# Assessment and pharmacological correction of abnormalities in chloride (Cl-), bicarbonate (HCO3-) and mucus transport in rectal biopsies of CF patients

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We aim to fill up the present gap in knowledge about HCO3- and mucus transport in native human intestine, and about possible effects of pharmacological correctors on these parameters in CF, by detailed electrophysiological and biochemical ex vivo...

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| **Ethical review** | Approved WMO |
| **Status** | Recruiting |
| **Health condition type** | Congenital and hereditary disorders NEC |
| **Study type** | Observational invasive |

## Summary

### ID

NL-OMON43631

**Source**
ToetsingOnline

**Brief title**
Anion and mucus secretion in CF patients

### Condition

- Congenital and hereditary disorders NEC

**Synonym**
Cystic Fibrosis, Mucoviscidosis

**Research involving**
Human

### Sponsors and support

**Primary sponsor:** Universitair Medisch Centrum Utrecht

**Source(s) of monetary or material Support:** Nederlandse Cystic Fibrosis Stichting

## Intervention

**Keyword:** Bicarbonate, CF, Correctors, Intenstinal Current Measurement

## Outcome measures

### Primary outcome

HCO3- and mucus transport in native human intestine, and about possible effects of pharmacological correctors on these parameters in CF patients and controls, by measuring:

1. Comparative current measurements in the presence and absence of HCO3- and carbonic anhydrase (Car) inhibitors.

2. pH-stat measurements of total HCO3- secretion to determine wether an electroneutral component contributes tot colonic HCO3- secretion.

3. Measuring HCO3- transport in the presence of bestrophin-2 inhibitors.

4. Cl-, HCO3- and mucustransport in biopsies of CF patients incubated with CFTR correctors.

5. HCO3- transport in distal colon incubated with PPAR-γ agonists or NHE3 inhibitor.

### Secondary outcome

Confocal microscopy will be applied to determine possible changes in expression

and localization of Best2 in the CF biopsies.

Mucus stasis will be assessed by Alcian Blue staining of the biopsies [11,14,17], and expression and (re)distribution of transporters (CFTR, Best2) and enzymes (Car4, Car2) will be examined by immunostaining and confocal microscopy [11,14].

# Study description

### Background summary

Accumulation of viscid mucus in the lung, intestine, pancreas, hepatobiliary tract, and reproductive tract is a hallmark of cystic fibrosis (CF; *mucoviscidosis*) and is the primary cause of defective mucociliary clearance in the airways and of luminal obstruction in the GI and reproductive tract. Recent studies in CF mouse models indicate that normal mucus release in intestinal epithelium requires concurrent bicarbonate (HCO3-) secretion and that aggregated mucus is a consequence of defective transepithelial HCO3- transport in CF [1]. Moreover the pancreatic phenotype of CF patients segregates well with mutations in the gene for CF transmembrane conductance regulator (CFTR) that severely disrupt CFTR-dependent HCO3- transport [2]. However, in contrast to the wealth of information about the chloride transport defect in native epithelia from both CF mice and CF patients emerging from in vivo and ex vivo assays (e.g. sweat test; measurements of nasal potential difference, NPD; intestinal current measurements in human rectal biopsies, ICM [3-9]), there is as yet a paucity of data about bicarbonate secretion and mucus release in native epithelia from both healthy individuals and CF patients. Whereas sweat tests and NPD measurements are only suitable to monitor possible defects in Cl- and Na+ transport, ICM performed in mini-Ussing chambers is the sole technique capable of measuring both electrogenic Cl- and HCO3- secretion. However sofar this technique has been developed and exploited by us [3-6] and others[7,8] solely for diagnostic purposes, and a possible contribution of HCO3- to the transepithelial anion current has not been evaluated yet or was even precluded by the use of HCO3- -free bath fluid in the Freiburg protocol [7,8].
Traditionally, intestinal bicarbonate secretion has been studied mainly or exclusively in the duodenum (DBS) [9,10], ignoring potential contributions from the colon. However, very recent studies in distal colon of CF mice and of mice lacking bestrophin-2 (Best2), a Ca2+-activated anion channel, have shown that

(1) a large part of the plateau phase of carbachol-induced anion currents is HCO3- -and Best2-dependent, and (2) Best2 is localized basolaterally in mouse and human colonic goblet cells and, in concert with apical Cl-/HCO3- exchangers, may enable Ca2+-stimulated transepithelial HCO3- secretion in parallel with mucin secretion [11] . In this way, HCO3- may promote mucin volume expansion and release by raising the local pH and sequestering Ca2+ [12]. In addition, colonic crypt cells may secrete HCO3- through basolateral NBC1 Na+ / HCO3- cotransporters and apical CFTR channels, and brushborder cells at the epithelial surface may cause net secretion of HCO3- by the apical SLC26a3 Cl-/HCO3- exchanger under conditions that the NHE3 Na+/H+ exchanger is inhibited by Ca2+-, cAMP-or cGMP-linked secretagogues [10,13].

Importantly, colonic HCO3- secretion is expected to be reduced or abolished in CF at multiple levels: ill-functioning of CFTR, acting itself as a HCO3- channel or as a Cl- shunt pathway to facilitate SLC26-mediated HCO3- secretion10 ; reduced expression and redistribution of Best2[11] ; and reduced expression of HCO3- -producing enzymes, i.e. the carbonic anhydrases (Car4, Car2) in mouse colon [14].

Sofar, studies of colonic HCO3- secretion and mucus release have been carried out exclusively in rodent models, and data on human colon are completely lacking. Furthermore, colonic HCO3- secretion has been assessed only by transepithelial current measurements, ignoring the contribution of electroneutral components.
Surprisingly, even less information is as yet available about the ability of the novel CFTR correctors emerging from high-throughput screens (e.g. the Vertex and Verkman correctors, including VX-809 that is presently tested in clinical trials) to correct defects in Cl-, HCO3- and mucin release in addition to their known ability to improve transepithelial Cl- secretion [15.] Unfortunately none of the outcome parameters monitored in the clinical trials sofar (NPD, sweat test, FEV1, etc.) provides direct information on HCO3- and mucin secretion in the CF affected epithelia. Therefore, additional studies focusing on such measurements in an easily accessible tissue, i.e. rectal biopsies, are urgently needed.

**Study objective**

We aim to fill up the present gap in knowledge about HCO3- and mucus transport in native human intestine, and about possible effects of pharmacological correctors on these parameters in CF, by detailed electrophysiological and biochemical ex vivo studies in rectal biopsies from CF patients and healthy subjects mounted in Ussing chambers
[3-6].
The following questions will be investigated:

1. How much of the basal and secretagogue-stimulated anion current in non-CF and CF biopsies is carried by Cl- and how much by HCO3-?

Comparative current measurements in the presence and absence of HCO3- and carbonic anhydrase (Car) inhibitors in the serosal bath will provide insight into the electrogenic component of HCO3- secretion in CF and non-CF biopsies. It is anticipated that electrogenic HCO3- secretion, in analogy to mouse distal colon, is reduced in CF, by multiple causes: defective transport through the CFTR channel itself; defective basolateral transport through the Best2 anion channel in goblet cells which is shown to be downregulated and redistributed in CF[11]; and downregulation of carbonic anhydrases in CF as a consequence of pathological reprogramming of gene expression caused by defective stimulation of the transcription factor PPAR-γ [14].

2. Does an electroneutral component contribute to colonic HCO3- secretion and is this component altered in CF and modulated by colonic secretagogues?
The electroneutral component (most plausibly reflecting SLC26a3-mediated Cl-/HCO3- exchange unbalanced by NHE3-mediated Na+/H+ exchange) can be infered from pH-stat measurements of total HCO3- secretion (electrogenic+ electroneutral) into the luminal bath followed by subtraction of the electrogenic component. We expect that this component is enhanced by secretagogues capable of inhibiting NHE3 but not SLC26a3, i.e. Ca2+-, cAMP- and cGMP-linked hormones, neurotransmitters and microbial enterotoxins [10,13] . Whether this component is reduced too in CF rectal biopsies is more difficult to predict considering the controversial evidence for a loss of cAMP-inhibition of NHE3 in CF intestine (reported to occur in the jejunum of CF mice but not in the jejunum of CF patients [16]). In addition, the effect of specific pharmacological inhibitors of NHE3 on colonic HCO3- and mucin secretion will be examined, encouraged by recent studies in Cftr-/- mice showing that the additional KO of NHE3 restored luminal hydration, mucin release and (most plausibly) HCO3- secretion and protected the mice against lethal intestinal obstruction [17].

3. Is the calcium-activated anion channel bestrophin-2 involved in HCO3- secretion in human colon, and down-regulated/redistributed in distal colon of CF patients?
In human distal colon, similar to mouse colon, Best2 is localized in the basolateral membrane of the mucin-secreting goblet cells[14], but it is unclear whether human Best2 serves as a HCO3- importer and is likewise downregulated by ~80% in CF colon, as reported for Cftr-/- mice[14]. Because the survival time of rectal biopsies (~24h) is too short to allow efficient siRNA-induced knockdown (KD) of Best2, the use of pharmacological inhibitors is the only way to evaluate the contribution of Best2 to HCO3- secretion in human colon. Surprisingly, the cyclo-oxygenase inhibitor indomethacin was recently shown to act as a rather specific inhibitor of Best2 anion channels[14], allowing us to assess the contribution of Best2 to carbachol/Ca2+-induced secretion under conditions in which we compensate for the simultaneous indomethacin-induced inhibition of cyclo-oxygenases/prostaglandin synthesis by adding PGE2 exogenously. This compensation is needed because endogenous prostaglandins in human rectal biopsies are supposed to partially activate CFTR through cAMP

signaling and to allow carbachol to stimulate Cl- secretion through the CFTR channel indirectly by Ca2+-stimulation of basolateral K+ channels that hyperpolarize the membrane and increase the driving force for apical Cl- exit [11,13]. Moreover confocal microscopy will be applied to determine possible changes in expression and localization of Best2 in the CF biopsies.

4. Can the defect in HCO3- secretion and mucin release in CF colon be corrected by pharmacological CFTR correctors?
Our recent success in preserving the morphology and function of human rectal biopsies for at least 24 h ex vivo will allow us to answer the important question whether pharmacological CFTR correctors that emerged from high-throughput screening (HTS) and are known to partially restore CFTR-mediated Cl- secretion in CF epithelial cells are also capable of restoring CF defects in epithelial HCO3- and mucin secretion. These experiments will reveal whether rescued F508del-CFTR in the apical membrane is able to conduct HCO3- itself or can normalize CFTR-dependent, SLC26A-mediated HCO3- transport.

5. Can the defect in HCO3- secretion and mucin release in CF human colon be corrected by PPAR-γ agonists in the absence of CFTR correction?
Recent studies in Cftr-/- mice have shown that the defect in heat-stable enterotoxin (STa; a cGMP-linked secretagogue)-induced colonic HCO3- and mucin secretion could be restored, and intestinal obstruction prevented, by in vivo treatment of the mice with the PPAR-* agonist rosiglitazone[14]. This correction was associated with the upregulation of the carbonic anhydrases Car4 and Car2, encoded by genes that are regulated by the PPAR-γ signaling pathway[14]. An important aim of this project is to verify whether PPAR-γ agonists are able to exert a similar action on human distal colon ex vivo. Aside rosiglitazone, we will also test the bicyclic fatty acid lubiprostone[18] (presently in clinical use against chronic obstipation) for its ability to cross-activate PPAR-* and to restore HCO3- and mucin secretion in CF biopsies.

**Study design**

1. Studying HCO3- secretion, mucus stasis and the expression and localization of key transporters and enzymes involved in HCO3- secretion in rectal biopsies of healthy subjects and CF patients.

Transepithelial current measurements in rectal suction biopsies from healthy individuals and CF patients (4 per patient) will be performed in the *Rotterdam* mini-Ussing chambers (aperture 1.13 mm2) connected to a DVC-1000 voltage clamp (WPI) and a Powerlab for digitalization and current recording (LabChart software), as described in detail in ref. 6. The serosal bath solution (Meyler buffer containing 10 mM glucose) is circulated with a carbogen gas-lift system (95% O2, 5% CO2) at pH 7.4, and the unbuffered solution at the luminal side (154 mM NaCl containing 10 µM amiloride to inhibit ENaC channels) is circulated with 100% O2. Following a 1h equilibration period, the

electrogenic component of basal and secretagogue-stimulated anion (i.e. HCO3- + Cl-) transport will be assessed by short-circuit current (Isc) measurements, and total HCO3- secretion (i.e. electrogenic+electroneutral) is monitored by measuring luminal alkalinization using a continuous pH-stat titration method (Radiometer, Copenhagen). By this approach the luminal pH is maintained at 7.4 by addition of an isotonic solution containing 2 mM HCl. Subsequently, the secretagogues are washed out by multiple changes of the bath fluid, and the Isc measurements are repeated after isotonic replacement of HCO3- serosally by Na-HEPES (pH 7.4) and Na-gluconate, bilateral addition of the Car inhibitor methazolamide (100 µM), and a shift to 100% O2 gassing. The electrogenic component of HCO3- transport is assessed by comparing the Isc responses in the first and second period, i.e. in the presence and absence of serosal HCO3-.
To evaluate the HCO3- secretory response to different intracellular signaling pathways, the following secretagogues will be tested consecutively: carbachol (Ca2+-linked)-wash out-guanylin (cGMP-linked)-STa (cGMP-linked)-wash out-forskolin+ IBMX (cAMP-linked)-genistein (improving the gating of F508del-CFTR)-carbachol.

In separate experiments, the contribution of the Ca2+-dependent anion channel bestrophin-2 to HCO3- secretion will be estimated by comparing Isc and pH-stat responses to carbachol in the absence or presence of the Best2 inhibitor indomethacin [11]. PGE2 will be added to mask indomethacin-inhibition of endogenous PGE formation.

Mucus stasis will be assessed by Alcian Blue staining of the biopsies, and expression and (re)distribution of transporters (CFTR, Best2) and enzymes (Car4, Car2) will be examined by immunostaining and confocal microscopy.

We estimate that completion of part 1 of this study will require the participation of 10 healthy controls and 8 F508del CF patients (5 biopsies/patient).

2. Ex vivo repair of CF defects in colonic HCO3- secretion and mucus release by pharmacological approaches.

We have recently defined tissue preservation conditions in which human rectal biopsies mounted in Ussing chamber inserts and maintained at 370C under carbogen gassing remain fully functional for at least 24h. We aim to exploit these findings in this project by testing long-term rescue effects of 3 classes of potential pharmacological correctors on rectal biopsies from homozygous F508del CF patients ex vivo:

(i) Correctors of F508del misprocessing that are known to improve CFTR-mediated Cl- transport but have not been tested yet for their ability to improve HCO3- and mucus transport in CF. The Vertex corrector VRT-809, previously found to enhance CFTR-mediated Cl- secretion in human bronchial epithelial cells [15], and presently evaluated in clinical trials, will be

tested first as a positive control. Subsequently, 4 recently developed EPIX F508del-CFTR correctors (donated by the CFFT-USA) will be investigated in the same test protocol. Following the Isc and pH-stat measurements in fresh biopsies described under (1), biopsies will be incubated for 24h in the presence of the CFTR corrector or vehicle (2 biopsies/condition), followed by repetition of the Isc and pH-stat measurements and Alcian Blue staining.

(ii) Potential correctors of the defect in PPAR-gamma signaling in CF, i.e. the synthetic PPAR-* ligand rosiglitazone [14] and the bicyclic fatty acid and PGE1 derivative lubiprostone [18], both in clinical use for treatment of type-2 diabetes and chronic obstipation, respectively.

(iii) S3226 (provided by Sanofi Aventis), a novel inhibitor of NHE3 that may mimic the beneficial effects of NHE3-KO on CF pathology (e.g. improvement of intestinal HCO3- secretion) as shown previously in Cftr-/- mice [17] (see ii for test protocol).

We estimate that completion of part 2 of this study (involving the testing of 8 different pharmacological correctors) will require the participation of 32 F508del CF patients. Power analysis shows that the demonstration of a statistically significant improvement of transepithelial anion secretion in the Ussing chamber (up to a Isc value >10% of healthy controls) requires the testing of each corrector or vehicle in 2 biopsies from at least 4 CF patients.

Recruitment of healthy controls and CF patients:
Following written informed consent and with approval from the Medical Ethical Committee of the UMCU, rectal biopsies (5/subject) from adult homozygous F508del CF patients and healthy volunteers will be collected by a suction biopsy device (Meeker Instruments, Utrecht) and processed exactly as described previously. [18]

### Study burden and risks

Burden and risk of rectal biopsy are minimal.

Risk of bleeding due to rectal biopsy in literature:
N= 389, comp. 2 (bleeding 0.5%) [19]

## Contacts

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# Trial sites

## Listed location countries

Netherlands

# Eligibility criteria

**Age**
Adults (18-64 years)
Elderly (65 years and older)

## Inclusion criteria

For patients: CF (F508del homozygotes)
For controls: non-CF, healthy

## Exclusion criteria

For patients: non CF
For controls: CF or CF carrier

# Study design

## Design

**Study type:** Observational invasive

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|---|---|
| Masking: | Open (masking not used) |
| Control: | Uncontrolled |
| Primary purpose: | Prevention |

### Recruitment

NL

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|---|---|
| Recruitment status: | Recruiting |
| Start date (anticipated): | 08-05-2014 |
| Enrollment: | 42 |
| Type: | Actual |

## Ethics review

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|---|---|
| Approved WMO Date: | 11-04-2012 |
| Application type: | First submission |
| Review commission: | METC NedMec |
| Approved WMO Date: | 22-06-2016 |
| Application type: | Amendment |
| Review commission: | METC NedMec |

## Study registrations

### Followed up by the following (possibly more current) registration

No registrations found.

### Other (possibly less up-to-date) registrations in this register

No registrations found.

## In other registers

| Register | ID |
| --- | --- |
| CCMO | NL35551.041.11 |