Effect of nasogastric administration of sodium bicarbonate on carbon 13 isotopic enrichment of carbon dioxide in serum of horses

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Objective—To determine the effect of administration of commercially available sodium bicarbonate ($NaHCO_3$) on carbon 13 (^{13}C) isotopic enrichment of carbon dioxide (CO_2) in serum of horses.

Animals—7 healthy Thoroughbreds.

Procedures—Sodium bicarbonate (450 g) was administered via nasogastric intubation to horses. Horses had been fed a diet obtained from the same source and had access to water from the same source for 3 months before the study. Blood samples were collected immediately before and at 2, 4, 6, and 24 hours after administration of $NaHCO_3$. The concentration of total CO_2 in serum was measured by use of a commercial analyzer. The ^{13}C enrichment of bicarbonate in serum was estimated by measurement of ^{13}C enrichment of CO_2 released by acidification of the serum. The ^{13}C enrichment of commercially available $NaHCO_3$ was also determined and compared with that of CO_2 in serum of horses before administration of $NaHCO_3$.

Results—Commercially available $NaHCO_3$ had a ^{13}C enrichment significantly different from that of carbon dioxide in serum of horses before treatment. Administration of $NaHCO_3$ increased the concentration of total CO_2 from pretreatment values. The ^{13}C enrichment of CO_2 in serum was only transiently and minimally affected after administration of $NaHCO_3$.

Conclusions and Clinical Relevance—Administration of $NaHCO_3$ was not detected by measuring ^{13}C enrichment of CO_2 in serum of horses. (*Am J Vet Res* 2004;65:307–310)

Administration of **sodium bicarbonate ($NaHCO_3$)** to Standardbred or Thoroughbred racehorses is prohibited by rules of racing in many jurisdictions.[1] Regulatory authorities have therefore sought to develop methods of detecting administration of $NaHCO_3$ to horses.[1] Presently, administration of $NaHCO_3$ is detected by measurement of **total carbon dioxide (TCO_2)** or bicarbonate concentrations in serum, base excess, or blood pH.[1] Use of these variables to detect $NaHCO_3$ administration is problematic because bicarbonate is normally present in serum, the variable concentration of bicarbonate in horses being prepared for racing renders interpretation of high values controversial, and because of methodological concerns regarding laboratory measurement of high TCO_2 concentrations in serum. Presently used methods for detecting administration of $NaHCO_3$ rely on detection of the alkalinizing effect of the compound rather than the presence of exogenous bicarbonate. A useful means of detecting administration of $NaHCO_3$ in horses would be one in which the presence of exogenous bicarbonate could be detected.

Carbon exists in 3 isotopic forms: **carbon 12 (^{12}C), 13 (^{13}C)**, and 14. The common isotope is ^{12}C, with ^{13}C, a nonradioactive isotope of carbon, constituting approximately 1.1% of naturally occurring carbon.[2] This abundance of ^{13}C varies between 1.06% and 1.12% reflecting the enrichment or depletion of ^{13}C relative to ^{12}C.[3] The ^{13}C enrichment of CO_2 derived from plant-based foods differs from that of carbonates with plant- derived foods having a lower enrichment.[3] The ^{13}C enrichment of an individual is strongly influenced by the ^{13}C enrichment of the diet, consequently ^{13}C enrichment of CO_2 from humans is markedly lower than that of naturally occurring carbonates.[3] Moreover, ingestion of substrates with ^{13}C enrichment markedly different to that of the individual results in rapid and detectable changes in ^{13}C enrichment of CO_2 from that individual.[3]

Therefore, we hypothesized that ^{13}C enrichment of commercially available $NaHCO_3$ would differ from that of bicarbonate in serum of horses. Furthermore, we speculated that if the ^{13}C enrichment of commercially available $NaHCO_3$ differed from that in serum of horses, then administration of commercially available $NaHCO_3$ would be detectable as a change in ^{13}C enrichment of CO_2 in serum of horses. The purpose of the study reported here was to determine the effect of administration of commercially available $NaHCO_3$ on ^{13}C enrichment of CO_2 in serum of horses.

Materials and Methods

Animals—Seven healthy Thoroughbreds (3 female and 4 geldings) from 2 to 7 years old and weighing from 426 to 541 kg were used in the study. Horses were maintained in a research herd used for exercise physiology studies and were conditioned by repeated (4 times weekly) exercise. Horses had been housed at the research facility, fed hay and corn obtained from the same source, and drank water from the same source for a minimum of 3 months before study initiation. Horses were not exercised for 2 days before or during the 24-hour study.

Experimental design—The experiment was a single longitudinal study involving repeated collection of blood samples for measurement of ^{13}C enrichment of serum-derived CO_2 in horses before and after intragastric administration of commercially available $NaHCO_3$. Horses were housed in box stalls, which was their usual housing, without access to feed for 14 hours before administration of $NaHCO_3$. Feed was withheld for an additional 6 hours after

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$NaHCO_3$ administration. Water was available at all times. Sodium bicarbonate[a] (450 g) dissolved in 3 L of water was administered to each horse via nasogastric intubation between 9 and 10 AM. Blood was collected from a jugular vein into evacuated serum separator tubes[b] approximately 5 minutes before (pretreatment) and 2, 4, 6, and 24 hours after administration of $NaHCO_3$. Serum was separated by centrifugation. Samples were stored at 4°C after clot formation.

Analysis of samples—All samples were handled anaerobically. Concentrations of TCO_2 in serum were measured within 24 hours of sample collection by use of an automated analyzer using appropriate calibration solutions.[c,d] The ^{13}C enrichment of CO_2 released by acidification of serum and of samples of the commercially available bicarbonate was determined by use of isotope ratio mass spectrometry performed by a commercial laboratory by means of a described method.[4,d] Enrichment of CO_2 in serum was compared with that of Pee Dee Belemite limestone, which is the conventional standard for such analyses, and expressed as δ (per mil). The units are thus expressed as differences (δ) from Pee Dee Limestone. The analytical precision of the assay was 0.1 mil.

Statistical analyses—Data were analyzed by a 1-way, repeated-measures ANOVA. Posthoc testing was performed by use of the Dunnett test. The ^{13}C enrichment of CO_2 from 7 samples of the commercially available $NaHCO_3$ was compared with that in CO_2 in serum of horses before administration of $NaHCO_3$ by use of a Student *t* test for unpaired samples. Examination of the association between TCO_2 concentration and ^{13}C enrichment was performed by use of linear regression.

Results

Administration of $NaHCO_3$ increased ($P < 0.05$) the concentration of TCO_2 from pretreatment values (mean ± SE, 28.0 ± 0.8 mmol/L to 36.6 ± 0.5 at 6 hours; **Fig 1**). There was a minimal and variable effect of $NaHCO_3$ administration on ^{13}C enrichment of CO_2 in serum of horses after treatment (**Fig 2**). . Mean ± SE ^{13}C values were –21.00 ± 0.05 (δ ^{13}C), –20.97 ± 0.14, –21.15 ± 0.36, –21.45 ± 0.07, and –21.50 ± 0.07 at pretreatment and 2, 4, 6, and 24 hours after treatment, respectively. Enrichment of serum-derived CO_2 6 and 24 hours after administration of $NaHCO_3$ was significantly less than values obtained before administration of $NaHCO_3$. There was no correlation between ^{13}C enrichment of CO_2 and the concentration of TCO_2 in serum of horses after $NaHCO_3$ administration (**Fig 3**). Commercially available $NaHCO_3$ had a ^{13}C enrichment significantly ($P < 0.01$) different from that of CO_2 in serum of horses

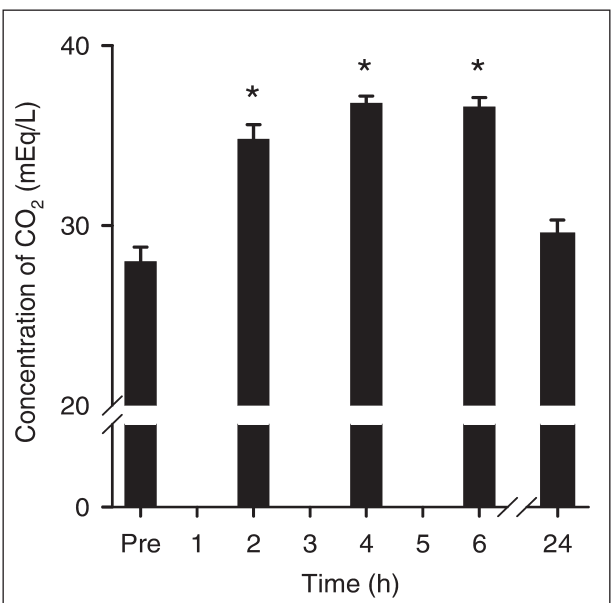

Figure 1—Mean ± SE concentrations of total carbon dioxide (TCO_2) in serum after administration of commercially available sodium bicarbonate ($NaHCO_3$; 450 g) via nasogastric intubation in 7 horses. *Significantly ($P < 0.05$) different from values obtained before administration of $NaHCO_3$ (Pre).

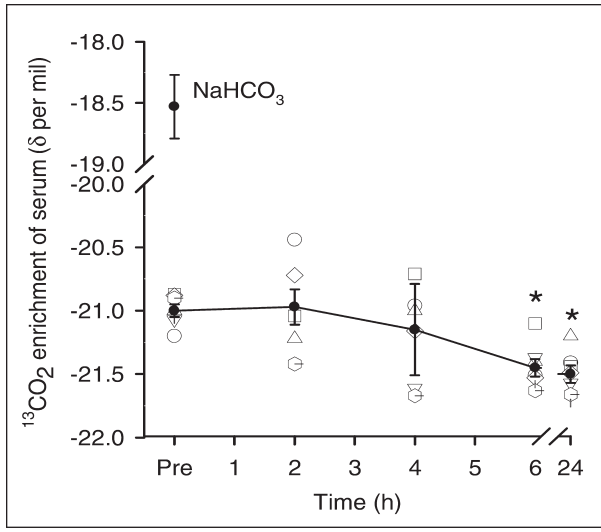

Figure 2—Mean ± SE (closed circles) values for carbon 13 (^{13}C) enrichment of CO_2 in serum after administration of commercially available $NaHCO_3$ (450 g) via nasogastric intubation in 7 horses. Enrichment of CO_2 is depicted as the difference (δ, delta) in parts per thousand of the sample from Pee Dee Belemite limestone standard. Responses of individual horses are represented as open symbols. *Significantly ($P < 0.05$) different from values obtained before administration of $NaHCO_3$ (Pre).

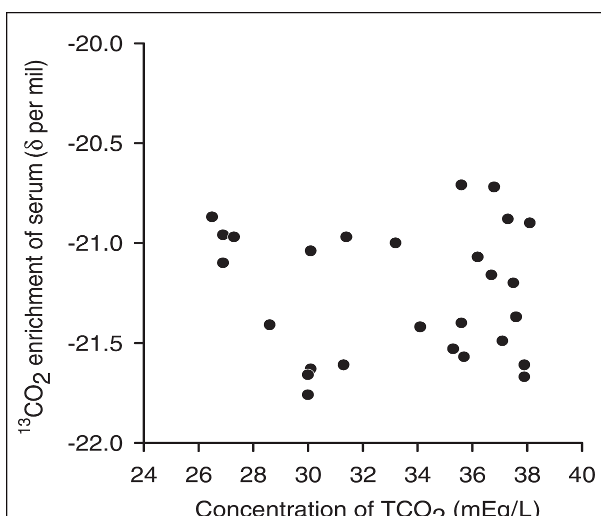

Figure 3—Scatterplot of ^{13}C enrichment of CO_2 and the concentration of TCO_2 in serum after administration of commercially available $NaHCO_3$ (450 g) via nasogastric intubation in 7 horses. There was no significant association between the variables ($r = 0.02$).

before treatment (mean ± SE, –18.53 ± 0.26 and –21.00 ± 0.05 δ ^{13}C, respectively; Fig 2).

Discussion

Administration of $NaHCO_3$ to horses caused small changes in ^{13}C enrichment of CO_2 in serum. However, the change in ^{13}C enrichment of CO_2 in serum of horses was directionally opposite to that anticipated on the basis of the enrichment of $NaHCO_3$ that was administered. The commercially available $NaHCO_3$ that was administered had a significantly different enrichment than that of CO_2 from serum of horses before treatment. Furthermore, the increased concentration of TCO_2 in serum of horses administered $NaHCO_3$ was not associated with a proportional change in the ^{13}C enrichment of CO_2 in serum. Although additional studies are indicated, results of the study reported here do not support the measurement of ^{13}C enrichment of CO_2 in serum as a means of detecting or confirming administration of $NaHCO_3$ to horses.

The changes in ^{13}C enrichment of CO_2 in serum observed in this study were small, compared with changes detected in humans after ingestion of meals containing substrates having ^{13}C enrichment markedly different to that of the individual.[3] Changes in enrichment detected in horses in our study were less than 0.5 mils which is similar to changes in ^{13}C enrichment of CO_2 in humans fed their customary meal.[3] Changes in ^{13}C enrichment in humans is minimized by withholding food for 8 hours before and during the test period and by rest.[3] In our study, feed was withheld from horses for 14 hours before and 6 hours after administration of $NaHCO_3$. Furthermore, because exercise may alter ^{13}C enrichment of serum-derived CO_2, the horses had been confined to box stalls and not exercised for 2 days before study initiation and horses were not exercised during the study. The horses had consumed hay, corn, and water from the same sources for 3 months before study initiation, thereby preventing the effect of sudden changes in diet on ^{13}C enrichment. Thus, the small changes in ^{13}C acute changes in enrichment observed at 6 and 24 hours in horses in this study were likely caused by changes in substrate oxidation induced by withholding feed and subsequent feeding. However, it must be emphasized that the magnitude of the changes is small and opposite in direction to that expected to be induced by administration of the bicarbonate.

Ingestion of a substrate with a ^{13}C enrichment markedly different to the enrichment of the individual before ingestion causes an increase or decrease in the ^{13}C enrichment of the individual, depending on the difference in enrichment of the substrate and the individual. For example, ingestion of a substance with a ^{13}C enrichment markedly greater than that of the individual causes an increase in the enrichment of the individual.[3] Therefore, administration of $NaHCO_3$ with ^{13}C enrichment greater (ie, less negative) than that of the horse could be expected to increase the ^{13}C enrichment of the horse. This effect was not observed in our study.

The inability to detect administration of $NaHCO_3$ to horses in the study reported here may have been a consequence of the rapid turnover of bicarbonate in horses. The dose of $NaHCO_3$ administered to horses provided approximately 5,400 mEq of bicarbonate (450 g of $NaHCO_3 \times 12$ mEq/g). The bicarbonate content of a 450-kg horse is approximately 4,500 mEq, assuming a distribution space of 0.33 L of bicarbonate/kg. Assuming that all of the administered bicarbonate equilibrated instantly with endogenous bicarbonate, the final ^{13}C enrichment of CO_2 in serum would have been –19.6 δ. This difference was not detected in the present study.

The assumption that all the bicarbonate that was administered was absorbed and completely equilibrated with endogenous bicarbonate instantaneously is unrealistic. Absorption of bicarbonate requires minutes to hours, as indicated by the time required to reach peak TCO_2 concentrations in this and other studies.[5,6] Therefore, absorption of bicarbonate from the intestinal tract would have occurred at the same time as elimination of bicarbonate from the respiratory tract, and the combined effect of these processes would likely have further reduced the change in ^{13}C enrichment of CO_2 in serum of these horses.

Bicarbonate is the quantitatively most important means of CO_2 transportation in mammals, including horses. Carbon dioxide produced by aerobic respiration in tissues diffuses into the plasma. A small proportion (< 10%) is transported as dissolved CO_2, whereas the majority diffuses into red blood cells where it is hydrated in a reaction catalyzed by carbonic anhydrase to form bicarbonate via carbonic acid.[7] The bicarbonate produced in this reaction is exchanged across the red cell membrane for chloride, thereby entering the plasma. The reaction is reversed in the lungs, and CO_2 is exhaled.

A 450-kg horse at rest produces approximately 140 mmol of CO_2/min or 8,400 mmol of (mEq) CO_2/h, of which approximately 6,000 mmol of CO_2/h is transported as bicarbonate.[7] Thus, the total bicarbonate flux in 1 hour is greater than the amount of bicarbonate (5,400 mEq) that was administered to horses in our study. Because endogenous bicarbonate has substantially different ^{13}C enrichment from that of exogenous bicarbonate, the large flux of endogenous bicarbonate would have reduced the effect of exogenous bicarbonate on ^{13}C enrichment of CO_2 in serum to the extent that it was not detectable in the present study. Given the small amount of bicarbonate that was administered, compared with the total bicarbonate flux during the 6 hours of the study, the administration of exogenous bicarbonate was not expected to detectably alter ^{13}C enrichment of CO_2 in serum of these horses.

The small change in ^{13}C enrichment of CO_2 in serum of horses detected 6 and 24 hours after administration of $NaHCO_3$ may have been attributable to the effect of withholding feed. That the changes in ^{13}C enrichment of CO_2 in serum were not detected when increases in the concentration of TCO_2 were greatest and opposite to that expected to be induced by administration of $NaHCO_3$ indicated that the observed changes in ^{13}C enrichment of CO_2 in serum were not directly attributable to $NaHCO_3$ administration.

Inability to detect administered bicarbonate as a change in the ^{13}C enrichment of CO_2 in serum is consistent with the prolonged alkalinizing effect of the bicarbonate observed in the study reported here. Although the amount of bicarbonate administered in 450 g of $NaHCO_3$ is small, compared with the bicarbonate flux, the amount of sodium is substantial. There is approximately 5,400 mEq of sodium in 450 g of $NaHCO_3$, and the sodium content of extracellular fluid of a 450-kg horse is approximately 21,000 mEq. Therefore, administration of 450 g of $NaHCO_3$ has the potential to increase total body sodium content by as much as 25%. Although absorption of sodium occurs concurrent with urinary excretion of sodium, administration of $NaHCO_3$ causes substantial and physiologically important increases in the concentration of sodium in serum.[5,6,8] The increase in the concentration of sodium in serum, without an increase in the concentration of chloride in serum, causes an increase in the strong ion difference (the difference between the serum concentrations of strong anions and strong cations).[6] Increases in the strong ion difference induce alkalosis with consequent increases in blood pH and base excess. Thus, the alkalinizing effect of $NaHCO_3$ administration occurs because of the large amount of sodium administered and not the small amount of bicarbonate.

[a]Sodium bicarbonate, Arm & Hammer, Princeton, NJ.

[b]Vacutainer, Becton, Dickinson & Co, Parsippany, NJ.

[c]Beckman synchron EL-ISE, Beckman Instruments, Brea, Calif.

[d]NERL four component standard, Casco-NERL Diagnostics, East Providence, RI.

[e]Metabolic solutions, Nashua, NH.

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