

Spontaneous alteration of blood pH by a bicarbonate buffer system during experimental hypercalcaemia in cows

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Abstract

Introduction: Maintaining mineral homeostasis as well as the secretion and metabolism of mineralotropic hormones is important for healthy of periparturient dairy cows. To increase the activity of mineralotropic hormones, blood pH can be adjusted. The purpose of this study was to investigate changes in blood pH and the mechanism of action of this change in induced hypercalcaemic cows. **Material and Methods:** Six non-lactating Holstein cows were used in a 2 × 2 crossover design. To induce hypercalcaemia, calcium borogluconate was administered subcutaneously to experimental cows and normal saline was administered subcutaneously to control cows. Blood and urine samples were collected serially after administration. Whole blood without any anticoagulant was processed with a portable blood gas analyser. Plasma concentration and urinary excretion of calcium were measured. **Results:** In hypercalcaemic cows, both blood and urine calcium levels were significantly increased at 8 h compared to those at 0 h ($P < 0.05$), and a spontaneous increase in blood pH was also observed. The calcium concentration in plasma was highest at 2 h after administration (3.02 ± 0.27 mmol/L). The change in pH correlated with that in bicarbonate ($r = 0.781$, $P < 0.001$) rather than that in partial pressure of CO₂ ($r = 0.085$, $P = 0.424$). **Conclusion:** Hypercalcaemia induced a spontaneous change in blood pH through the bicarbonate buffer system and this system may be a maintainer of calcium homeostasis.

Keywords: calcium, blood pH, cattle, hypercalcaemia, bicarbonate buffer system.

Introduction

In dairy cows, disturbance of calcium (Ca) homeostasis causes productivity loss and production diseases (2, 7, 14). In the body, endocrine factors are mainly responsible for maintaining the balance of Ca, and their target organs include the intestines, the bones and kidneys (2, 8). However, to facilitate homeostatic processes, the entire environment of the body may be adjusted, including blood pH and the respiratory system (1, 4, 9, 10).

Many endocrine factors are known to control mineral levels, and the most well-known is the parathyroid hormone (PTH), the main function of which is to maintain optimal Ca levels in blood and to regulate Ca uptake or excretion by the bones and kidneys (1, 7, 8, 13, 14). Parathyroid hormone activity is optimal when

blood pH is mildly acidic (1, 9, 10). Its antagonist calcitonin is secreted from the thyroid gland in a hypercalcaemic state and inhibits reabsorption of Ca and inorganic phosphate (iP) in the kidneys, thereby increasing the amount of Ca and iP excretion *via* urine (3, 5). Calcitonin also suppresses the action of osteoclasts releasing Ca from the bones, thereby reducing the Ca concentration in blood (3, 5). In addition, according to a previous study, the activity of calcitonin is known to decrease as blood pH decreases (11).

Our previous study showed a spontaneous decrease in plasma pH in cows that had hypocalcaemia; such a drop in pH is considered to maximise the effect of PTH (3). Although the mechanism of pH change was not explained as the pH was measured using plasma, our study showed that there is a mechanism which regulates the body environment so as to maximise the

effectiveness of endocrine factors. Therefore, the purpose of this study was to determine whether a spontaneous change in blood pH in dairy cows is induced by hypercalcaemia and what mechanism underlies such a change.

Material and Methods

Experimental design. The study was conducted on six non-pregnant, non-lactating Holstein Friesian cows with parities of 1.33 ± 0.82 (mean \pm SD), body weight of 710 ± 39 kg and body condition score of 3.33 ± 0.12 . Hay and concentrated feed were provided twice a day, and the cows were kept in a space with free access to drinking water and mineral blocks (Daehan New Pharm Co., Hwaseong, South Korea). The experimental animals were all kept under the same conditions, and no animals were moved nor other treatments administered throughout the experimental period. Three cows were subcutaneously injected with calcium; magnesium; dextrose (C.M.D., pH 3.56; Samyang Anipharm Co., Seoul, South Korea) containing 300 mg of calcium borogluconate, 30 mg of magnesium chloride, and 50 mg of dextrose per 1 mL and these were the induced hypercalcaemic group (HYC). The remaining three cows were injected with 0.9% normal saline (pH 5.5; Handong Co., Seoul, South Korea) and their purpose was to be the control group (CON). In both groups, 1 L of solution was subcutaneously administered per cow on both sides of the cervical region (500 mL into each region) over 5 min. Ten days later, this protocol was repeated, changing the groups. This study was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-190522-3).

Sample collection. Blood and urine samples were collected at 0, 1, 2, 4, 8, 12, 18, 24, and 48 h after treatment. Blood samples were collected with a syringe connected to a 14G \times 5.25 EQUIVET HiFlow Long-Term IV catheter (Kruuse, Langeskov, Denmark) previously installed. Blood for plasma chemistry analysis was aliquoted into a BD Vacutainer Lithium Heparin tube (Becton Dickinson, Franklin Lakes, NJ, USA), and the remaining whole blood in the syringe was used for blood gas analysis immediately after sampling. Urine samples were collected at every sampling time point through a BARDEX Urethral Catheter, (Medline, Northfield, IL, USA) which was introduced into the urethra and from there into the urinary bladder. Blood and urine samples were centrifuged at $2,000 \times g$ for 15 min to collect the plasma and harvest the clear supernatant, respectively, and then stored at -70°C until analysis. Frozen plasma and urine samples were thawed at room temperature prior to each test.

Laboratory analyses. Immediately after blood sampling, whole blood without any anticoagulant was used for blood gas analysis with an i-STAT portable blood gas analyser (Abbott, Abbott Park, IL, USA), and

the parameters including pH, ionised Ca (iCa), bicarbonate (HCO_3^-) and partial pressure of carbon dioxide (pCO_2) were analysed. For measurement of minerals in thawed plasma and urine samples, a BS-400 auto-analyser (Mindray, Shenzhen, China) was used. All samples were tested on one day under the same conditions. The plasma parameters and urine mineral analyses included determination of Ca, magnesium (Mg) and creatinine (crea) levels. To estimate the amounts of macrominerals in urine, Ca and Mg levels were calculated along with their ratios to crea.

Statistical analyses. All results from the study were analysed using SigmaPlot 12.5 (Systat Software Inc., Chicago, IL, USA). One-way repeated-measures ANOVA followed by multiple comparisons with the Holm-Šidák method was used to identify significant differences within the group. Student's *t*-test was performed to compare the differences between the groups, and the Pearson method was used to confirm the correlations between the parameters.

Results

In the HYC group, the plasma Ca levels were significantly higher than their 0 h values until up to 8 h afterwards ($P < 0.05$). They were also significantly higher than those seen in the CON group from 1 to 8 h ($P < 0.05$, Fig. 1A). Furthermore, the HYC group had significantly different Ca/crea ratios from those of the CON group from 1 h to 12 h ($P < 0.05$), and they were higher from 1 h to 8 h after administration than at 0 h ($P < 0.05$, Fig. 1B). Mg levels were significantly higher in the HYC group from 1 h to 4 h after administration ($P < 0.001$, Fig. 1C), this group's Mg/crea ratios were significantly different from 1 h to 4 h from those of the CON group, and they were significantly higher at 2 h and 4 h than at 0 h ($P < 0.05$, Fig. 1D).

Blood pH in the HYC group showed a significant difference at 1 h and 2 h after administration from its 0 h value ($P < 0.05$), and significant differences between the two experimental groups were confirmed at 1 h and 4 h after administration ($P < 0.05$, Fig. 1E). HCO_3^- also differed significantly between the groups at 1 h and 2 h after administration ($P < 0.05$, Fig. 1F), as did pCO_2 at 2 h ($P < 0.05$, Fig. 1G).

In the correlation analysis, pH was significantly correlated with HCO_3^- ($r = 0.781$, $P < 0.001$); however, pCO_2 was only weakly correlated with pH ($r = 0.085$, Table 1). In particular, the correlation between pH and HCO_3^- was higher in the HYC group ($r = 0.820$, $P < 0.001$, Table 2). Levels of iCa in blood gas analysis were highly associated with the Ca concentration in the blood ($r = 0.917$, $P < 0.001$; Fig. 2H; Table 1), and the latter was highly correlated with Ca/crea and pH in all samples ($r = 0.825$ and 0.466 , respectively; $P < 0.001$; Table 1), especially in the HYC group ($r = 0.846$ and 0.688 , respectively; Table 2).

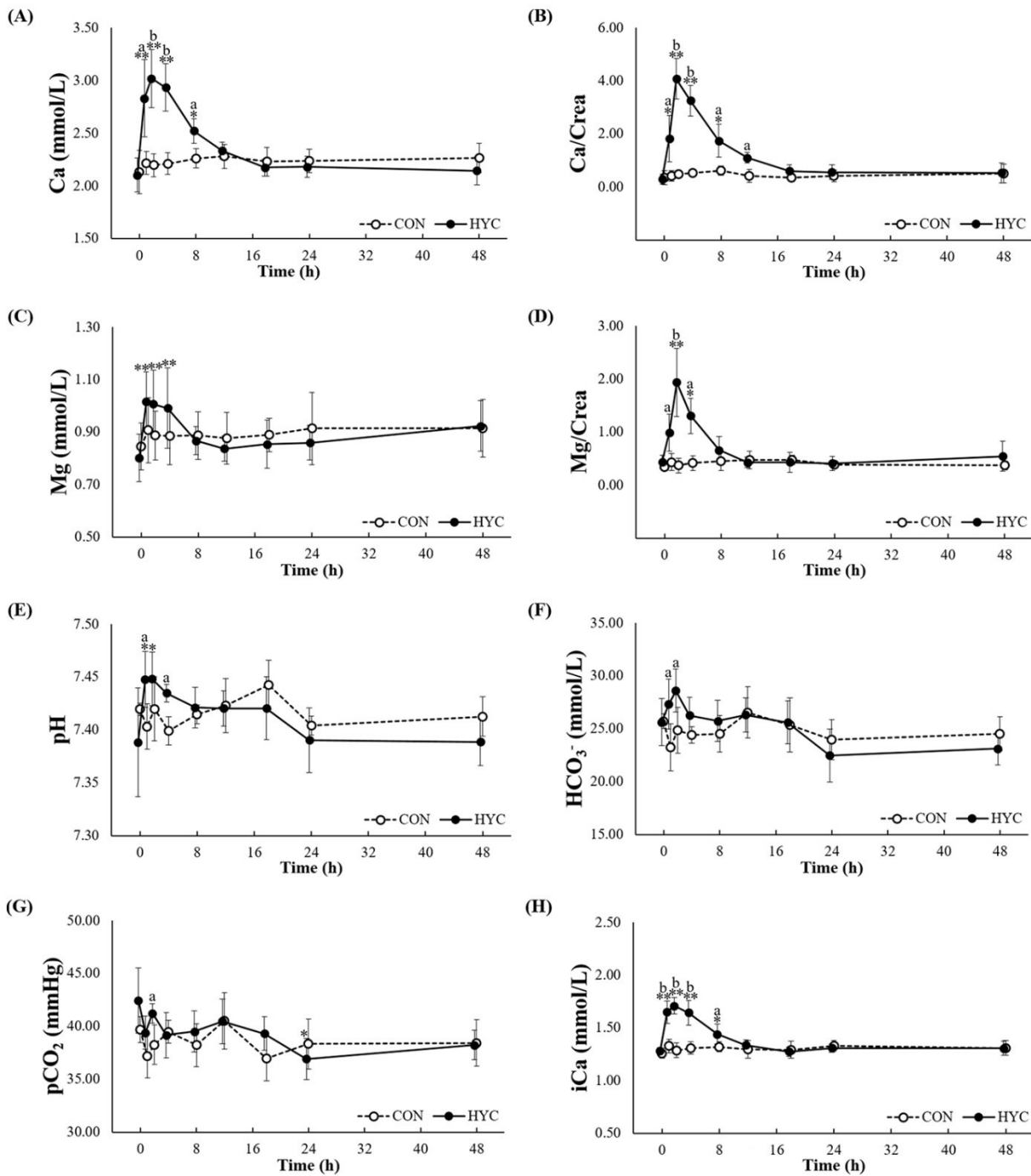


Fig 1. Changes in plasma mineral concentrations, urinary mineral excretions, and blood gas parameters in the control group (CON) and induced hypercalcaemic group (HYC)

A – Ca concentration in plasma; B – Ca excretion through urine (Ca/creatinine (crea)); C – Mg concentration in plasma; D – Mg excretion through urine (Mg/crea); E – blood pH; F – bicarbonate (HCO₃⁻); G – partial pressure of carbon dioxide (pCO₂); H – ionised Ca. Data are expressed as means ± standard deviation. * – significant difference compared to 0h (P < 0.05); ** – significant difference compared to 0 h (P < 0.001); ^a – significant difference compared to CON (P < 0.05); ^b – significant difference compared to CON (P < 0.001)

Table 1. Results of Pearson correlation analysis of parameters in blood analyses in all individuals

| | | Test (n) | Mg | Ca/crea | Mg/crea | iCa | pH | HCO ₃ ⁻ | pCO ₂ |
|-------------------------------|----------------|----------|------------|------------|------------|------------|------------|-------------------------------|------------------|
| Ca | <i>r</i> value | 90 | 0.599 | 0.825 | 0.698 | 0.917 | 0.466 | 0.490 | 0.220 |
| | (P value) | | (<0.001)** | (<0.001)** | (<0.001)** | (<0.001)** | (<0.001)** | (<0.001)** | (0.037)* |
| Mg | <i>r</i> value | 90 | | 0.381 | 0.438 | 0.410 | 0.340 | 0.262 | 0.004 |
| | (P value) | | | (<0.001)** | (<0.001)** | (<0.001)** | (0.001)* | (0.013)* | (0.971) |
| Ca/crea | <i>r</i> value | 90 | | | 0.891 | 0.857 | 0.370 | 0.382 | 0.161 |
| | (P value) | | | | (<0.001)** | (<0.001)** | (<0.001)** | (<0.001)** | (0.131) |
| Mg/crea | <i>r</i> value | 90 | | | | 0.712 | 0.345 | 0.380 | 0.181 |
| | (P value) | | | | | (<0.001)** | (<0.001)** | (<0.001)** | (0.088) |
| iCa | <i>r</i> value | 90 | | | | | 0.309 | 0.348 | 0.175 |
| | (P value) | | | | | | (0.003)* | (<0.001)** | (0.098) |
| pH | <i>r</i> value | 90 | | | | | | 0.781 | 0.085 |
| | (P value) | | | | | | | (<0.001)** | (0.424) |
| HCO ₃ ⁻ | <i>r</i> value | 90 | | | | | | | 0.687 |
| | (P value) | | | | | | | | (<0.001)** |

r – Pearson's correlation coefficient values; * – significant difference between the hypercalcaemic group and the control group at P < 0.05; ** – significant difference between the hypercalcaemic group and the control group at P < 0.001; Ca/crea – calcium/creatinine in urine; Mg/crea – magnesium/creatinine in urine; iCa – ionised calcium; HCO₃⁻ – bicarbonate; pCO₂ – partial pressure of carbon dioxide

Table 2. Results of Pearson correlation analysis of Ca concentration with pH in the induced hypercalcaemic group

| | | Test (n) | iCa | Ca/crea | pH | HCO ₃ ⁻ | pCO ₂ |
|-------------------------------|----------------|----------|------------|------------|------------|-------------------------------|------------------|
| Ca | <i>r</i> value | 45 | 0.933 | 0.846 | 0.688 | 0.672 | 0.206 |
| | (P value) | | (<0.001)** | (<0.001)** | (<0.001)** | (<0.001)** | (0.174) |
| iCa | <i>r</i> value | 45 | | 0.860 | 0.542 | 0.549 | 0.194 |
| | (P value) | | | (<0.001)** | (<0.001)** | (<0.001)** | (0.201) |
| Ca/crea | <i>r</i> value | 45 | | | 0.511 | 0.486 | 0.125 |
| | (P value) | | | | (<0.001)** | (<0.001)** | (0.412) |
| pH | <i>r</i> value | 45 | | | | 0.820 | 0.041 |
| | (P value) | | | | | (<0.001)** | (0.788) |
| HCO ₃ ⁻ | <i>r</i> value | 45 | | | | | 0.603 |
| | (P value) | | | | | | (<0.001)** |

r – Pearson's correlation coefficient values; * – significant difference between the hypercalcaemic group and the control group at P < 0.05; ** – significant difference between the hypercalcaemic group and the control group at P < 0.001; Ca/crea – calcium/creatinine in urine; iCa – ionised calcium; HCO₃⁻ – bicarbonate; pCO₂ – partial pressure of carbon dioxide

Discussion

Administration of Ca borogluconate caused a significant increase in plasma Ca concentration and urinary Ca excretion. Simultaneously, blood pH was more basic in the HYC group. The changing pattern of blood pH was similar to that of HCO₃⁻, and the two values showed a strong correlation. Even though an acidic solution was administered, mildly alkaline blood pH was confirmed in the HYC group. This alteration was the opposite of the results of our previous study inducing the opposite state, hypocalcaemia, in which the deficit of Ca caused more acidic plasma pH, and probably resulted in the maximisation of the effect of PTH (9, 10, 12). As estimated in previous studies, there is a mechanism by which a decrease in blood pH results in an increase in the activity of PTH (9, 10, 13). The relationship between calcitonin, which lowers Ca levels in blood in a hypercalcaemic state, and blood pH has only been verified experimentally in hypocalcaemic status or *in vitro* (6, 11). Our *in vivo* results suggest that a spontaneous increase in blood pH after inducing

hypercalcaemia strengthens the action of calcitonin. We additionally posit that the spontaneous increase in blood pH in the HYC group was due to the HCO₃⁻ buffer system, citing the verified correlation between pH and HCO₃⁻ in support. Thus, blood Ca concentration higher than the normal range activates a series of homeostatic processes and activates the HCO₃⁻ buffer system to change blood pH, creating an optimal environment for the processes to operate efficiently. By maximising the effect of calcitonin, Ca reabsorption in the kidneys is suppressed and excretion of Ca through urine is increased, thereby returning Ca levels in the blood to their normal state. In the HYC group, Ca/crea and Mg/crea increased significantly. Levels of Ca were higher in the HYC group for 8 h after administration of the drug compared to the pre-dosing levels. This was presumed to be due to the action of calcitonin, as its secretion is dependent on blood Ca levels and it controls the excretion of macrominerals *via* urine (3, 5). In addition to the effect of calcitonin, as blood pH increases, the binding of iCa to albumin increases, leading to a decrease in iCa levels in the blood to

an extent which varies depending on the concentration of albumin in the blood (9, 10).

In conclusion, this study confirmed the change in blood pH concomitantly with the change of Ca concentration in blood during the induction of hypercalcaemia and recovery to normocalcaemia. Furthermore, our results suggest that this pH change was caused by the alteration of HCO_3^- concentration. Although mineralotropic hormones were not measured in the course of the experiment, changes in blood pH and HCO_3^- are presumed to be mechanisms that maintain Ca homeostasis. Therefore, in further studies, measurement of mineralotropic hormones at varied breeding stages and larger numbers of experimental animals are needed to clarify the mechanism.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Animal Rights Statement: This study was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-190522-3).

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