Alkalosis-induced hypoventilation in cystic fibrosis: The importance of efficient renal adaptation

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The lungs and kidneys are pivotal organs in the regulation of body acid–base homeostasis. In cystic fibrosis (CF), the impaired renal ability to excrete an excess amount of HCO_3^- into the urine leads to metabolic alkalosis [P. Berg et al., *J. Am. Soc. Nephrol.* 31, 1711–1727 (2020); F. Al-Ghimlas, M. E. Faughnan, E. Tullis, *Open Respir. Med. J.* 6, 59–62 (2012)]. This is caused by defective HCO_3^- secretion in the β-intercalated cells of the collecting duct that requires both the cystic fibrosis transmembrane conductance regulator (CFTR) and pendrin for normal function [P. Berg et al., *J. Am. Soc. Nephrol.* 31, 1711–1727 (2020)]. We studied the ventilatory consequences of acute oral base loading in normal, pendrin knockout (KO), and CFTR KO mice. In wild-type mice, oral base loading induced a dose-dependent metabolic alkalosis, fast urinary removal of base, and a moderate base load did not perturb ventilation. In contrast, CFTR and pendrin KO mice, which are unable to rapidly excrete excess base into the urine, developed a marked and transient depression of ventilation when subjected to the same base load. Therefore, swift renal base elimination in response to an acute oral base load is a necessary physiological function to avoid ventilatory depression. The transient urinary alkalization in the postprandial state is suggested to have evolved for proactive avoidance of hypoventilation. In CF, metabolic alkalosis may contribute to the commonly reduced lung function via a suppression of ventilatory drive.

CFTR | acid–base | cystic fibrosis | kidney | hypoventilation

Adequate regulation of acid–base homeostasis is essential to maintain normal cellular function and metabolism, and several intra- and extracellular mechanisms maintain blood pH within a narrow range. CO_2/HCO_3^- is the most important buffer system in plasma qua its large buffering capacity along with the body's ability to regulate HCO_3^- and CO_2 elimination via the kidneys and lungs (1).

It is well-established that mammals hypoventilate in response to metabolic alkalosis, whereby they retain CO_2 and thus restore plasma pH (2–5). This respiratory compensation to an alkali load is rapid but remains incomplete (6, 7). On the other hand, the textbook conception asserts that renal adaptation to acid–base disorders is a slow process that requires hours to days before reaching full effect (1). Renal compensation to alkalemia is normally attributed to reduced HCO_3^- reabsorption and decreased H^+ secretion in the proximal tubule (1), but recent studies highlight the importance of a markedly up-regulated HCO_3^- secretion in the cortical collecting duct of the renal tubular system during base loading (8, 9). Here, HCO_3^- secretion is governed by base-secreting β-intercalated cells, where HCO_3^- is secreted via the apical Cl^-/HCO_3^- exchanger, pendrin (SLC26A4) (10).

Metabolic alkalosis is the most prevalent acid–base disorder among patients admitted to the intensive care unit (11) and hospitalized patients in general (12), and elevated arterial blood pH is associated with increased mortality in both surgical and medical patients (13). Given the diverse etiology, high incidence, and potentially fatal outcome, a better understanding of the physiological response to metabolic alkalosis stands paramount for both diagnostics and treatment. Metabolic alkalosis can develop due to several pathological conditions, primarily diseases or syndromes affecting renal acid–base handling (14). As an example, patients suffering from cystic fibrosis (CF) have an increased risk of developing acute (15) and chronic (16) metabolic alkalosis.

CF is caused by loss-of-function mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel and affects multiple organs. This includes recurrent lung infections, poor lung function, and gastrointestinal malabsorption (17). Recent studies have found decreased pendrin abundance and function in CF, leading to deficient renal HCO_3^- excretion, explaining the characteristic metabolic alkalosis in these patients (8, 9, 18). Renal inability to correct an alkalosis could cause excessive hypoventilation, which in combination with poor lung function could entail dire respiratory consequences. Corroborating this, a clinical study found that pronounced metabolic alkalosis contributes to acute hypercapnic respiratory failure in adults with exacerbations of CF (19). However, the potential connection between an impaired renal HCO_3^- excretion in CF and respiratory failure has not been examined.

Significance

In conditions when our blood experiences alkalosis, a pronounced kidney response is initiated causing a marked increase of urine base excretion. We recently discovered the cellular physiology of this, which employs fast activation of the base-secreting β-intercalated cells of the collecting duct. This function is fully dependent on the cystic fibrosis transmembrane conductance regulator (CFTR), the anion channel defective in CF. Hence, CF patients and mice cannot efficiently excrete an acute base load. Here, we find that the inability of fast urinary base removal in the CF mouse model causes a marked inhibition of ventilation. This study provides physiological insights into acid–base regulation and may have important implications for CF patients who are already burdened with reduced lung function.

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Understanding how the lungs and kidneys respond when challenged to eliminate excess base is not only relevant in a pathological setting. The purpose of the alkaline tide, a long-known normophysiological process, has remained unresolved. The alkaline tide describes the acute rise in urine pH observed in the postprandial state (20, 21) that attends the alkalinization of plasma pH when gastric acid secretion is stimulated upon ingestion of a meal. Recent evidence suggests urine pH and base excretion increase via a secretin-mediated pendrin- and CFTR-dependent increase of β-intercalated cell HCO_3^- secretion in the collecting ducts of the kidney (8). The molecular mechanism was recently discovered and reviewed (22), but the physiological relevance of this response remains unclarified. Here, we speculate that it serves to diminish the need for respiratory retention of CO_2 to compensate postprandial alkalosis.

To study the interplay between respiratory and renal compensation to an acute gastric alkali load, we assessed how impaired renal HCO_3^- excretion, due to the absence of either pendrin or CFTR expression, affects the ventilatory response to an acute alkaline challenge. We hypothesized that when pendrin knockout (KO) and CFTR KO mice were subjected to an acute base load, their inability to increase renal HCO_3^- excretion would exacerbate the metabolic alkalosis, causing depression of ventilatory drive and thus hypoventilation. To address this hypothesis, we employed simultaneous measurements of metabolic rate and ventilation using barometry and intermittently closed respirometry. This enabled continuous measurements of ventilation and air convection requirement (ACR) before and after an acute base load. These experiments were further supplemented by metabolic cage studies and blood gas analyses.

Results

Variation of Ventilation, CO_2 Production, and ACR. As expected from nocturnal animals, ventilation and CO_2 production increased markedly during nighttime (Fig. 1*B*). The air convection requirement for CO_2 (ACR_{CO2}), namely the minute ventilation relative to the CO_2 production (ultimately expressing milliliter air ventilated per milliliter CO_2 excreted [mL_{air}/mL_{CO2}]), is a stable parameter reflecting the respiratory system's ability to maintain stable arterial pCO_2 ($PaCO_2$) values at any metabolic rate (Fig. 1*B*). It permits quantitative comparison of ventilation, where a drop of ACR_{CO2} reflects hypoventilation and an increase of $PaCO_2$. The mean minute ventilation was highly dependent on the metabolic rate, expressed as CO_2 production (Fig. 1*C*).

Gastric $NaHCO_3$ Gavage Imposes a Significant and Dose-Dependent Alkaline Challenge. Systemic acid–base status was assessed in C57BL/6J mice 1, 2, and 4 h after either a control gavage, a moderate dose of $NaHCO_3$ (200 mM; ∼4 mmol $NaHCO_3$/kg body weight [BW]), or a high dose of $NaHCO_3$ (400 mM; ∼8 mmol $NaHCO_3$/kg BW). Compared to control-treated animals, plasma pH and standard HCO_3^- were markedly increased 1 and 2 h after administration of the high dose (Fig. 2 *A* and *B*). Both parameters were also elevated, but to a lesser extent, in the mice receiving the moderate dose, most pronounced 1 h after the gavage. Compared with control gavage-treated mice, venous pCO_2 was increased only in the mice receiving the high dose (Fig. 2*C*). Plasma Cl^- also decreased in a dose- and time-dependent manner in the $NaHCO_3$-loaded mice (Fig. 2*D*).

In conclusion, both $NaHCO_3$ gavage doses imposed a significant alkaline challenge, which was reflected in higher venous

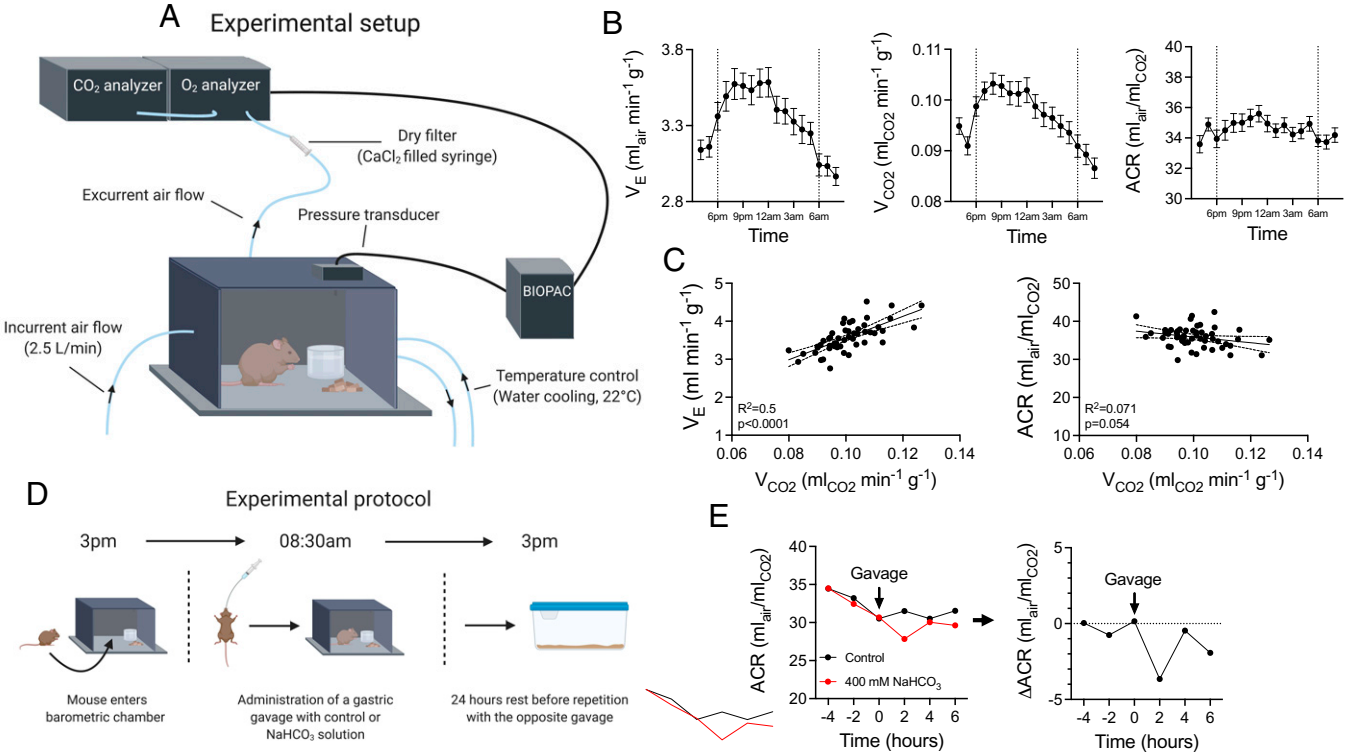

Fig. 1. Ventilation is dependent on CO_2 production while the ACR for CO_2 stays constant. (*A*) Graphic presentation of the experimental setup for barometric measurements. (*B*) Summary $\dot{V}_E$, $\dot{V}_{CO2}$, and ACR curves for all WT mice during baseline (17 h). (*C*) Mean $\dot{V}_E$ as a function of mean $\dot{V}_{CO2}$, and mean ACR as a function of mean $\dot{V}_{CO2}$ for all WT mice during a 17-h baseline recording. Note that ACR_{CO2} has a smaller variation and is largely independent of CO_2 production. (*D*) A graphical presentation of the experimental protocol used to assess the effect of an $NaHCO_3$ gavage on ventilation. (*E*, *Left*) Paired measurements from the same mouse receiving a control gavage (black) or $NaHCO_3$ gavage (red). (*E*, *Right*) The control gavage adjusted Δ trace. The correlations between mean $\dot{V}_E$ and $\dot{V}_{CO2}$ as well as between mean ACR_{CO2} and $\dot{V}_{CO2}$ were assessed using simple linear regression. Error bars indicate mean ± SEM.

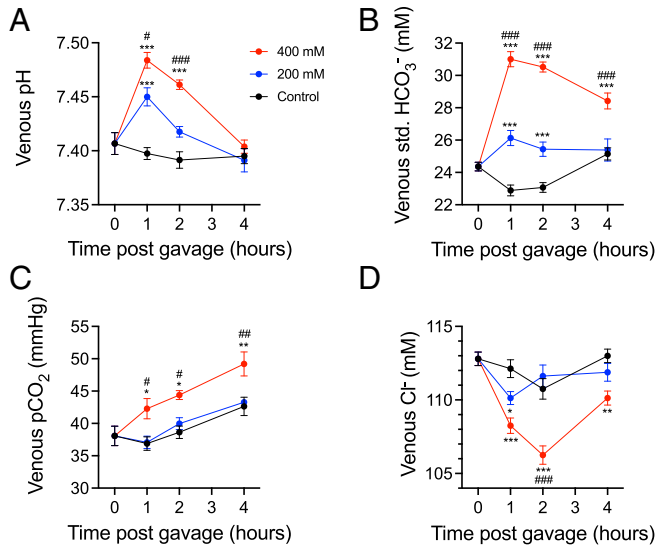

Fig. 2. $NaHCO_3$ gavage induces an acute dose-dependent alkalosis. Tail-vein samples drawn from lightly isoflurane-anesthetized mice showing venous pH (*A*), standard (std.) HCO_3^- (*B*), pCO_2 (*C*), and Cl^- (*D*) at baseline and 1, 2, and 4 h after a control gavage (black), a 200 mM $NaHCO_3$ gavage (blue), and a 400 mM $NaHCO_3$ gavage (red); $n = 10$ for time point 0 and $n = 7$ or 8 for each following time point. Differences were tested by two-way ANOVA followed by Tukey posttest for multiple comparisons. $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ vs. 200 mM. Error bars indicate mean ± SEM.

pH and standard HCO_3^-. Further, a clear dose dependency between $NaHCO_3$ load and plasma alkalinity was found. Following the tested $NaHCO_3$ gavage loads, blood alkalinity appears to peak around 1 h after the intervention, and blood pH is normalized 4 h after the challenge.

A 400 mM $NaHCO_3$ Gavage Induces Hypoventilation in Wild-Type Mice. Before subjecting the pendrin KO and CFTR KO mice to the barometric protocol, we sought to elucidate whether a ventilatory response to the alkaline challenge could be identified in wild-type (WT) mice by administering a control gavage or a moderate (200 mM; ∼4 mmol/kg BW) or high dose (400 mM; ∼8 mmol/kg BW) of $NaHCO_3$. The experimental protocol and an illustration of how ΔACR and $\Delta\dot{V}_E$ were calculated are shown in Fig. 1 *D* and *E*.

The moderate $NaHCO_3$ dose did not suppress ACR_{CO2} or minute ventilation during the 6 h after gavage (Fig. 3), which is consistent with the unaltered pCO_2 in C57BL/6J mice receiving a 200 mM $NaHCO_3$ gavage (Fig. 2*C*). This shows that WT mice can handle a moderate alkaline challenge without significant respiratory compensation. In contrast, the high dose of $NaHCO_3$ produced a significant depression of both ACR_{CO2} and minute ventilation during the first 2 h post gavage followed by a gradual recovery (Fig. 3). This is coherent with the increased pCO_2 in C57BL/6J mice receiving a 400 mM $NaHCO_3$ gavage (Fig. 2*C*). HCO_3^--induced ΔACR and $\Delta\dot{V}_E$ were calculated as the paired control gavage-subtracted responses to the $NaHCO_3$ gavage for each mouse. The 400 mM $NaHCO_3$ gavage induced a significant decrease of ΔACR and $\Delta\dot{V}_E$ compared with the 200 mM $NaHCO_3$ gavage.

In summary, WT mice proved capable of handling moderate doses of $NaHCO_3$ without compromising ventilation. When challenged with a doubling of the dose, respiratory compensation became evident. This signifies that acute compensation to a moderate base load is not handled by the pulmonary system and that increased renal base excretion may partake to a significant extent.

Pendrin and CFTR KO Mice Have Reduced Net Base Excretion after an $NaHCO_3$ Gavage. Next, we investigated the renal response to an $NaHCO_3$ gavage (112 mM, 2.24 mmol/kg BW) in the pendrin KO and CFTR KO mouse model.

Compared with pendrin WT mice, pendrin KO mice had significantly lower urine pH at each of 3 h post the gavage (Fig. 4*A*). In concordance with the alkaline urine, pendrin WT mice had a positive net base excretion following the gavage, while the KO mice proved completely unable to excrete HCO_3^- and had a negative net base excretion (Fig. 4*A*).

Similarly, CFTR KO mice had a lower urine pH post gavage compared with CFTR WT mice, albeit the difference was not as pronounced as for the pendrin KO mice. Accordingly, CFTR WT mice had a much higher net base excretion compared with CFTR KO mice after gavage (Fig. 4*B*). These results confirm and extend that mice lacking either pendrin or CFTR have severely diminished renal base excretion capabilities when challenged with an acute base load, as previously demonstrated for urine pH and HCO_3^- excretion only (8).

Metabolic and Ventilatory Baseline Characteristics in the Pendrin and CFTR Mice. Prior to investigating the respiratory response to an $NaHCO_3$ gavage in the pendrin and CFTR mouse models, metabolic and ventilatory baseline characteristics were established during the baseline period. The quantitative values of each parameter in both mouse models were in concordance with previous studies (23–26). The mean ACR_{CO2} of WT mice was ∼34 mL_{air}/mL_{CO2}, corresponding to an $PaCO_2$ of ∼31 mmHg (*SI Appendix*, *Methods*). This aligns well with directly measured $PaCO_2$ in conscious mice with an in-dwelling carotid catheter (23).

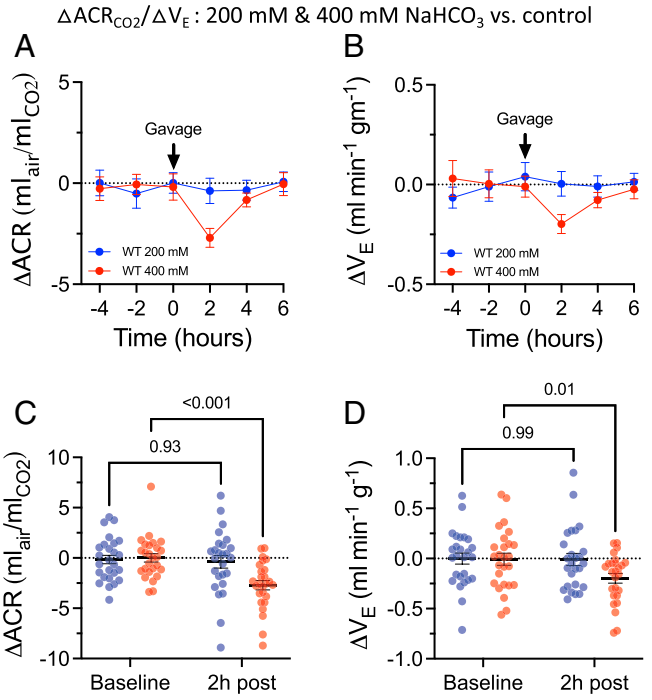

Fig. 3. Compared with a control gavage, a 400 mM $NaHCO_3$ gavage induces hypoventilation in WT mice. This effect is absent in mice receiving a 200 mM $NaHCO_3$ gavage. Summary ΔACR_{CO2} (*A*) and $\Delta\dot{V}_E$ (*B*) curves for WT mice receiving a 400 mM HCO_3^- gavage (red, $n = 26$) and 200 mM HCO_3^- gavage (blue, $n = 25$). Difference between ΔACR_{CO2} (*C*) and $\Delta\dot{V}_E$ (*D*) at baseline and 2 h post gavage for the 400 mM group and 200 mM group. Note that the 400 mM HCO_3^- gavage induces a significant decrease in ACR_{CO2} and $\dot{V}_E$ while the 200 mM HCO_3^- gavage does not. Differences were tested by two-way ANOVA followed by Bonferroni's multiple-comparisons test. Error bars indicate mean ± SEM.

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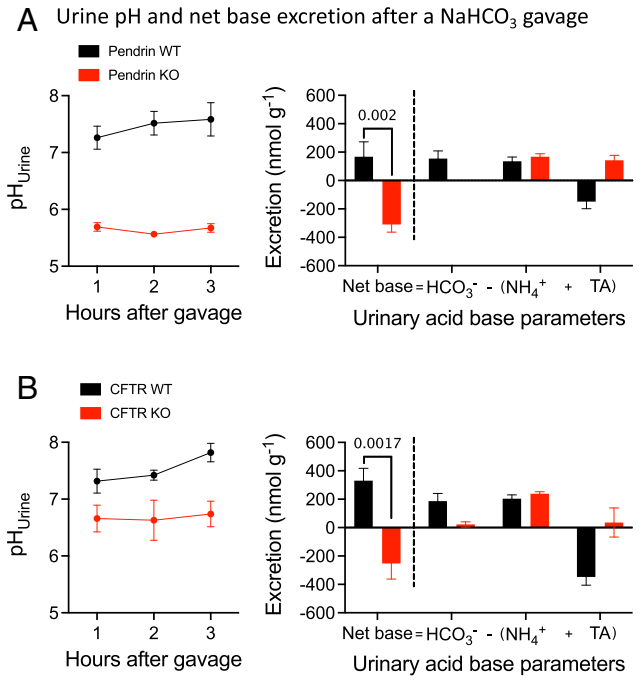

Fig. 4. $NaHCO_3$ gavage induces an acute urine alkalization and pronounced increased net base excretion in pendrin WT and CFTR WT mice while these effects are absent in pendrin KO and CFTR KO mice. (*A*) Summary pH_{urine} curves for pendrin WT (black, $n = 8$) and pendrin KO (red, $n = 7$) mice 3 h post a 112 mM $NaHCO_3$ gavage and pooled averages of ammonium (NH_4^+), TA, HCO_3^-, and net base excretion for the 3 h post gavage. (*B*) Summary pH_{urine} curves for CFTR WT (black, $n = 7$) and CFTR KO (red, $n = 5$) mice 3 h post a 112 mM $NaHCO_3$ gavage and pooled averages of NH_4^+, TA, HCO_3^-, and net base excretion for the 3 h post gavage. Note the marked significant difference in net base excretion between WT and KO mice in both mouse models. Differences in net base excretion were tested by unpaired *t* tests. The urine samples from CFTR WT and CFTR KO mice were collected in previously published experiments (8) and analyzed for urinary acid–base parameters. Error bars indicate mean ± SEM.

Pendrin KO and WT mice presented with comparable ventilatory characteristics throughout the baseline period (*SI Appendix*, Table S1), while CFTR KO mice had a significantly higher V_T, $\dot{V}_E$, and $\dot{V}_{O2}$ throughout the baseline period compared with CFTR WT mice (*SI Appendix*, Table S2). This difference could very likely be explained by the significantly lower BW of the CFTR KO mice (*SI Appendix*, Table S3). Accordingly, when adjusting for weight, no differences between CFTR WT and KO mice were found during the baseline period (*SI Appendix*, Table S4). Mean V_T, $\dot{V}_E$, $\dot{V}_{O2}$, and ACR_{CO2} as a function of BW are depicted in *SI Appendix*, Fig. S1.

A 200 mM $NaHCO_3$ Gavage Induces Ventilatory Depression in Pendrin and CFTR KO Mice While This Effect Is Completely Absent in WT Mice. Next, we wanted to elucidate whether the impaired ability to increase renal net base excretion (Fig. 4) in pendrin and CFTR KO mice affected the respiratory response to an oral base load.

After a 200 mM $NaHCO_3$ gavage, both pendrin and CFTR KO mice showed significant ventilatory depression (i.e., a lowered ACR_{CO2}) 2 h after gavage compared with baseline, while this effect was absent in the WT group (Fig. 5). $\Delta\dot{V}_E$ is depicted in *SI Appendix*, Fig. S2.

The mean HCO_3^--induced ventilatory depression in pendrin KO mice amounted to 3.1 mL_{air}/mL_{CO2}, which corresponds to an approximate 8% decrease as compared with the mean baseline ACR_{CO2} of pendrin KO mice. In CFTR KO mice, the magnitude of the ventilatory depression was 4.8 mL_{air}/mL_{CO2}, which corresponds to an approximate 13% decrease compared with the mean baseline ACR_{CO2} of CFTR KO mice. Because the HCO_3^--induced ΔACR_{CO2} and $\Delta\dot{V}_E$ were similar between CFTR WT and pendrin WT mice, the combined group of WT mice was compared with CFTR KO mice and pendrin KO mice (*SI Appendix*, Fig. S3). The HCO_3^--induced ventilatory depressions of CFTR KO mice and pendrin KO mice were of similar magnitude (4.8 vs. 3.1 mL_{air}/mL_{CO2}, respectively, $P = 0.29$) and both were significantly different from the response observed in WT mice. Also, the HCO_3^--induced $\Delta\dot{V}_E$s were similar in the two KO models and showed the same qualitative pattern (*SI Appendix*, Fig. S3).

In summary, these data show that pendrin and CFTR KO mice exhibit significant functional hypoventilation in response to a moderate, acute base load, while WT mice do not. This indicates that a loss of pendrin or CFTR, and thus a markedly reduced capacity for renal base excretion, imposes a significant burden on the respiratory component of the systemic compensation to an acute base load.

Discussion

A comprehensive understanding of the integrated systemic response to acute metabolic alkalosis that includes renal function has yet to be attained. This study aimed to 1) investigate the acute compensatory mechanisms to an acute gastric base load, and 2) elucidate how impaired renal HCO_3^- excretion, as seen in CF, affects ventilation following an acute base load.

First, our results demonstrate that WT mice handle an acute moderate base load (4 mmol/kg BW) without respiratory compensation despite an increase of both plasma pH and standard HCO_3^-. This is illustrated by the absence of changes in

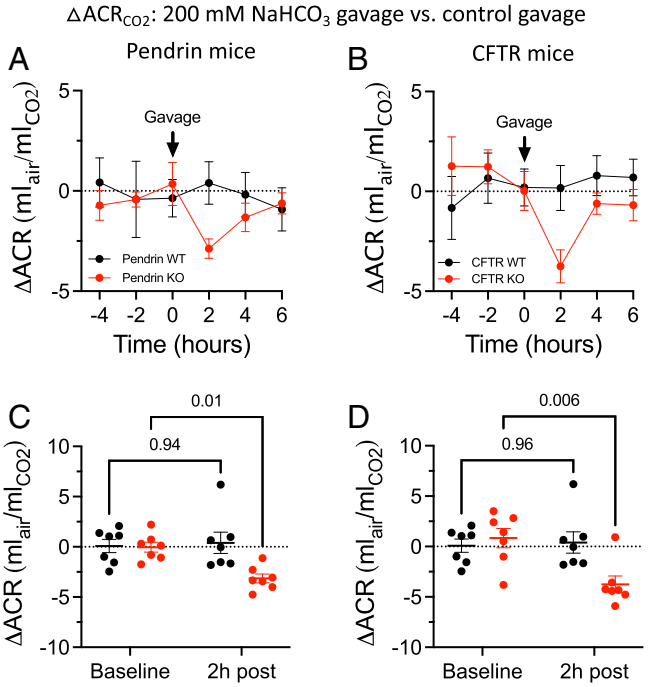

Fig. 5. Significant hypoventilation is induced by a 200 mM $NaHCO_3$ gavage in pendrin KO mice and CFTR KO mice. (*A* and *B*) Summary ΔACR_{CO2} curves for KO (red, $n = 7$) and WT mice (black, $n = 7$). (*C* and *D*) Differences between ΔACR_{CO2} at baseline and 2 h post gavage for KO and WT mice. Note that both pendrin KO mice and CFTR KO mice have a significant decrease in ΔACR_{CO2} 2 h post the gavage, while WT mice do not. Differences were tested by two-way ANOVA followed by Bonferroni's multiple-comparisons test. Error bars indicate mean ± SEM.

ACR_{CO2}, $\dot{V}_E$, and pCO_2. Second, our results show that a lack of pendrin or CFTR, and thus inability to acutely increase urinary HCO_3^- excretion (8, 9, 18), causes a marked depression of ACR_{CO2} in mice when given an acute $NaHCO_3$ gavage. Compared with WT mice, both pendrin and CFTR KO mice responded with a significant ventilatory depression in the first 2 h after receiving a moderate dose of $NaHCO_3$ gavage (4 mmol/kg BW). In contrast, ventilation and ACR_{CO2} of the WT mice were unaffected by the moderate dose but, when subjected to a doubling of the dose, these mice also responded with significant ventilatory depression.

As diurnal variation could affect our results, we also produced an experimental series of pendrin WT and pendrin KO mice exposed to a high dose of $NaHCO_3$ (8 mmol/kg BW) at 1600 hours instead of 0830 hours (*SI Appendix*, Fig. S4). In this setting, the high-$NaHCO_3$ dose also produced a depression of ventilation and ACR_{CO2} in WT mice of similar magnitude as found in Fig. 3. Also, in this series, pendrin KO mice tended to have a larger HCO_3^--induced ventilatory depression than WT mice ($P = 0.056$).

Combined, our results indicate that acutely increased renal base excretion is fully capable of correcting a moderate alkaline challenge without the need for a ventilatory response. When this ability to increase urinary HCO_3^- is impaired, such as in CF, even a moderate dose of alkali triggers a marked ventilatory depression.

A remarkable finding of this study is that renal adaptation via increased urine HCO_3^- excretion occurs immediately and that the threshold for renal activation appears to be reached before respiratory compensation initiates. Thus, while a gavage load of 4 mmol $NaHCO_3$/kg BW does not elicit respiratory compensation in WT mice, we have previously found that an $NaHCO_3$ dose as low as 2.24 mmol/kg BW triggers a drastic rise of urine base excretion in WT mice (8). This acute increase of urinary base excretion is fully dependent on pendrin and CFTR function and therefore adequate function of the β-intercalated cells of the collecting duct (8). These findings indicate that the distal part of the kidneys, more specifically the β-intercalated cells, responds acutely to a systemic alkalotic disturbance. This is in stark contrast to the classic "textbook" understanding of how an acute alkalosis is compensated (Introduction).

An important question would be how these findings can be integrated into a meaningful physiological understanding. The most frequent physiological acid–base disturbance is postprandial alkalosis, coined "the alkaline tide." Intriguingly, in snakes and other reptiles, this postprandial alkalization causes a decrease of ventilation relative to CO_2 production (ACR_{CO2}), and therefore a rise in $PaCO_2$ (27). Seen in this context, our results suggest that the increase in urine HCO_3^- excretion following an acute gastric $NaHCO_3$ challenge, or a meal, could serve to ameliorate the need for respiratory compensation in this state.

Another question would be what clinical impact our findings pose. Patients suffering from CF have an increased risk of developing metabolic alkalosis (16), and alkalosis has been attributed to a role in the acute hypercapnic failure observed in the end stage of this disease (19). Our findings provide a concrete mechanistic explanation for this observation. In addition, elevated $PaCO_2$ is strongly associated with increased mortality among CF patients (28). Numerous factors could explain the increased $PaCO_2$ in patients with CF, including decreased lung function and decreased alveolar gas exchange, but also metabolic alkalosis. To what extent each factor contributes is currently undetermined. However, our results show that in a mouse model of CF, metabolic alkalosis causes excessive respiratory hypoventilation. Whether the impaired renal base excretion in CF indeed is a contributing factor to the increased overall mortality remains uncertain, but we advocate that more attention should be directed toward managing the acid–base status of CF patients.

Another relevant clinical setting is chronic kidney disease (CKD) patients receiving HCO_3^--rich dialysate (29). To our knowledge, nothing is known about CFTR function in CKD patients, but recent evidence suggests that in the case of acidosis, a common complication to CKD, these patients also have reduced pendrin expression (30). In combination with reduced kidney function, this would reduce the capacity for renal base excretion. Indeed, some CKD patients develop metabolic alkalosis during dialysis (31–34), but it has never been studied whether this combination of an acute alkalic load and impaired renal excretion capabilities affects ventilation. Respiratory depression in CKD patients receiving hemodialysis has been described (35, 36), pointing toward a potential correlation. If true, our results could aid in the understanding of this utmost unfortunate complication during dialysis treatment.

In this study, we induced metabolic alkalosis by $NaHCO_3$ gavage loading (Fig. 2). The alkalosis achieved by this method is presumably mediated through several concurring physiological mechanisms. First, basolateral export of HCO_3^- into the bloodstream by parietal cells will occur following a likely increase of apical H^+ secretion due to alkalization of the ventricular pH. Second, HCO_3^- will be absorbed in the duodenum along with water. Hence, the degree of alkalosis will reflect the overall balance of HCO_3^- absorption, cellular buffering, and secretion of HCO_3^- in the intestine, pancreas, liver, and kidney. Despite that the CF mouse model used in this study is a transgenic mouse with intestinal correction of CFTR (37), differences in this balance can be expected between CFTR KO and WT mice. Hence, CF mice may have their aggravated and prolonged alkalosis due to impairment of both renal and gastrointestinal HCO_3^- handling.

It is conceivable that by introducing a metabolic alkalosis through a gastric gavage a feedforward signal from the gut to the kidney is initiated. In this understanding, the activation of renal base excretion is driven by an alkalosis sensed in the gut, rather than the systemic acid–base disturbance. In this regard, we and others have previously found that the gastrointestinal hormone secretin activates renal HCO_3^- excretion (8, 38). Here, we also found that mice receiving an $NaHCO_3$ gavage have higher plasma secretin than mice receiving a control gavage (8), but it is plausible that other pathways are involved. To further elucidate what triggers the increase in renal base excretion, other ways to introduce an acute metabolic alkalosis could be explored, for example intravenous infusion of alkaline saline or an experimentally induced respiratory alkalosis. Nonetheless, we believe that a gastric administration of excess base more closely mirrors physiological conditions.

It is possible that respiratory compensation does occur in WT mice when challenged with the moderate dose of $NaHCO_3$ but that the magnitude of the effect precludes us from finding it with our current experimental setup. A close inspection of Fig. 3*A* does indeed suggest that ACR_{CO2} could be slightly decreased 2 to 4 h post the gavage.

It should be noted that similar to the β-intercalated cells of the kidney, CFTR and pendrin also colocalize and cofunction in the airway epithelia (39). Here, pendrin-dependent HCO_3^- transport likely serves the purpose of maintaining an alkaline airway surface liquid layer to prevent bacterial infection (40). Whether the loss of airway expression of pendrin and CFTR could confound our results is undetermined, but we believe that it is very unlikely that airway HCO_3^- transport contributes to systemic acid–base homeostasis or ventilation control.

In conclusion, this study demonstrates a rapid renal compensation in response to an oral base load. When the renal mechanisms governing HCO_3^- secretion in the distal tubule are impaired, excessive respiratory compensation is necessary. This challenges the conventional paradigm regarding renal adaptation to an acute alkalosis under both normal and pathological

conditions. Our findings provide a sound explanation for the physiological relevance of the postprandial alkaline tide in the urine, an observation more than 150 y old. Lastly, our results have highly relevant implications for clinicians treating patients with CF and other patients suffering from a concurrent decrease in lung and kidney function.

Materials and Methods

Animals. Littermates from heterozygous breeding pairs were used in experiments with genetically modified mice. Animals were bred in the animal facility of the Department of Biomedicine, Aarhus University. Mice of both sexes were used with similar body mass and age between the groups (*SI Appendix*, Table S3). Ten-week-old C57BL/6J mice (Janvier) were used to investigate the time course of plasma pH and standard HCO_3^- changes following acute gastric base load. C57BL/6J mice were acclimatized in the animal facility of the Department of Biomedicine, Aarhus University for 1 wk before the experiments.

The pendrin KO mouse, with a genetic background of 129S1/SvImJ, was purchased from The Jackson Laboratory (JAX stock no. 018424) (41).

The CFTR KO mouse (CFTR S489X-; FABP-hCFTR), with a genetic background of C57BL/6 and 129P2/OlaHsd, was also purchased from The Jackson Laboratory (JAX stock no. 002364) (37). The CFTR KO mouse expresses human CFTR under the activity of the rat fatty acid–binding protein 2 intestinal promoter. This corrects the otherwise lethal intestinal phenotype of these mice (37).

All mice had ad libitum access to normal drinking water and standard rodent chow and were kept on a 12-h light/dark cycle throughout the experiments and during housing in the animal facility.

Acute Base Load via Gavage. In all experiments, mice were given either an $NaHCO_3$ gavage solution or a control solution with a volume of 20 μL/g BW. For the assessment of systemic acid–base parameters and barometric measurements, the $NaHCO_3$ solution consisted of distilled water with 2% mass glucose and either 200 or 400 mM $NaHCO_3$ (equivalent to a dose of 4 or 8 mmol $NaHCO_3$/kg BW, respectively).

For the measurements of urine pH and urine acid–base parameters, the $NaHCO_3$ solution consisted of distilled water with 2% mass glucose and 112 mM $NaHCO_3$ (equivalent to a dose of 2.24 mmol $NaHCO_3$/kg BW). In all experiments, the control solution consisted of distilled water with 2% mass glucose.

Assessment of Systemic Acid–Base Parameters. Blood was sampled with glass capillaries (CLINITUBES; Radiometer) from lightly anesthetized mice (isoflurane anesthesia, tail-vein samples) and analyzed with an ABL80 Flex blood gas analyzer (Radiometer) immediately after collection. Blood was drawn at baseline and 60, 120, and 240 min after a gastric gavage of either 4 or 8 mmol $NaHCO_3$/kg BW or control solution.

Measurements of Urine pH and Urine Acid–Base Parameters. Pendrin WT and KO mice were acutely administered 2.24 mmol $NaHCO_3$/kg BW. Following gavage, mice were placed in metabolic cages for 3 h, while urine was collected in funnels covered with parafilm to allow for precise, immediate urine collections (42). Urine samples were collected every hour with a micropipette and kept at −20 °C until further analysis. Urine pH was measured each hour while the remaining acid–base parameters were measured in one sample comprising the weighted average of all the urine collected in the 3-h period. Urine samples collected from CFTR WT and KO mice undergoing the same protocol in a previously published study (8) were also included and net base excretion was measured.

Urine pH was measured with a micro pH electrode (pH-400; Unisense). Urine [HCO_3^-] was measured using a custom-built infrared CO_2 sensor–based system (43). Urine [NH_4^+] was measured via an Orion High-Performance Ammonia Ion-Selective Electrode (Thermo Scientific; 9512HPBNWP) with the use of ammonia pH-adjusting Ionic Strength Adjuster (Thermo Scientific; 951211) as previously done (9, 44). Urine [titratable acid] ([TA]) was measured by titration using the method of Chan (45) modified to small-volume samples (9, 44). Net base concentration was calculated as [HCO_3^-] − ([TA] + [NH_4^+]) and multiplied by the corresponding 3-h weight-adjusted diuresis to yield net base excretion.

Barometric Measurements of Ventilation and Metabolism.

Experimental setup. Metabolic rate and ventilation were measured using intermittent-closed respirometry. The setup comprised a total of four chambers (1.1 L) for measurements. Each chamber (one at a time) was flushed with compressed air at a rate of 2.5 standard liters per minute for 2 min controlled by a flow controller (Sierra Instruments). Hereafter, the chamber was sealed by solenoid valves and the next chamber was flushed, resulting in a 2-min flushing and a 6-min sealed cycle for each chamber.

When a chamber was closed, ventilation was measured by a barometric method based upon the original method developed by Drorbaugh and Fenn (46). When the same chamber was subsequently flushed with air, the accumulated CO_2 and consumed O_2 during the closed period were measured, resulting in the above-mentioned intermittent, closed system (23, 47, 48).

Each chamber was connected to an air inlet, an air outlet, and a temperature-regulating system (maintaining an in-chamber temperature of ∼22 °C at all times). Also, temperature, relative humidity (Humitter; Vaisala), and pressure variations caused by ventilation (First Sensor; HCLA02X5DB) were monitored inside the chamber.

To measure O_2 consumption and CO_2 production in each closed cycle, the excurrent air, when flushed, passed through a drying filter containing $CaCl_2$ to a calibrated gas-analyzing system (Ametek Applied Electrochemistry) consisting of an O_2 and a CO_2 sensor and analyzer (P-61B, N-22M, CD-3A, and S-3A/I). Before the start of each experiment and after gavage the sensors were calibrated with dried outside air (20.95% O_2 and 0.04% CO_2) and with a mixture of 5% CO_2 in dried outside air. The gas mixture for calibration was delivered by a precision Wösthoff gas-mixing pump. Ventilation frequency and tidal volume were calculated from the volume-related pressure changes inside the chamber during each closed cycle.

All data were collected by a data-acquisition system (BIOPAC; MP150) at 100 Hz. After the end of the experiment, data were exported to Wolfram Mathematica (version 7; Wolfram Research) and analyzed in a script that automatically identified and integrated each CO_2 and O_2 top and bottom relative to their baselines and calculated O_2 uptake ($\dot{V}_{O2}$, mL_{O2}/min) and CO_2 production ($\dot{V}_{CO2}$, mL_{CO2}/min) using the closed period of the chambers. The script also detected each breathing-related chamber pressure fluctuation and calculated median tidal volume (V_T, mL) and median respiratory frequency (f_R, per minute) in each closed period. Minute ventilation ($\dot{V}_E$, mL/min) was calculated from V_T and f_R. The respiratory quotient was calculated as $\dot{V}_{CO2}/\dot{V}_{O2}$. Subsequently, $\dot{V}_{O2}$, $\dot{V}_{CO2}$, V_T, and $\dot{V}_E$ were weight-adjusted.

Quantitative comparison of ventilation in different animals requires determination of ACR for CO_2 or O_2 (i.e., ACR_{CO2}, mL_{air}/mL_{CO2} or ACR_{O2}, mL_{air}/mL_{O2}) and was calculated for each cycle. ACR_{CO2} is the minute ventilation relative to the CO_2 excretion ($\dot{V}_E/\dot{V}_{CO2}$) and reflects $PaCO_2$ (see *SI Appendix*, *Methods* for further details).

A presentation of the experimental setup and ventilatory data recordings is in Fig. 1 *A* and *B*.

Experimental protocol. Conscious, unrestrained mice were weighed and placed in the four separate respirometry chambers. The chambers were translucent on one side, allowing natural incident light.

After an overnight baseline measurement period of 17.5 h (1500 to 0830 hours), the mice were weighed and acutely administered either 4 or 8 mmol $NaHCO_3$/kg BW or the control solution. For a subset of experiments, mice underwent an 8-h baseline recording from 0800 to 1600 hours before the gavage.

Following gavage, the mice were returned to their chambers and postgavage measurements ensued over the next 6.5 h. The mice underwent the above-mentioned protocol twice separated by a 24-h resting period, thus yielding an $NaHCO_3$ gavage recording and a control gavage recording for each mouse (see Fig. 1*D* for a schematic representation of the protocol). To investigate the effect of an $NaHCO_3$ gavage vs. a control gavage, the paired measurements were subtracted ($NaHCO_3$ vs. control), yielding Δ traces showing the differential effect of the two gavage maneuvers (Fig. 1*E*).

For every experimental cycle, two mice received a control gavage and two mice received an $NaHCO_3$ gavage. In experiments with KO and WT mice, each cycle included two WT and two KO mice.

However, one pendrin WT mouse did not yield results due to technical problems with the setup, one CFTR KO mouse was euthanized due to accidental administration of the gavage load into the lungs instead of the gastric ventricle, one CFTR "WT" mouse was excluded because it was found to be heterozygous in the postmortem control genotyping, and one pendrin KO mouse was excluded due to an extreme ventilatory depression (*SI Appendix*, Fig. S5).

Statistical Analysis. Prism 9.2.1 (GraphPad Software) was used for the graphical depiction of results and statistical analysis. The distribution of data was assessed by plotting QQ plots and, when relevant, model validation was performed by plotting residual plots and homoscedasticity plots. If the datasets could not be characterized as normally distributed and log transformations could not properly adjust for this, nonparametric tests were used.

Statistical differences were assessed as follows: A comparison between two groups was performed with Student's *t* test or Mann–Whitney *U* test where relevant. Multiple comparisons between more than two groups were controlled for using one-way ANOVA. A comparison between two groups with more than one intervention was performed by two-way ANOVA followed by

correction for multiple comparisons. A comparison of data collected time-dependently was performed by two-way ANOVA followed by correction for multiple comparisons. Regression analysis of two variables was performed using simple linear regression. Regression analysis of more than two variables was performed using multiple linear least-squares regression. Differences in sex distribution were tested with Fisher's exact test

All tests were two-sided and performed at a significance level of 5%. The statistical test used is specified in each figure legend. All values are presented as mean values ± SEM unless stated otherwise.

Study Approval. Handling and experiments on mice were approved by Danish animal welfare regulations (Dyreforsøgstilsynet, Approval no. 2016-15-0201-01129).

Data Availability. All study data are included in the article and/or *SI Appendix*.

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1. L. L. Hamm, N. Nakhoul, K. S. Hering-Smith, Acid-base homeostasis. *Clin. J. Am. Soc. Nephrol.* **10**, 2232–2242 (2015).
2. S. Javaheri, N. S. Shore, B. Rose, H. Kazemi, Compensatory hypoventilation in metabolic alkalosis. *Chest* **81**, 296–301 (1982).
3. M. Feldman, N. M. Alvarez, M. Trevino, G. L. Weinstein, Respiratory compensation to a primary metabolic alkalosis in humans. *Clin. Nephrol.* **78**, 365–369 (2012).
4. E. Oppersma *et al.*, The effect of metabolic alkalosis on the ventilatory response in healthy subjects. *Respir. Physiol. Neurobiol.* **249**, 47–53 (2018).
5. S. Javaheri, H. Kazemi, Metabolic alkalosis and hypoventilation in humans. *Am. Rev. Respir. Dis.* **136**, 1011–1016 (1987).
6. J. H. Galla, Metabolic alkalosis. *J. Am. Soc. Nephrol.* **11**, 369–375 (2000).
7. J. L. Seifter, H. Y. Chang, Disorders of acid-base balance: New perspectives. *Kidney Dis.* **2**, 170–186 (2017).
8. P. Berg *et al.*, Impaired renal HCO_3^- excretion in cystic fibrosis. *J. Am. Soc. Nephrol.* **31**, 1711–1727 (2020).
9. P. Berg *et al.*, Impaired renal HCO_3^- secretion in CFTR deficient mice causes metabolic alkalosis during chronic base-loading. *Acta Physiol. (Oxf.)* **231**, e13591 (2021).
10. I. E. Royaux *et al.*, Pendrin, encoded by the Pendred syndrome gene, resides in the apical region of renal intercalated cells and mediates bicarbonate secretion. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4221–4226 (2001).
11. K. Mæhle, B. Haug, H. Flaatten, E. Nielsen, Metabolic alkalosis is the most common acid-base disorder in ICU patients. *Crit. Care* **18**, 420 (2014).
12. J. E. Hodgkin, F. F. Soeprono, D. M. Chan, Incidence of metabolic alkalemia in hospitalized patients. *Crit. Care Med.* **8**, 725–728 (1980).
13. L. E. Anderson, W. L. Henrich, Alkalemia-associated morbidity and mortality in medical and surgical patients. *South. Med. J.* **80**, 729–733 (1987).
14. M. Emmett, Metabolic alkalosis: A brief pathophysiologic review. *Clin. J. Am. Soc. Nephrol.* **15**, 1848–1856 (2020).
15. S. Mauri *et al.*, Acute metabolic alkalosis in cystic fibrosis: Prospective study and review of the literature. *Miner. Electrolyte Metab.* **23**, 33–37 (1997).
16. F. Al-Ghimlas, M. E. Faughnan, E. Tullis, Metabolic alkalosis in adults with stable cystic fibrosis. *Open Respir. Med. J.* **6**, 59–62 (2012).
17. D. A. Stoltz, D. K. Meyerholz, M. J. Welsh, Origins of cystic fibrosis lung disease. *N. Engl. J. Med.* **372**, 1574–1575 (2015).
18. M. Varasteh Kia *et al.*, Downregulation of the Cl^-/HCO_3^-exchanger pendrin in kidneys of mice with cystic fibrosis: Role in the pathogenesis of metabolic alkalosis. *Cell. Physiol. Biochem.* **45**, 1551–1565 (2018).
19. A. E. Holland, J. W. Wilson, T. C. Kotsimbos, M. T. Naughton, Metabolic alkalosis contributes to acute hypercapnic respiratory failure in adult cystic fibrosis. *Chest* **124**, 490–493 (2003).
20. H. B. Jones, Contributions to the chemistry of the urine. On the variations in the alkaline and earthy phosphates in the healthy state, and on the alkalescence of the urine from fixed alkalies. *Philos. Trans. R. Soc. Lond.* **135**, 335–349 (1845).
21. C. E. Brunton, The influence of foodstuffs on the rate of urinary acid excretion. *J. Physiol.* **78**, 65–79 (1933).
22. P. Berg *et al.*, The molecular mechanism of CFTR- and secretin-dependent renal bicarbonate excretion. *J. Physiol.* **599**, 3003–3011 (2021).
23. N. K. Iversen, H. Malte, E. Baatrup, T. Wang, The normal acid-base status of mice. *Respir. Physiol. Neurobiol.* **180**, 252–257 (2012).
24. P. Frappell, C. Lanthier, R. V. Baudinette, J. P. Mortola, Metabolism and ventilation in acute hypoxia: A comparative analysis in small mammalian species. *Am. J. Physiol.* **262**, R1040–R1046 (1992).
25. P. L. Milton, H. Dickinson, G. Jenkin, R. Lim, Assessment of respiratory physiology of C57BL/6 mice following bleomycin administration using barometric plethysmography. *Respiration* **83**, 253–266 (2012).
26. L. Friedman *et al.*, Ventilatory behavior during sleep among A/J and C57BL/6J mouse strains. *J. Appl. Physiol.* **97**, 1787–1795 (2004).
27. T. Wang, E. Rindom, The physiological response to digestion in snakes: A feast for the integrative physiologist. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **254**, 110891 (2021).
28. E. Kerem, J. Reisman, M. Corey, G. J. Canny, H. Levison, Prediction of mortality in patients with cystic fibrosis. *N. Engl. J. Med.* **326**, 1187–1191 (1992).
29. C. Basile, L. Rossi, C. Lomonte, The choice of dialysate bicarbonate: Do different concentrations make a difference? *Kidney Int.* **89**, 1008–1015 (2016).
30. P. H. Imenez Silva *et al.*, Acidosis and alkali therapy in patients with kidney transplant is associated with transcriptional changes and altered abundance of genes involved in cell metabolism and acid-base balance. *Nephrol. Dial. Transplant.* **36**, 1806–1820 (2021).
31. M. J. Blank, S. Q. Lew, Hypoventilation in a dialysis patient with severe metabolic alkalosis: Treatment by hemodialysis. *Blood Purif.* **9**, 109–113 (1991).
32. L. Huber, F. J. Gennari, Severe metabolic alkalosis in a hemodialysis patient. *Am. J. Kidney Dis.* **58**, 144–149 (2011).
33. D. Sethi, J. R. Curtis, D. L. Topham, P. E. Gower, Acute metabolic alkalosis during haemodialysis. *Nephron* **51**, 119–120 (1989).
34. M. E. Ostermann, Y. Girgis-Hanna, S. R. Nelson, J. B. Eastwood, Metabolic alkalosis in patients with renal failure. *Nephrol. Dial. Transplant.* **18**, 2442–2448 (2003).
35. H. Okada *et al.*, Daytime hypertension, sleep apnea and metabolic alkalosis in a haemodialysis patient—The result of sodium bicarbonate abuse. *Nephrol. Dial. Transplant.* **14**, 452–454 (1999).
36. C. R. F. Palacios, A. Altayeh, Q. Qian, Acute hypercapnic respiratory failure associated with hemodialysis. *Dial. Transplant.* **40**, 83–85 (2011).
37. L. Zhou *et al.*, Correction of lethal intestinal defect in a mouse model of cystic fibrosis by human CFTR. *Science* **266**, 1705–1708 (1994).
38. A. L. Viteri, J. W. Poppell, J. M. Lasater, W. P. Dyck, Renal response to secretin. *J. Appl. Physiol.* **38**, 661–664 (1975).
39. D. Kim *et al.*, Pendrin mediates bicarbonate secretion and enhances cystic fibrosis transmembrane conductance regulator function in airway surface epithelia. *Am. J. Respir. Cell Mol. Biol.* **60**, 705–716 (2019).
40. J. Simonin *et al.*, Airway surface liquid acidification initiates host defense abnormalities in cystic fibrosis. *Sci. Rep.* **9**, 6516 (2019).
41. L. A. Everett *et al.*, Targeted disruption of mouse Pds provides insight about the inner-ear defects encountered in Pendred syndrome. *Hum. Mol. Genet.* **10**, 153–161 (2001).
42. I. S. Jensen, C. K. Larsen, J. Leipziger, M. V. Sørensen, Na(+) dependence of K(+)-induced natriuresis, kaliuresis and Na(+) /Cl(−) cotransporter dephosphorylation. *Acta Physiol. (Oxf.)* **218**, 49–61 (2016).
43. F. Trepiccione *et al.*, Measurement of total CO_2 in microliter samples of urine and other biological fluids using infrared detection of CO_2. *Pflugers Arch.* **469**, 1267–1275 (2017).
44. N. Ayasse *et al.*, Benzamil-mediated urine alkalization is caused by the inhibition of H^+-K^+-ATPases. *Am. J. Physiol. Renal Physiol.* **320**, F596–F607 (2021).
45. J. C. Chan, The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. *Clin. Biochem.* **5**, 94–98 (1972).
46. J. E. Drorbaugh, W. O. Fenn, A barometric method for measuring ventilation in newborn infants. *Pediatrics* **16**, 81–87 (1955).
47. M. B. S. Svendsen, P. G. Bushnell, J. F. Steffensen, Design and setup of intermittent-flow respirometry system for aquatic organisms. *J. Fish Biol.* **88**, 26–50 (2016).
48. H. L. Christensen *et al.*, The choroid plexus sodium-bicarbonate cotransporter NBCe2 regulates mouse cerebrospinal fluid pH. *J. Physiol.* **596**, 4709–4728 (2018).

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