutamine in the absence of glucose. Similarly, no measurable amount of alanine was formed from glutamine in the presence or absence of glucose, as determined by HPLC (data not shown). Decreasing extracellular [HCO_3^-] from 25 to 12.5 mmol/L at a constant pH of 7.4 decreased ($P < .05$) the formation of [^{14}C]glutamate and ammonia from [U-^{14}C]glutamine by 23% to 24%, but had no effect ($P > .05$) on the production

of $^{14}CO_2$ or [^{14}C]aspartate. Rates of production of $^{14}CO_2$, [^{14}C]glutamate, [^{14}C]aspartate, and ammonia from [^{14}C]glutamine were all decreased ($P < .05$) in the absence of added HCO_3^- as compared with the presence of 12.5 or 25 mmol/L HCO_3^-. The decrease in production of measured glutamine metabolites in lymphocytes incubated in the absence of added HCO_3^- was much greater for glutamate and ammonia (69% to 76%) than for CO_2 and aspartate (41% to 43%).

Glutamate and aspartate levels in cell extracts plus incubation medium are listed in Table 2. In the absence of glutamine, decreasing extracellular [HCO_3^-] had no effect ($P > .05$) on the accumulation of glutamate or aspartate. This indicates that HCO_3^- did not affect endogenous production of glutamate or aspartate in lymphocytes in the absence of added glutamine. However, in the presence of 1 mmol/L glutamine, decreasing extracellular [HCO_3^-] markedly decreased ($P < .05$) the accumulation of glutamate in cell extracts plus incubation medium in a concentration-dependent manner. As noted for [^{14}C]aspartate production from [U-^{14}C]glutamine, decreasing [HCO_3^-] from 25 to 0 mmol/L significantly decreased ($P < .05$) aspartate accumulation in the presence of 1 mmol/L glutamine.

Reducing extracellular [HCO_3^-] from 25 to 0 mmol/L at a constant pH of 7.4 decreased ($P < .05$) glutamine utilization and slightly increased ($P < .05$) glutamine concentration in lymphocytes (Table 3). The inhibiting effects of extracellular [HCO_3^-] on glutamine metabolism in lymphocytes incubated in the presence of 1 mmol/L glutamine were also observed in cells in the presence of 0.5 or 2 mmol/L glutamine (Table 4). Decreasing medium [HCO_3^-] from 25 to 15.7 mmol/L at a constant CO_2 level of 1.25 mmol/L also suppressed ($P < .05$) glutamine utilization and glutamate and ammonia production in lymphocytes (Table 5).

Effects of HCO_3^- on Glucose Metabolism

The effects of extracellular [HCO_3^-] on glucose metabolism in lymphocytes are shown in Table 6. The major end products of glucose in lymphocytes were L-lactate, pyruvate, and CO_2. No measurable amount of D-lactate or alanine was formed in the presence of 5 mmol/L glucose plus 1 mmol/L glutamine. Decreasing extracellular [HCO_3^-] from 25 to 12.5 mmol/L at constant pH 7.4 had no effect ($P > .05$) on production of $^{14}CO_2$, lactate, or pyruvate from [U-^{14}C]glucose. A further decrease in [HCO_3^-] from 12.5 to 0 mmol/L had no effect ($P > .05$) on production of $^{14}CO_2$ or lactate, but significantly decreased ($P < .05$) production of pyruvate from glucose by 39%. This resulted in an increased lactate to pyruvate ratio ($P < .05$) in the absence of added HCO_3^-, as compared with the presence of 12.5 and 25 mmol/L HCO_3^-.

Table 1. Effects of Extracellular [HCO_3^-] at Constant pH on Glutamine Metabolism in Rat Mesenteric Lymph Node Lymphocytes

Medium [HCO_3^-] (mmol/L)	Products From [U-^{14}C]glutamine (nmol/h/10^6 cells)			
	$^{14}CO_2$	[^{14}C]glutamate	[^{14}C]aspartate	Ammonia
25	2.42 ± 0.18[a]	5.07 ± 0.36[a]	1.07 ± 0.15[a]	7.78 ± 0.64[a]
12.5	2.38 ± 0.17[a]	3.83 ± 0.32[b]	0.99 ± 0.08[a]	5.97 ± 0.47[b]
0	1.37 ± 0.09[b]	1.22 ± 0.06[c]	0.63 ± 0.03[b]	2.38 ± 0.22[c]

NOTE. Rat mesenteric lymphocytes (5 × 10^6/mL) were incubated at 37°C for 1 hour in the presence of 1 mmol/L [U-^{14}C]glutamine plus 5 mmol/L glucose. Incubation media containing 25, 12.5, and 0 mmol/L HCO_3^- were equilibrated with 95% O_2/5% CO_2, 97.5% O_2/2.5% CO_2, and 100% O_2, respectively, to obtain a constant pH of 7.4, with CO_2 at 1.25, 0.625, and 0 mmol/L, respectively. Data are the mean ± SEM (n = 8). Means sharing different superscripts within a column are significantly different ($P < .05$) as analyzed by 1-way ANOVA.

Table 2. HPLC Analysis of Glutamate and Aspartate (nmol/h/10^6 cells) in Cell Extracts Plus Incubation Media of Lymphocytes

Medium [HCO_3^-] (mmol/L)	No Glutamine		+1 mmol/L Glutamine	
	Glutamate	Aspartate	Glutamate	Aspartate
25	2.40 ± 0.05	1.37 ± 0.21	8.95 ± 0.21[a]	2.74 ± 0.13[a]
12.5	2.37 ± 0.07	1.26 ± 0.26	6.62 ± 0.15[b]	2.89 ± 0.14[a]
0	2.35 ± 0.08	1.18 ± 0.32	4.93 ± 0.14[c]	2.01 ± 0.12[b]

NOTE. Rat mesenteric lymphocytes (5 × 10^6/mL) were incubated at 37°C for 1 hour in the presence of 5 mmol/L glucose with or without 1 mmol/L glutamine. The medium contained 0 or 1 mmol/L glutamine. Incubation media containing 25, 12.5, and 0 mmol/L HCO_3^- were equilibrated with 95% O_2/5% CO_2, 97.5% O_2/2.5% CO_2, and 100% O_2, respectively, to obtain a constant pH of 7.4, with CO_2 at 1.25, 0.625, and 0 mmol/L, respectively. Data are the mean ± SEM (n = 8). Means sharing different superscripts within a column are significantly different ($P < .05$) as analyzed by 1-way ANOVA.

Effects of [HCO_3^-] on the Potential Production of ATP From Glutamine and Glucose

The effects of [HCO_3^-] on the potential production of ATP from glutamine and glucose in lymphocytes are shown in Table 7. Formation of aspartate from glutamine was the major pathway for production of glutamine-derived ATP in lymphocytes, as compared with oxidation of glutamine to CO_2 via the Krebs cycle, regardless of the presence or absence of added HCO_3^-. There was no difference in the contribution of glutamine to ATP production in lymphocytes incubated in the presence of 12.5 and 25 mmol/L

Table 3. Effects of Extracellular [HCO_3^-] at Constant pH on Glutamine Utilization and Cellular Glutamine Concentration (nmol/h/10^6 cells) in Rat Mesenteric Lymph Node Lymphocytes

Medium [HCO_3^-] (mmol/L)	Glutamine Utilization	Cellular Glutamine Concentration
25	7.53 ± 0.68[a]	3.19 ± 0.16[a]
12.5	6.25 ± 0.41[b]	3.34 ± 0.18[a]
0	2.37 ± 0.31[c]	4.16 ± 0.24[b]

NOTE. Rat mesenteric lymphocytes (40 × 10^6/mL) were incubated at 37°C for 1 hour in the presence of 1 mmol/L glutamine plus 5 mmol/L glucose. Incubation media containing 25, 12.5, and 0 mmol/L HCO_3^- were equilibrated with 95% O_2/5% CO_2, 97.5% O_2/2.5% CO_2, and 100% O_2, respectively, to obtain a constant pH of 7.4, with CO_2 at 1.25, 0.625, and 0 mmol/L, respectively. Data are the mean ± SEM (n = 8). Means sharing different superscripts within a column are significantly different ($P < .05$) as analyzed by 1-way ANOVA. Glutamine utilization by lymphocytes was determined from the disappearance of glutamine from the medium at the end of the 1-hour incubation period.

Table 4. Effects of Extracellular [HCO_3^-] at Constant pH on Glutamine Utilization and Metabolism in Rat Mesenteric Lymph Node Lymphocytes

Medium [HCO_3^-] (mmol/L)	Medium [Gln] (mmol/L)	Glutamine Utilization (nmol/h/10^6 cells)	Products From Glutamine (nmol/h/10^6 cells)			
			$^{14}CO_2$	Glutamate	Aspartate	Ammonia
25	0.5	4.36 ± 0.35	1.67 ± 0.14	3.43 ± 0.25	0.67 ± 0.05	4.78 ± 0.53
12.5	0.5	3.44 ± 0.27*	1.43 ± 0.20	2.67 ± 0.23*	0.64 ± 0.06	3.60 ± 0.44*
25	2.0	11.4 ± 0.15	4.91 ± 0.42	8.52 ± 0.69	1.66 ± 0.08	12.8 ± 1.15
12.5	2.0	9.25 ± 0.12*	4.58 ± 0.37	7.04 ± 0.57*	1.52 ± 0.12	9.86 ± 1.03*

NOTE. Rat mesenteric lymphocytes at concentrations of 12.5 and 25 × 10^6/mL were incubated at 37°C for 1 hour in the presence of 0.5 and 2 mmol/L [U-^{14}C]glutamine, respectively. The medium contained 5 mmol/L glucose. Incubation media containing 25 and 12.5 mmol/L HCO_3^- were equilibrated with 95% O_2/5% CO_2 and 97.5% O_2/2.5% CO_2, respectively, to obtain a constant pH of 7.4, with CO_2 at 1.25 and 0.625 mmol/L, respectively. Data are the mean ± SEM (n = 8). Rates of glutamine utilization and metabolism were higher ($P < .01$) in the presence of 2 mmol/L glutamine than 0.5 mmol/L glutamine at 25 or 12.5 mmol/L HCO_3^-, as analyzed by paired *t* test.

*$P < .01$: Significantly different from the value for 25 mmol/L HCO_3^- at 0.5 or 2 mmol/L glutamine, as analyzed by paired *t* test.

HCO_3^-, because of no alterations in the production of aspartate and CO_2 from glutamine. However, decreasing extracellular [HCO_3^-] from 12.5 to 0 mmol/L resulted in decreased ATP production from glutamine ($P < .05$). On the other hand, glycolysis was a more important pathway for the production of glucose-derived ATP than the oxidation of glucose via the Krebs cycle. Altering [HCO_3^-] from 25 to 0 mmol/L had no effect on the potential production of ATP from glucose. In the absence of HCO_3^-, glucose contributed more ATP than glutamine for lymphocytes, due to the decreased rate of glutamine metabolism in these cells.

DISCUSSION

HCO_3^-/CO_2 is one of the major buffer systems in the body.[21] It has also been recognized that HCO_3^- contributes to the regulation of acid/base balance both by directly regulating ammonia production from glutamine in the kidneys[22] and by modulating glutamine metabolism in hepatocytes and enterocytes[14] and therefore the provision of glutamine for renal ammoniagenesis. In kidney slices, decreasing extracellular [HCO_3^-] increases glutamine oxidation[22] and its metabolism to ammonia plus α-ketoglutarate via glutamate dehydrogenase.[14] In contrast, reducing extracellular [HCO_3^-] decreases production of glutamate and ammonia from glutamine via glutaminase in hepatocytes,[14] probably due to a direct effect of HCO_3^- on the activity of hepatocyte mitochondrial glutaminase.[23] This results in an increased release of glutamine from the liver to sustain an increased supply of glutamine to the kidneys during acidosis.[5] Similarly, HCO_3^- has been shown to decrease glutamine metabolism in enterocytes,[14] important glutamine-using cells,[8] thereby partially sparing glutamine for renal ammoniagenesis. This may explain the previous observation that utilization of glutamine by the gut decreases during metabolic acidosis.[5] Thus, regulation of mammalian glutamine metabolism may be signaled by an alteration in extracellular [HCO_3^-]. These findings demonstrate exquisite interorgan cooperations in the regulation of acid/base balance, which is vital to the survival of organisms.[21]

A novel finding from this study is that reducing extracellular [HCO_3^-] from 25 to 12.5 mmol/L at a constant pH of 7.4 (Table 1) or from 25 to 15.7 mmol/L at a constant CO_2 level of 1.25 mmol/L (Table 5) decreases glutamine utilization and metabolism. The decreased production of [^{14}C]glutamate and [^{14}C]aspartate from [U-^{14}C]glutamine in the absence of HCO_3^- was not due to possible label dilution, since analysis of amino acids by HPLC also showed decreased formation of glutamate and aspartate in lymphocytes incubated in the presence of 1 mmol/L glutamine (Table 2). To the best of our knowledge, this study demonstrates for the first time that extracellular [HCO_3^-] regulates glutamine metabolism in lymphocytes. Our results are consistent with previous studies on the effect of extracellular [HCO_3^-] on glutamine metabolism in hepatocytes and enterocytes,[14] and further suggest an important role for HCO_3^- in pH homeostasis as both an important buffering component and as a key regulator of glutamine metabolism in renal and extrarenal tissues. Note that decreasing [HCO_3^-] from 25 to 12.5 mmol/L decreased the production of ammonia and glutamate from glutamine, but had no effect on that of aspartate and CO_2 in lymphocytes. This suggests that the major site of the HCO_3^- effect on glutamine metabolism in lymphocytes is at the step of mitochondrial glutaminase, as previously reported for rat hepatocytes.[14,23]

Table 5. Effects of Extracellular [HCO_3^-] at Constant CO_2 Level on Glutamine Utilization and Metabolism and Cellular Glutamine Concentrations in Rat Mesenteric Lymph Node Lymphocytes

Medium [HCO_3^-] (mmol/L)	Glutamine Utilization (nmol/h/10^6 cells)	Products From Glutamine (nmol/h/10^6 cells)				Cellular Glutamine (nmol/10^6 cells)
		$^{14}CO_2$	Glutamate	Aspartate	Ammonia	
25	7.24 ± 0.58	2.53 ± 0.27	5.37 ± 0.49	1.27 ± 0.11	7.56 ± 0.66	3.28 ± 0.18
15.7	6.03 ± 0.54*	2.29 ± 0.24	4.49 ± 0.35*	1.14 ± 0.22	6.22 ± 0.51*	3.50 ± 0.22

NOTE. Rat mesenteric lymphocytes (25 × 10^6/mL) were incubated at 37°C for 1 hour in the presence of 1 mmol/L [U-^{14}C]glutamine plus 5 mmol/L glucose. Incubation media containing 25 and 15.7 mmol/L HCO_3^- were equilibrated with 95% O_2/5% CO_2 to obtain a constant CO_2 level of 1.25 mmol/L, with pH at 7.4 and 7.2, respectively. Data are the mean ± SEM (n = 8).

*$P < .01$: Significantly different from the value for 25 mmol/L HCO_3^- within each glutamine concentration, as analyzed by paired *t* test.

Table 6. Effects of Extracellular [HCO_3^-] on Glucose Metabolism in Rat Mesenteric Lymph Node Lymphocytes

Medium [HCO_3^-] (mmol/L)	Products From [U-^{14}C]glucose (nmol/h/10^6 cells)			Lactate to Pyruvate Ratio
	$^{14}CO_2$	L-Lactate	Pyruvate	
25	1.11 ± 0.08	9.43 ± 0.39	1.65 ± 0.14[a]	5.73 ± 0.14[a]
12.5	1.18 ± 0.09	9.03 ± 0.43	1.47 ± 0.15[a]	6.59 ± 0.69[a]
0	1.09 ± 0.09	10.1 ± 0.47	1.01 ± 0.10[b]	10.8 ± 1.20[b]

NOTE. Rat mesenteric lymphocytes (5 × 10^6/mL) were incubated at 37°C for 1 hour in the presence of 5 mmol/L [U-^{14}C]glucose plus 1 mmol/L glutamine. Incubation media containing 25, 12.5, and 0 mmol/L HCO_3^- were equilibrated with 95% O_2/5% CO_2, 97.5% O_2/2.5% CO_2, and 100% O_2, respectively, to obtain a constant pH of 7.4, with CO level at 1.25, 0.625, and 0 mmol/L, respectively. Data are the mean ± SEM (n = 8). Means sharing different superscripts within a column are significantly different ($P < .05$) as analyzed by 1-way ANOVA.

Interestingly, altering extracellular [HCO_3^-] from 25 to 0 mmol/L at a constant pH of 7.4 had no significant effect on glucose utilization by lymphocytes on the basis of formation of lactate, a major product of glucose in lymphocytes,[3,6,7] and of CO_2 from glucose (Table 6). It is noteworthy that decreasing extracellular [HCO_3^-] from 12.5 to 0 mmol/L decreased pyruvate production, thereby resulting in an increased lactate to pyruvate ratio in lymphocytes (Table 6). This suggests that HCO_3^- affects the cellular redox state in lymphocytes, as recently reported for hepatocytes.[15] It is possible that HCO_3^- affects the transfer of reducing equivalents from the cytosol into the mitochondria, but has no effect on activities of enzymes involved in glycolysis. Since pyruvate is only a minor product of glucose in lymphocytes,[3,6,7] the overall rate of glucose utilization by these cells was not significantly affected by HCO_3^-. Thus, because the rate of production of lactate and CO_2 from glucose was not altered in the presence or absence of extracellular HCO_3^- (Table 6), glucose was a more important fuel than glutamine for lymphocytes in the presence of low extracellular [HCO_3^-] or its absence (Table 7). This may help to explain the previous observation that glucose was a more important fuel than glutamine for splenic lymphocytes incubated in the presence of low (5 mmol/L) [HCO_3^-].[24] However, in the presence of 12.5 and 25 mmol/L HCO_3^-, glutamine was quantitatively as important an energy substrate for lymphocytes as glucose (Table 7).

Although the mechanisms underlying the effects of [HCO_3^-] on glutamine metabolism and pyruvate production from glucose are not known, our data suggest that not all aspects of cellular metabolism are influenced by HCO_3^-. Our results may have relevance to a better understanding of the regulation of acid/base balance in vivo. There are large amounts of lymphocytes in the body, which weigh approximately 1.5 kg in an adult man,[9] equivalent to the liver mass. Also, the activity and number of lymphocytes are increased in disease states such as infection and lymphomas. Thus, since lymphocytes actively metabolize glutamine[3,6,7] and have a large mass,[9] these cells have been suggested to play an important role in glutamine utilization in the body.[19] In metabolic acidosis associated with reduced extracellular [HCO_3^-], extraction of glutamine by the kidneys is markedly increased to support the enhanced ammoniagenesis.[5] Under such an acidotic condition, although glutamine supplies from skeletal muscle and liver increase,[5] a decrease in glutamine utilization by lymphocytes may help to spare glutamine for renal ammoniagenesis. This suggests a hitherto unrecognized role for the regulation of lymphocyte glutamine metabolism by HCO_3^- in pH homeostasis in the body. Because blood [HCO_3^-] often decreases in metabolic acidosis, as observed in diabetic ketoacidosis from 25 to less than 5 mmol/L,[10] our present findings with lymphocytes incubated in the presence of 0 to 25 mmol/L HCO_3^- may have physiological and clinical implications.

In conclusion, this study demonstrates for the first time that reducing extracellular [HCO_3^-] from 25 to 12.5 mmol/L at a constant pH of 7.4 or from 25 to 15.7 mmol/L at a constant CO_2 level of 1.25 mmol/L decreases glutamine utilization and metabolism in lymphocytes. Decreased glutamine utilization by lymphocytes may help to spare glutamine for renal ammoniagenesis in metabolic acidosis characterized by decreased extracellular [HCO_3^-]. We suggest that HCO_3^- contributes to the regulation of acid/base balance in the body by serving as an important buffer component and as a regulator of glutamine metabolism in lymphocytes, in addition to its effects on the kidneys, hepatocytes, and enterocytes.

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Table 7. Effects of Extracellular [HCO_3^-] on the Potential Production of ATP From Glutamine and Glucose in Rat Mesenteric Lymph Node Lymphocytes

Medium [HCO_3^-] (mmol/L)	Potential ATP Production From Glutamine or Glucose					
	Glutamine to CO_2	Glutamine to Aspartate	Total From Glutamine	Glucose to CO_2	Glucose to Lactate	Total From Glucose
25	6.50 ± 0.93[a]	9.63 ± 1.35[a]	16.1 ± 1.15[a]	7.03 ± 0.51	9.43 ± 0.39	16.5 ± 0.55
12.5	6.70 ± 0.72[a]	8.91 ± 0.72[a]	15.6 ± 1.06[a]	7.47 ± 0.57	9.03 ± 0.43	16.4 ± 0.47
0	3.58 ± 0.34[b]	5.67 ± 0.27[b]	9.23 ± 0.52[b]	6.90 ± 0.57	10.1 ± 0.47	16.9 ± 0.52*

NOTE. Rat mesenteric lymphocytes (5 × 10^6/mL) were incubated at 37°C for 1 hour in the presence of 1 mmol/L [U-^{14}C]glucose plus 5 mmol/L glucose or 5 mmol/L [U-^{14}C]glucose plus 1 mmol/L glucose. Data are the mean ± SEM (n = 8). Means sharing different superscripts within a column are significantly different ($P < .05$) as analyzed by 1-way ANOVA.

*$P < .01$: Significantly different from the value for total ATP production from glutamine as analyzed by paired t test.

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