Original Article

Ventilatory pattern and energy expenditure are altered in cystic fibrosis mice

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Abstract

Background: Altered ventilatory pattern and increased energy expenditure are facets of the complex cystic fibrosis (CF) phenotype. It is not known whether these are inherent attributes of CF, secondary consequences of lung infection or other disease complications.

Methods: Studies were performed in congenic C57BL/6J, F508del (Cftrtm1kth) and CF gut-corrected (F508del) mice. Ventilatory patterns were measured using whole-body plethysmography. Indirect calorimetry was used to determine oxygen consumption, carbon dioxide production and resting energy expenditure.

Results: CF mice (F508del and F508del gut-corrected) have a significantly faster respiratory rate and increased ventilatory pattern variability as compared to non-CF mice. F508del but not CF gut-corrected mice had significantly increased energy expenditure per gram body weight.

Conclusions: CF mice exhibit a faster, more variable ventilatory pattern. These changes were present in the absence of detectable infection or illness due to gastrointestinal obstruction. Increased resting energy expenditure does not completely account for these differences.

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1. Introduction

Cystic fibrosis (CF) patients exhibit increases in both respiratory rate [1–3] and energy expenditure [4]. Changes in ventilatory pattern are thought to be related to the development of lung infection and disease progression; however, alterations in energy expenditure are associated with abnormal breathing patterns in other chronic pulmonary diseases [5]. In CF, elevated resting energy expenditure has been reported to be independent of pulmonary function and nutritional status. The degree to which changes in ventilatory pattern are apparent prior to the development of lung infection and gut obstruction is not known. If alterations in ventilatory pattern manifest in the absence of infection then other mechanisms must contribute to their underlying etiology. To begin to address this knowledge gap, we tested the hypothesis that the absence of functional Cftr in mice will result in differences in respiratory rate, ventilatory pattern variability and energy expenditure.

Increased breathing rate in humans with CF is one component of ventilatory pattern that has been reported, but the mechanisms are not completely understood [1]. Munder et al. [6] reported an increase in respiratory rate in CF mice with a hypomorphic mutation when compared to their wild type littermates. Development of infection and progression of lung disease are known to alter ventilatory patterns [7], but these may not be the only contributors to changes in breathing pattern in CF. There is evidence of a developmental role for CFTR in the lung [8] and diaphragm [9], which may influence pulmonary mechanics. Also, nitric oxide was reported to be decreased in the lungs of CF patients [10–12], and low levels of nitric oxide and subsequent

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reduction in airway relaxation have been linked to airway obstruction [13].

Another potential contributor to ventilatory pattern is energy homeostasis, in which elevated energy expenditure may be a “CF-specific” intrinsic defect affecting development of pulmonary disease and other co-morbidities. However, it is unknown if a relationship exists between energy expenditure and ventilatory pattern in CF.

As the CF mouse rarely shows signs of pulmonary infection, unless manipulated to do so, it provides a tool to examine the inherent differences in ventilatory pattern and energy expenditure in the absence of confounding factors such as infection. In addition, the capability to provide functional Cfr by transgene expression in the intestinal tract of CF mice permits characterization of the ventilatory pattern phenotype in the absence of illness due to gut obstruction. Further, CF mouse models are pancreatic sufficient and do not have apparent malabsorption [17] and thus serve as a model for the study of energy balance independent of malnutrition. Here we describe elevated respiratory rate and increased ventilatory pattern variability in CF mouse models, all in the absence of pulmonary infection, malnutrition and distress from gastrointestinal complications. Further, changes in resting energy expenditure do not completely account for these differences. The data provide compelling evidence that altered ventilatory pattern is an inherent feature of CF.

2. Methods

2.1. Mouse models

Two well-described mouse models of cystic fibrosis were examined: 1) F508del (Cftr\textsuperscript{tm1kth}) the murine version of the most common human CF mutation [18], and 2) the so-called “gut-corrected” mice (Tg(FABPCFTR)), in which the mice are homozygous for a mutation in the endogenous Cfr gene but expressing human CFTR from the rat fatty acid binding protein 1 promoter to prevent intestinal obstruction [19]. To make a better comparison between our strains of mice we substituted the F508del mutation (Cftr\textsuperscript{m1kth}) for the null mutation reported for the original gut-corrected mouse [19]. The F508del gut-corrected mouse with the F508del mutation displays a phenotype comparable to that reported for the null mutation [19,20]. Both of these CF mouse models are congenic on the C57BL/6J background, thus wild type C57BL/6J were used as controls. Each mouse model strain is backcrossed to the C57BL/6J background every five generations to account for possible genetic drift between strains. The Institutional Animal Care and Use Committee of Case Western Reserve University approved the experimental protocols. All mice used in the study were adults (at least 6 weeks old). Non-CF mice (n=11; 73% male) weighed 25±5 g, F508del mice (n=11, 45% male) weighed 13±4 g and FABP-F508del mice (n=6; 60% male) weighed 26±2 g. All mice were allowed unrestricted access to chow (Harlan Teklad 7960; Harlan Teklad Global Diets, Madison, WI) and sterile water with an osmotic laxative, Colyte (Schwarz Pharma, Milwaukee, WI), and were maintained on a 12 h light, 12 h dark cycle at a mean ambient temperature of 22 °C.

2.2. Whole-body plethysmography

Respiratory patterns were recorded in spontaneously breathing mice in a temperature equilibrated whole-body plethysmograph (sampling rate=200 Hz following an acclimatization period as described previously [21]. Pressure changes in the chamber were converted to signals representing ventilatory pattern, passed through a pre-amplifier (Max II, Buxco Electronics), acquired (Power1401, CED, Cambridge, UK) and stored with respiratory acquisition software (Spike 2, CED) for further analysis of breathing-pattern dynamics. Ventilatory pattern was quantified for 60 s epochs. Average values of three epochs are reported. Augmented breaths, sighs, swallows, obvious movements and gasps were excluded from the analysis, and the animals were recorded under comparable conditions.

2.3. Indirect calorimetry

Oxygen consumption (VO\textsubscript{2}), carbon dioxide production (VCO\textsubscript{2}), and respiratory exchange ratio (RER) were determined using an Oxymax indirect calorimetry system (Columbus Instruments, Columbus, Ohio). Non-CF (n=4), F508del (n=4) and CF gut-corrected (n=3) mice were weighed and placed individually into enclosed plastic indirect calorimetry chambers, and given access to ad libitum water and food supply. The instrument was calibrated against a standard gas mixture containing defined quantities of oxygen, carbon dioxide, and nitrogen. After brief acclimatization and instrument calibration, collection of experimental data began and proceeded for ~24 h under 12:12-h light–dark cycle at room temperature. Total energy expenditure was computed using a modified Weir equation (TEE = (3.815 + 1.232 × RER) × VO\textsubscript{2}).

2.4. Statistical analysis

Linear and information theory-based analytical techniques were applied to stationary, three artifact-free epochs (60 s) of the raw plethysmography signal as described previously [22,23]. Respiratory cycle length (T\textsubscript{TOT}) was determined for each breath. Mean respiratory rate (RR) and coefficient of variation of respiratory cycle length (CV − T\textsubscript{TOT} = SD/mean) were calculated for each epoch and averaged to yield a value for each mouse. Autocorrelation functions were computed for each epoch [22,23] and showed characteristic relationships with the cycle length. We report the autocorrelation coefficient at the average respiratory cycle length as a measure of the strength of linear correlations in the time series.

An index of nonlinear complexity [22,23] was generated by comparing the absolute value of statistically significant differences
between the sample entropy of surrogate data sets and that of original data. As the breathing pattern is overall highly periodic, we computed the nonlinear complexity from adjacent points (separated by 5 ms) to one cycle length. Surrogate data sets (n = 19) were constructed by shuffling the raw plethysmography recording while maintaining the amplitude distribution and the autocorrelation function of the original data set. This preserves linear correlations but destroys nonlinear relationships in the time series.

Data are presented as means±standard deviation. A one-way ANOVA was performed across the three groups (non-CF, F508del and F508del gut-corrected); if statistical significance was found (p < 0.05), then an adjustment for multiple comparisons (Student–Newman–Keuls Method) was performed to determine significance among groups.

3. Results

3.1. Respiratory rate

We used whole-body plethysmography to characterize breathing patterns in non-CF (C57Bl/6J) and CF (F508del and F508del gut-corrected) mice (Fig. 1A). The F508del CF mice showed a significantly faster respiratory rate as compared to non-CF mice (227±50 vs. 179±28 breaths per minute; p=0.017; Fig. 1B). Similarly, CF F508del gut-corrected mice had breathing frequencies (270±58 breaths per minute; Fig. 1B) that were significantly faster than non-CF mice (p=0.001) and F508del gut-corrected (0.06±0.01 s; p<0.001) mice. Likewise, expiratory time tended to be longer in non-CF mice (0.21±0.04 s) than F508del (0.18±0.04 s; p=0.054) and F508del gut-corrected (0.15±0.02 s; p=0.014) mice.

3.2. Ventilatory pattern

F508del CF mice exhibited greater variability in their ventilatory patterns. Coefficient of variation (CV) of respiratory cycle length (Fig. 1C) was significantly higher in both F508del (0.27±0.08; p=0.026) and F508del gut-corrected mice (0.39±0.07; p<0.001) than in non-CF mice (0.19±0.05). This increased variability was evident in the CV of the duration of inspiration and expiration. CV of inspiratory time in non-CF mice (0.15±0.05) was significantly lower than F508del (0.22±0.06; p=0.011) and F508del gut-corrected (0.39±0.03; p<0.001) mice. Likewise, CV of expiratory time was lower in non-CF mice (0.26±0.10) than F508del (0.39±0.11; p=0.046) and F508del gut-corrected (0.49±0.10; p<0.001) mice.

To further quantify differences in ventilatory pattern variability the autocorrelation coefficient was measured at a time lag corresponding to the average respiratory cycle length (Fig. 2A). The autocorrelation coefficient tended to be lower in F508del (0.33±0.19; p=0.061) and F508del gut-corrected (0.24±0.11; p=0.035) mice as compared to non-CF controls (0.47±0.17). A measure of nonlinear complexity of the ventilatory waveform
was similar among the non-CF control mice (0.17±0.03 bits), F508del mice (0.17±0.07 bits) and F508del gut-corrected mice (0.11±0.02 bits) (Fig. 2B, ANOVA p=0.073).

3.3. Energy expenditure

As alterations of respiratory pattern may be related to differences in energy expenditure, we measured carbon dioxide production and oxygen consumption, and calculated total energy expenditure per kilogram body weight over a 24-hour period. Overall patterns are shown in Fig. 3. We found that F508del CF mice had significantly elevated average 24-hour total energy expenditure (F508del CF: 25.0 ± 3.0 vs. non-CF: 19.1±0.9 Kcal/kg/h, p = 0.007, Fig. 4). Interestingly, the F508del gut-corrected mice had 24-hour total energy expenditure (19.4 ± 0.8 Kcal/kg/h) which was significantly lower than F508del CF mice (p = 0.006), and appeared more similar to non-CF mice (p = 0.842). These relationships persisted when dark (active) and light (sleep) periods were analyzed separately (Fig. 4).

4. Discussion

4.1. Rationale and results

Pulmonary pathology is the primary cause of CF-related morbidity and mortality, and identifying an animal model that spontaneously exhibits a pulmonary phenotype consistent with CF human disease continues to be a high priority. Although spontaneous pulmonary infections have not been reported in CF mice, these animals do exhibit altered airway mechanics including impaired relaxation, apparently due to decreased nitric oxide production [13]. In addition, CF mice have increased airway resistance and decreased compliance [24], further demonstrating functional changes in the murine CF airway in the absence of infection and, presumably, inflammation. These findings suggest that although the CF mouse may not fully recapitulate CF pulmonary disease, it may still have significant utility in understanding intrinsic properties of CF pulmonary physiology.

The overall goal of the present study was to investigate correlates of human CF pulmonary phenotypes in CF mice. The mice utilized in this study were congenic in order to minimize...
There are a number of potential mechanisms that might explain the observed differences in ventilatory pattern and energy expenditure. First, F508del mice exhibit a relatively strong propensity for intestinal obstruction, and are very small in both length and weight when compared to non-CF littermates [20]. In order to determine if the increased respiratory rate observed in these mice was attributable to their overall health rather than a specific pulmonary manifestation, we examined the breathing rates of the F508del gut-corrected mice. These mice are completely protected from the obstruction phenotype of the F508del mouse, and overall are healthier [20]. In our study, the F508del gut-corrected mice also exhibited an increased respiratory rate compared to non-CF mice, indicating that this phenotype is not a consequence of distress resulting from intestinal dysfunction.

Second, energy homeostasis can alter respiratory patterns, and we hypothesized that differences in metabolic rate might underlie the increase in respiratory frequency. F508del mice had higher resting energy expenditure than non-CF mice. Changes in cardiac output may alter energy expenditure. We did not quantify cardiac output, but studies in CF patients have shown a cardiac limitation to exercise, but rather heart rate and ventilatory responses consistent with a pattern of ventilatory limitation [26,27]. Thus, it is unlikely that altered cardiac output in the CF mice is a key determinant of energy expenditure. However, we did not measure heart rate or LV stroke volume in our mice. Thus, our data cannot exclude the possibility that changes in circulation may contribute to the observed differences in energy expenditure.

It is also possible that increased work by respiratory muscles may contribute to this elevated energy expenditure. Cftr dysfunction in respiratory and skeletal muscles has been shown to contribute to altered metabolism and breathing rate [9,28]. The causal relationship between these differences in energy expenditure and ventilatory pattern remains to be determined. Interestingly, the higher resting energy expenditure observed in the F508del mice was not apparent in the F508del gut-corrected mice. Both CF mouse models (F508del and F508del gut-corrected) have increased respiratory rates, however only the F508del mice have elevated energy expenditure levels. Taken together, these findings suggest that increased energy expenditure is not solely responsible for the increased respiratory rate.

Systemic factors such as anemia [29] and fever [30] can increase respiratory rate. Further, anemia is a common complication of CF with an incidence that increases with age [31]. Body temperature and hemoglobin levels were not measured in this study. Thus, we cannot exclude the possibility that changes in these physiologic parameters may contribute to the observed differences in ventilatory pattern.

Differences in pulmonary structure and function may also alter ventilatory pattern. The pulmonary and respiratory system in CF patients is characterized by many features, including mucus plugging, impaired mucociliary clearance, infection, inflammation, airway hyper-reactivity, reduced airway compliance, bronchiectasis and fibrosis. While mucus plugging appears to be an early manifestation of the CF airways, the temporal characteristics of the other traits are not clear as few studies are carried out in very young patients and thus are difficult to assess in the absence of lung infection. The airways of the CF mouse, in contrast, do not appear to display mucus plugging or impaired clearance, and no signs of bronchiectasis have been reported.

Fig. 4. Average total energy expenditure per kg body weight is shown for the total 24-hour period, and for the dark (active) and light (rest) phases for non-CF (C57BL/6J) (black bars), CF F508del (white bars) and CF F508del gut-corrected (gray bars) mice. Data represent average values± standard deviation. *p<0.05 compared to non-CF (C57BL/6J) mice; #p<0.05 compared to CF F508del gut-corrected mice.
Older CF mice do, however, develop signs of altered lung architecture, mount an inflammatory response that is slow to resolve, and even show signs of airway hyperreactivity [32,33]. However, the mice used in this study were young adults, suggesting that these changes were unlikely to fully explain the observed differences in ventilatory rate and pattern. In addition, the mice utilized in this study showed no evidence of acute infection despite routine monitoring with post-mortem cultures of bronchoalveolar lavage fluid. It is possible, however, that alterations in airway physiology may contribute to the observed phenotype. For example, iNOS expression is decreased in murine and human CF epithelial cells [34]. Studies in CF mice [13] identified decreased nitric oxide (NO) levels, impaired smooth muscle relaxation and increased airway resistance, which can potentially contribute to changes in frequency and ventilatory pattern.

Finally, CFTR is widely, if not ubiquitously expressed, and the F508del mice used in this study had systemic reduction of functional Cftr. Thus, it is possible that this reduced function in extra-pulmonary cell types may contribute to the observed phenotype. Bonora et al. [35] found that CF mice have a normal ventilatory response to hyperecapnia and decreased ventilatory response to hypoxia, suggesting that neuronal ventilatory drive may be altered. However, these studies were conducted in a different mouse strain (129/BC) with a different Cftr mutation (S489X) than the mice used in our study. The importance of Cftr function in neurons or muscle cells responsible for orchestration of the ventilatory pattern will be the focus of future studies.

4.3. Clinical implications of ventilatory pattern in CF

In addition to having a faster breathing rate, both F508del and F508del gut-corrected mice exhibit a higher coefficient of variation of respiratory cycle length than non-CF mice. Moreover, the autocorrelation coefficient measured at the average respiratory cycle length was lower for the CF than for the non-CF mice. The decrease in autocorrelation coefficient, a measure of linear correlation within the ventilatory waveform, is consistent with the observed increase in coefficient of variation of respiratory cycle length. Nonlinear determinants of variability can also contribute to differences in ventilatory pattern, and changes in nonlinear variability have been described with the development of respiratory disease [21]. However, no significant differences in nonlinear complexity were detected. Taken together, these findings suggest that changes in linear structure contribute to the greater breath-to-breath variation in CF mice.

The identification of differences in respiratory pattern in CF mice has potential clinical implications. More frequent, variable breaths might lead to heterogeneity in the inflation of distal lung units with the possibility of promoting regions of alveolar collapse and subsequently more anaerobic conditions for bacterial growth. More frequent breaths would also have a desiccating effect on already reduced airway surface liquid, further exacerbating the CF airway phenotype. The relationship between increased respiratory rate and CF disease progression remains to be determined. Even in the absence of a clinical mechanism, ventilatory pattern differences could be a useful marker to quantify disease severity or measure the efficacy of pharmaceutical interventions, with the potential advantage that respiratory rate measurements may be more sensitive to subclinical changes in overall health.

4.4. Conclusions

Increased respiratory rate has been observed in CF patients with advanced stages of lung disease and has been thought to be attributable to infection, fibrosis, or exercise [1–3]. We have observed increased respiratory rates in CF mice at rest and in the absence of detectable infection or fibrosis. It is unlikely that increased resting energy expenditure is solely responsible for these differences, as F508del gut-corrected mice breathe fast, but do not have increased energy expenditure. These data suggest that altered ventilatory pattern variability, including markedly increased respiratory rate, may be in part an inherent result of the underlying genetic defect in CF, rather than a symptomatic reaction to acute pulmonary pathology. In addition, tracking changes in ventilatory pattern may hold promise as a novel biomarker to augment current well-established screening and surveillance methods for CF lung disease progression.

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