An important function of the colon is absorption of sodium and water.[1] In the human descending colon, a large fraction of electrogenic transepithelial transport is due to sodium reabsorption. This process takes place by a mechanism, which involves a transcellular pathway dependent on apical epithelial sodium channels (ENaC) and the basolateral Na, K-ATPase.[2-4] This absorptive pathway is a target for aldosterone action.[13] Aldosterone increases electrogenic sodium absorption, decreases colonic crypt permeability,[6] and prevents back-leakage.[7]

Besides contributing to conservation of sodium chloride and water, the normal function of electrogenic sodium absorption in the distal colon is important for the proper dehydration of feces. The derangement of electrogenic sodium absorption is a major cause of nonsecretory diarrhea in inflammatory bowel disease.[8] Impaired electrogenic sodium absorption has been demonstrated both in ulcerative colitis[9] and in microscopic colitis,[10] probably caused by inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β.[11,12] Anti-inflammatory treatment is associated with an improvement in electrogenic sodium absorption.[10,13]

Electrogenic ion transport is dependent on oxidative energy metabolism[14] and demands a continuous oxygen supply.[15] Despite the well-known oxygen dependence of epithelial ion transport, there are relatively few papers published on colonic epithelium oxygen consumption under conditions preserving vectorial ion transport, and these have studied
nonhuman species, for example, rabbits\textsuperscript{16} and rats\textsuperscript{17}. We have recently reported data for the human colon, indicating that a significant fraction of epithelial oxygen consumption is associated with electrogenic sodium transport.\textsuperscript{18}

In the present report, we characterize the relationship between amiloride-sensitive electrogenic sodium transport and epithelial oxygen consumption in the human colon. Additionally, we assessed whether the age of the subject influences the effect of amiloride on short-circuit current or oxygen consumption.

**MATERIALS AND METHODS**

**Recruitment of volunteers**

We carried out this study in compliance with the 6\textsuperscript{th} Revision (2008) of the Declaration of Helsinki. The protocol was approved by the Committee on Bioethics of our Faculty of Medical Sciences. We secured written informed consent from patients who were scheduled to undergo extirpation of left colon adenocarcinoma. We obtained samples from 10 patients (4 female) with an age range of 62–77 years.

**Preparation of mucosal samples**

We took a 3-cm long segment (ring) of the sigmoid colon from each patient. Each selected segment was considered free of disease upon visual inspection by the operating surgeon. We rinsed the segments free of debris and placed them in an oxygen-saturated solution, kept at 4°C, and immediately carried to the laboratory. We dissected each segment to obtain an isolated mucosa preparation as previously described.\textsuperscript{18} We cut and immediately fixed a portion of the preparation for standard light microscopy to confirm the extent of dissection and the lack of signs of disease. Then, we mounted the remaining tissue as a flat sheet in a modified Ussing chamber.

**Ussing chamber**

We used a modified Ussing chamber, which could be hermetically closed to allow continuous monitoring of oxygen concentration through polarimetric oxygen probes, as previously described.\textsuperscript{17,18} The chamber has a window of 1 cm\textsuperscript{2}. Each hemichamber has a small magnetic bar in its bottom for continuous mixing of contents when placed on a magnetic stirrer (HI 300N, Hannah Instruments, Woonsocket, Rhode Island, USA). Each hemichamber has a bubble trap through which drugs may be injected, and a port for inserting a polarimetric oxygen probe (CellO × 325) connected to WTW Oxi 340 oxygen meter (WTW GmbH, Oberbayern, Germany). The probes allow continuous measurement of oxygen concentration and temperature. Knowing the volume of the chamber, we calculated the oxygen consumption rate from the change in oxygen concentration, taking into account the solubility of oxygen in solution at 37°C.

**Solution and amiloride**

We filled the chamber with a solution containing 145 mM NaCl, 1.6 mM K\textsubscript{2}HPO\textsubscript{4}. Units of concentration (mM) are lacking for KH\textsubscript{2}PO\textsubscript{4}, MgCl\textsubscript{2}, and CaCl\textsubscript{2} and 5 mM d-glucose (pH 7.40). We gassed the solution with 100% oxygen to saturation. For each experiment, we freshly dissolved amiloride (Sigma-Aldrich, Saint Louis, Missouri, USA) in dimethyl sulfoxide to yield a final concentration in the mucosal side of the chamber of 0.1 mmol/L.

**Mucosal electrophysiology**

Calomel electrodes were connected to each hemichamber through 3% agar-in Ringer bridges to record transepithelial potential difference. An amplifier, with correction for bridge asymmetry and solution resistivity, allowed passing current through Ag/AgCl\textsubscript{2} electrodes for clamping the transepithelial potential difference at 0 mV. Transepithelial resistivity (specific resistance) was calculated according to Ohm’s law.

**Experimental protocol**

We performed the experiments at 37°C, continuously recording short-circuit current, except for periodic releases to measure open-circuit transepithelial potential difference.

When the short-circuit current reached a plateau, we measured baseline oxygen consumption during a 30-min period. We then added amiloride. After the short-circuit current was again stable, we measured oxygen consumption for a second 30-min period.

**Statistical analysis**

We performed the statistical analysis with Prism for Windows, version 5.04 (Graph Pad, San Diego, CA, USA). We evaluated changes in electrophysiological variables and oxygen consumption with a two-sided Student’s t test for paired samples, after checking that the data did not significantly deviate from a Gaussian distribution with D’Agostino and Pearson omnibus normality test. We assessed the relationship between changes in oxygen consumption and short-circuit current caused by amiloride by linear regression analysis with a check for significant deviation from linearity. We also performed a regression analysis to check whether the age of the subjects had any influence on short-circuit current or oxygen consumption. We chose a significance level of 0.05 and report the data as mean ± standard error of mean.

**RESULTS**

None of the samples studied showed signs of neoplastic disease or inflammation by light microscopy. Figure 1 shows
a typical result, namely, a healthy epithelial sheet virtually free of submucosal tissue.

In Table 1, we show values of short-circuit current, transepithelial potential difference, transepithelial resistivity, and oxygen consumption rate at baseline and after amiloride addition. Amiloride caused a 48.2 ± 4.7 µA/cm² decrease in short-circuit current and a 1.86 ± 0.17 µmol/h/cm² decrease in oxygen consumption rate, representing reductions of approximately 80% and 26%, respectively. Transepithelial potential difference decreased by 75% and transepithelial resistivity increased by 16%. All of these changes were statistically significant (P < 0.0001).

We found no significant relationship with regression analysis between age of the subjects and baseline short-circuit current (P = 0.860), baseline oxygen consumption rate (P = 0.234), change in short-circuit current (P = 0.981), or change in oxygen consumption rate (P = 0.947) after addition of amiloride [Figure 2]. In no case did the relationship deviate significantly from linearity, nor was the slope significantly different from zero (all P > 0.05).

On the other hand, we found a significant linear correlation between the decrease in short-circuit current and the decrease in oxygen consumption rate after addition of amiloride, such that the latter decreased by 0.04 µmol/h/cm² for each µA/cm² of reduction in the former. The result of the regression analysis between oxygen consumption rate and short-circuit current is shown in Figure 3.

**DISCUSSION**

Baseline values of short-circuit current measured in this study are similar to those previously reported by us[18] and other authors.[19,20] Present data on baseline oxygen consumption rate are also in agreement with our previous work.[18] In that report, ouabain added to the serosal hemichamber caused a large decrease in short-circuit current and oxygen consumption rate, but the number of observations was too small for assessing correlation.[18]

| Table 1: Electrophysiological variables and oxygen consumption in the isolated mucosa of human sigmoid colon |
|--------------------------------------------|
| **Short-circuit current (µA/cm²)** | Baseline | Amiloride |
|--------------------------------------------|
| Short-circuit current (µA/cm²) | 58.5±4.9 | 10.0±1.1* |
| Transepithelial potential difference (mV) | 6.67±0.55 | 1.29±0.13* |
| Transepithelial resistivity (Ω·cm²) | 115.0±4.2 | 131.4±4.1* |
| Oxygen consumption (µmol/h/cm²) | 7.02±0.18 | 5.16±0.22* |

Amiloride was added to the mucosal hemichamber for a final concentration of 0.1 mmol/L. Values are mean±SEM. Data were analyzed with a two-sided Student’s t test for paired samples. *All differences between columns are significant at P<0.0001
Similarly, the magnitude of the effect of amiloride on short-circuit current is within the range reported by other researchers.\textsuperscript{(2,21)} We are not aware of reports on the effect of amiloride on epithelial oxygen consumption rate. Our present data show that amiloride causes a significant decrease in oxygen consumption rate, and that this decrease is highly correlated with the decrease in short-circuit current caused by the drug. This indicates that electrogenic sodium transport demands about one quarter of the total oxygen consumption of this epithelium. Therefore, this report corroborates the close relationship between sodium absorption and aerobic metabolism in the human sigmoid colon.

All isolated mucosa samples were obtained from the sigmoid colon of subjects older than 60 years. Because it is known that the sodium reabsorption capacity of the kidney declines with age,\textsuperscript{(21)} there might be some concern regarding whether present results are significantly affected by the age of the subject. However, within the age range of our participants (62–77 years), no significant relationship was found between age and short-circuit current or oxygen consumption rate, or their decreases caused by amiloride. This finding agrees with the results of Greig et al. regarding electrogenic transport in human sigmoid colon.\textsuperscript{(22)} They found no significant differences in subjects who were 20–40 years of age and in subjects older than 70 years in the expression of ENaC and Na-, K-ATPase, baseline transepithelial potential difference, response to amiloride, or response to a synthetic mineralocorticoid. They concluded that, unlike what is observed in the distal nephron, electrogenic sodium absorption does not decline with age in the normal human distal colon.\textsuperscript{(22)} Our own findings additionally suggest that the coupling between electrogenic transport and oxygen consumption is preserved even at advanced age.

From a clinical viewpoint, our results suggest that derangements in ion transport observed in inflammatory bowel diseases\textsuperscript{(9,10)} might be, at least in part, due to reduced oxygen availability for ATP synthesis, for example, due to increased utilization of oxygen for the generation of reactive oxygen species. This hypothesis may be tested in epithelial samples from patients with colitis.

We should point out some limitations of our study. First, our results come from a convenience sample. Second, the size of the sample did not allow us to test whether there are gender differences in electrogenic transport and oxygen consumption, influence of age, or effect of amiloride. Third, the age range of the patients was limited to just 15 years.

**CONCLUSION**

The present report shows that amiloride proportionally lowers sodium absorption and oxygen consumption in the human distal colon under conditions which preserve vectorial ion transport. These effects of amiloride, which corroborate a tight coupling between electrogenic transport and oxygen consumption, is not influenced by the age of the subjects.

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**REFERENCES**

1. Kunzelmann K, Mall M. Electrolyte transport in the mammalian colon: Mechanisms and implications for disease. Physiol Rev 2002;82:245-89.
2. Sandle GI, Wills NK, Alles W, Binder HJ. Electrophysiology of the human colon: Evidence of segmental heterogeneity. Gut 1986;27:999-1005.
3. Sellin JH, De Soignie B, Ion transport in human colon in vitro. Gastroenterology 1987;93:441-8.
4. Sandle GI, McGlone F. Segmental variability of membrane conductances in rat and human colonic epithelia. Implications for Na, K and Cl transport. Pflugers Arch 1989;414:706-12.
5. Sandle GI. Segmental heterogeneity of basal and aldosterone-induced electrogenic Na transport in human colon. Pflugers Arch 1989;414:706-12.
6. Moretó M, Cristià E, Pérez-Bosque A, Afzal-Ahmed I, Amat C, Naftalin RJ. Aldosterone reduces crypt colon permeability during low-sodium adaptation. J Membr Biol 2005;206:43-51.
7. Amasheh S, Milatz S, Krug SM, Bergs M, Amasheh M, Schulze JD, et al. Na+ absorption defends from paracellular back-leakage by claudin-8 upregulation. Biochem Biophys Res Commun 2009;378:45-50.
8. Martínez-Augustin O, Romero-Calvo I, Suárez MD, Zarzuelo A, Sánchez de Medina FS. Molecular bases of impaired water and ion movements in inflammatory bowel diseases. Inflamm Bowel Dis 2009;15:114-27.
9. Sandle GI. Pathogenesis of diarrhea in ulcerative colitis. New views on an old problem. J Clin Gastroenterol 2005;39(Suppl 2):S49-52.
10. Barmeyer C, Erko I, Fromm A, Bojarski C, Allers K, Moos V, et al. Ion transport and barrier function are disturbed in microscopic colitis. Ann N Y Acad Sci 2012;1258:143-8.
11. Amasheh S, Barmeyer C, Koch CS, Tavalali S, Mankertz J, Epplle HJ, et al. Cytokine-dependent transcriptional down-regulation of epithelial sodium channel in ulcerative colitis. Gastroenterology 2004;126:1711-20.
12. Barmeyer C, Amasheh S, Tavalali S, Mankertz J, Zeitz M, Fromm M, et al. IL-1beta and TNFalpha regulate sodium absorption in rat distal colon. Biochem Biophys Res Commun 2004;317:500-7.
13. Sandle GI, Hayslett JP, Binder HJ. Effect of glucocorticoids on rectal transport in normal subjects and patients with ulcerative colitis. Gut 1986;27:309-16.
14. Latella G, Caprilli R. Metabolism of large bowel mucosa in health and disease. Int J Colorectal Dis 1991;6:127-32.
15. Guebel DV, Torres NV. A computer model of oxygen dynamics in human colon mucosa: Implications in normal physiology and early tumor development. J Theor Biol 2008;250:389-409.
16. Durand J, Durand-Arzenska W, Wankmüller D. Coupling of active sodium transport to oxidative metabolism in the rabbit distal colon. J Physiol 1989;396:55-64.
17. Saravi FD, Saldeña TA, Carrera CA, Ibáñez JE, Cincunegui LM, Carra GE. Oxygen consumption and chloride secretion in rat distal colon isolated mucosa. Dig Dis Sci 2003;48:1767-73.
18. Carra GE, Ibáñez JE, Saravi FD. Electrogenic transport, oxygen...
consumption, and sensitivity to hypoxia of human colonic epithelium. Int J Colorectal Dis 2011;26:1205-10.

19. Stack WA, Filipowicz B, Hawkey CJ. Nitric oxide donating compounds stimulate human colonic ion transport in vitro. Gut 1996;39:93-9.

20. Taylor J, Hamilton KL, Butt AG. HCO3- potentiates the cAMP-dependent secretory response of the human distal colon through a DIDS-sensitive pathway. Pflugers Arch 2001;442:256-62.

21. Martin JE, Sheaff MT. Renal ageing. J Pathol 2007;211:198-205.

22. Greig ER, Mathialahan T, Boot-Handford RP, Sandle GI. Molecular and functional studies of electrogenic Na(+) transport in the distal colon and rectum of young and elderly subjects. Gut 2003;52:1607-15.

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