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Altered Intestinal Function in Patients With Chronic Heart Failure

Anja Sandek, MD,† Juergen Bauditz, MD,† Alexander Swidsinski, MD,† Sabine Buhner, PHD,† Jutta Weber-Eibel, MD,† Stephan von Haehling, MD,‡ Wieland Schroedl,§ Tim Karhausen,* Wolfram Doehner, MD, PHD,* Mathias Rauchhaus, MD, PHD,* Philip Poole-Wilson, MD, FMEDSCI,‡ Hans-Dieter Volk, MD, PHD,‖ Herbert Lochs, MD, PHD,† Stefan D. Anker, MD, PHD *‡

Berlin and Leipzig, Germany; and London, United Kingdom

Objectives We evaluated morphology and function of the gut in patients with chronic heart failure (CHF).

Background Intestinal translocation of bacterial endotoxin may contribute to the inflammatory state observed in patients with CHF. The morphology and function of the gut may be abnormal.

Methods We studied 22 patients with CHF (age 67 ± 2 years, left ventricular ejection fraction [LVEF] 31 ± 1%, New York Heart Association functional class 2.3 ± 0.1, peak Vo_2 15.0 ± 1.0 ml/kg/min) and 22 control subjects (62 ± 1 years, LVEF 68 ± 2%, peak Vo_2 24.7 ± 1.3 ml/kg/min). Bowel wall thickness was assessed by transcutaneous sonography, small intestinal permeability by the lactulose-mannitol test, passive carrier-mediated transport by D-xylose test, large intestinal permeability by sucralose test (5- and 26-h urine collection, high-performance liquid chromatography), and mucosal bacterial biofilm by fluorescence in situ hybridization in biopsies taken during sigmoidoscopy.

Results Chronic heart failure patients, compared with control patients, showed increased bowel wall thickness in the terminal ileum (1.48 ± 0.16 mm vs. 1.04 ± 0.08 mm), ascending colon (2.32 ± 0.18 mm vs. 1.31 ± 0.14 mm), transverse colon (2.19 ± 0.20 vs. 1.27 ± 0.08 mm), descending colon (2.59 ± 0.18 mm vs. 1.43 ± 0.13 mm), and sigmoid (2.97 ± 0.27 mm vs. 1.64 ± 0.14 mm) (all $p < 0.01$). Chronic heart failure patients had a 35% increase of small intestinal permeability (lactulose/mannitol ratio: 0.023 ± 0.001 vs. 0.017 ± 0.001, $p = 0.006$), a 210% increase of large intestinal permeability (sucralose excretion: 0.62 ± 0.17% vs. 0.20 ± 0.06%, $p = 0.03$), and a 29% decrease of D-xylose absorption, indicating bowel ischemia (26.7 ± 3.0% vs. 37.4 ± 1.4%, $p = 0.003$). Higher concentrations of adherent bacteria were found within mucus of CHF patients compared with control subjects ($p = 0.007$).

Conclusions Chronic heart failure is a multisystem disorder in which intestinal morphology, permeability, and absorption are modified. Increased intestinal permeability and an augmented bacterial biofilm may contribute to the origin of both chronic inflammation and malnutrition. (J Am Coll Cardiol 2007;50:1561–9) © 2007 by the American College of Cardiology Foundation

Chronic heart failure (CHF) is a condition with a high morbidity and mortality. Recent advances in understanding the pathophysiology of CHF have led to the conclusion that CHF is a multisystem disorder that affects not only the heart and circulation but also the musculoskeletal, neuroendocrine, metabolic, and immune systems. Chronic heart failure is now recognized as a state of chronic inflammation. Plasma levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-alpha, are long known to be related to disease severity in CHF patients (1) and to predict poor survival (2). The origin of this inflammatory state is not well understood (3), although several hypotheses have been put forward. One hypothesis (4) is that much of the inflammatory state arises from endotoxins entering the circulation from the gut.

Chronic heart failure leads to increased sympathetic activity, which contributes to a redistribution of blood flow away from

From the *Division of Applied Cachexia Research, Department of Cardiology, Charite, Campus Virchow-Klinikum, Berlin, Germany; †Department of Gastroenterology, Charite, Campus Mitte, Berlin, Germany; ‡Department of Clinical Cardiology, National Heart & Lung Institute, Imperial College London, London, United Kingdom; §Institute of Bacteriology and Mycology, Veterinary Faculty, Leipzig, Germany; and the ‖Department of Medical Immunology, Charite, Campus Mitte, Berlin, Germany. The first two authors contributed equally to this study.

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Abbreviations and Acronyms

CHF = chronic heart failure
ELISA = enzyme-linked immunosorbent assay
FISH = fluorescence in situ hybridization
hs CrP = high-sensitivity C-reactive protein
IL = interleukin
LPS = lipopolysaccharide
LVEF = left ventricular ejection fraction
NYHA = New York Heart Association
PI = permeability index
TNF = tumor necrosis factor
Vo_2 = oxygen consumption

the splanchnic circulation. In CHF patients, a decrease in intestinal mucosal pH has been observed at low levels of exercise, indicating intestinal ischemia (5). Inadequate mucosal perfusion increases intestinal mucosal permeability (6). Lipopolysaccharide (LPS) (endotoxin), a cell-wall component of gram-negative bacteria, can enter the circulation through the gut wall if barrier function is impaired in various diseases, such as burn injury, sepsis, liver cirrhosis, and ischemic reperfusion injury (7–10). In the circulation of CHF patients, LPS may activate monocytes and macrophages to release pro-inflammatory mediators, thus leading to an inflammatory state. Elevated plasma concentrations of LPS have been found in CHF patients during edematous decompensation (11). In acute heart failure, bioactive LPS levels are higher in hepatic veins than in the left ventricle (12). We hypothesized that intestinal barrier dysfunction with an increased paracellular permeability, a diminished transcellular transport activity, and an augmented intestinal bacterial biofilm are present in CHF patients. We report here the changes in function and morphology of the gastrointestinal system in these patients.

Methods

Patients. We studied 22 CHF patients and 22 control subjects (for demographic and clinical details see Table 1). The diagnosis of CHF was based on symptoms arising during exercise, clinical signs, and documented left ventricular impairment (left ventricular ejection fraction [LVEF] ≤ 40%) according to current guidelines (13). All patients were clinically stable (New York Heart Association [NYHA] functional class 2.3 ± 0.1) and receiving unchanged medication for at least 4 weeks before assessments. Patients were allowed to take aspirin 100 mg once daily, but not other nonsteroidal anti-inflammatory drugs or steroid hormones or antibiotics within at least 4 weeks before being studied. In CHF patients, medication consisted of angiotensin-converting enzyme inhibitors (77%), angiotensin receptor antagonists (23%), beta-blockers (86%), diuretics (86%), glycosides (18%), and statins (77%) in varying combinations. None of the control subjects was taking any medication except for calcium channel blocker in 1 subject, angiotensin-converting enzyme inhibitors in 2 subjects for mild arterial hypertension without evidence of left ventricular dysfunction, hormone replacement therapy in 1 subject, and L-thyroxin in 2 subjects. Subjects with clinical signs of infection, rheumatoid arthritis, renal failure, significant valvular heart disease, intestinal diseases, cancer, or a history of autoimmune disorders were excluded. None of the subjects had any known immune system disorders, and none received immune modulation therapy. The local ethics committee approved the study, and all subjects gave written informed consent.

Clinical assessments. Echocardiography was performed following standard procedures. Left ventricular ejection

Table 1 Baseline Data for Caucasian Patients and Control Subjects

	Control Subjects	CHF Patients	p Value
Number (women)	22 (11)	22 (4)	0.06
NYHA functional class		2.3 ± 0.1	
Ischemic etiology		17/22	
Ejection fraction (%)	68 ± 2	31 ± 1	<0.0001
Age (yrs)	62 ± 1	67 ± 2	0.054
BMI (kg/m^2)	26.2 ± 1.0	27.8 ± 1.0	0.3
Height (cm)	172 ± 9.9	174 ± 8.8	0.5
Weight (kg)	77.5 ± 3.3	84.4 ± 4.5	0.2
Peak Vo_2 (ml/min/kg)	24.7 ± 1.3	15.0 ± 1.0	<0.0001
Hemoglobin (g/dl)	13.4 ± 0.3	13.9 ± 0.4	0.4
Hematocrit (%)	39.8 ± 0.6	41.1 ± 1.1	0.3
White blood cells ($\times 10^9$/l)	6.7 ± 0.3	7.9 ± 0.4	0.02
hs C-reactive protein (μg/ml)	1.1 (0.5, 3.4)	2.1 (0.8, 3.6)	0.4
hs tumor necrosis factor-alpha (pg/ml)	2.5 ± 0.1	3.0 ± 0.2	0.04
hs interleukin-6 (pg/ml)	1.0 (0.8, 1.7)	3.0 (1.6, 3.4)	0.0002
Sodium (mmol/l)	141.4 ± 0.6	142.0 ± 1.1	0.6
Potassium (mmol/l)	4.3 ± 0.1	4.4 ± 0.4	0.8
Creatinine clearance (ml/min)	87.9 ± 4.0	74.8 ± 5.9	0.07
ASAT (U/l)	23.1 ± 1.5	30.9 ± 2.4	0.06
ALAT (U/l)	25.3 ± 2.9	28.2 ± 3.6	0.3

ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase; BMI = body mass index; CHF = chronic heart failure; hs = high sensitivity; NYHA = New York Heart Association.

fraction was measured using biplane Simpson's technique. Four CHF patients had elevated right ventricular pressure, with 2 of them showing widened liver veins as a sign of congestion. One of these patients had a small amount of free fluid in the abdomen. All subjects underwent a symptom-limited treadmill exercise testing (instantaneous breath-by-breath method) using the modified Naughton protocol (MedGraphics CPX/D, Medical Graphics Corporation, Cardiorespiratory Diagnostic Systems, St. Paul, Minnesota). The following variables were measured: peak oxygen consumption (peak V_{O_2}), total exercise time, ventilatory response to exercise (VE/VC_{O_2} slope), anaerobic threshold, peak heart rate (beats per minute), and peak systolic and diastolic blood pressures.

Gastrointestinal permeability was assessed using a sugar-drink test as previously described in detail (14). The test is based on the measurement of the urinary excretion of orally administered sugar-probe molecules. The lactulose/mannitol ratio (permeability index, PI) served as a marker for intestinal permeability to avoid differences in variable gastric emptying, intestinal transit time during the first 5 h, and renal clearance (15,16). No drugs influencing gut function, including laxatives and antidiarrhoeal agents, were taken by the subjects. Subjects were asked to refrain from alcohol for at least 2 days before the study and from nicotine in the morning before and during the test.

We performed 2 separate tests within an interval of at least 4 days of each other. During the first test, which was completed by 21 patients and 20 control subjects, each subject ingested capsules containing 5-g sucralose after an overnight fast and drank a solution containing 10-g lactulose, 5-g mannitol, and 20-g sucrose dissolved in 100 ml of water. Subjects were fasting during the first 5 h and were encouraged to drink water 2 h after the test started. Three urine samples were taken: a before-test sample, a 5-h urine collection sample during the first 5 h after starting the test, and a 21-h urine collection sample of the following period until 26 h after consumption. Gastroduodenal permeability was analyzed by excretion of sucrose in the 5-h sample, serving as a marker for gastroduodenal permeability (14). Small intestinal permeability was assessed by the lactulose/mannitol ratio reflecting passive noncarrier-mediated transport (15,17). In the 5-h sample of the second oral sugar test, we further assessed passive carrier-mediated transport in the small intestine by absorption of orally administered D-xylose. This test was completed in 22 patients and 21 control subjects and performed within 6 days of sigmoidoscopy. Large intestinal permeability was measured by sucralose recovery in the third urine sample 5 to 26 h after consumption (18). In both tests, sodium acid was used as a preservative for urine. Total urine volume was recorded on completion of the test, and a 10-ml aliquot of each urine sample was stored at −20°C until analysis.

For urine analysis, protein content was removed using sulfosalicylic acid. Urine was then desalted with amberlite mixed bed-3 resin. Sugars were separated using meso-erythritol, 2-deoxy-D-glucose and turanose as internal standards, analyzed and quantified by high-performance liquid chromatography with pulsed electrochemical detection (Dionex, Idstein, Germany; chromatography module: 250 × 40 mm Carbopac PA-1 column [Dionex]; eluent 150 mM NaOH/500 mM NaAC gradient; flow of 1 ml/min). Results were expressed as the percentage recovery of the ingested dose of sugar.

Transcutaneous abdominal sonography (12-MHz linear-array transducer, HDI 5000, Philips, Belgium) was performed to assess intrabdominal free fluid and to measure bowel wall thickness in the middle segment of the sigmoid (in Subjects #1 to #44), descending, transverse, and ascending colon (Subjects #7 to #44). Measurement of the terminal ileum (Subjects #15 to #44) was performed 5 cm proximal to the ileocecal valve. Because of obesity, the transverse colon was not assessed in 1 patient and the transverse and ascending colon in 1 other patient. Patients were scanned under identical conditions after overnight fasting. Measurement of bowel wall thickness was carried out in true cross and longitudinal sections of the relaxed bowel by assessment of the anterior bowel wall. Overall thickness of the bowel wall was measured from the first mucosal interface echo to the first serosal echo. Each measurement was repeated 3 times at different positions of the intestinal wall, and the mean was calculated. Arterial blood flow velocities in the superior mesenteric artery and the inferior mesenteric artery were assessed in all subjects by an 8-5-MHz vector-array transducer. We did not detect any stenoses of the mesenteric arteries in CHF patients or in control subjects. All recordings were performed in a standardized way, and readings were analyzed by the same experienced physician (J.B.), who was blinded as to the subjects' study group. The intraobserver coefficient of variation for intestinal ultrasound measurements repeated on consecutive days is 5%. Accuracy of measurement is <0.2 mm for all segments.

Flexible sigmoidoscopy was performed without sedation after a glycerol enema, except in 1 patient who declined this part of the study. Biopsies of the sigmoid mucosa were taken for fluorescence in-situ hybridization (FISH) as previously described (18). In brief, biopsies were fixed in Carnoy solution (19,20) and subjected to FISH evaluation on glass slides. Oligonucleotide probes were synthesized with a Cy3 or Cy5 (carbocyanine) reactive fluorescent dye at the 5' end (MWG Biotech, Ebersberg, Germany). A set of 38 FISH probes at domain and group levels together with 1 species-specific probe for *Helicobacter pylori* was used (19). Density of bacterial biofilm, spatial arrangement, and associative behavior of bacteria were investigated on 4-μm-thick sections of biopsies. All analyses were carried out by 2 different investigators in parallel who were unaware of the respective subject's study group. One patient sample could not be analyzed because the amount of material from the biopsy was too small. Digital pictures of bacteria on the microscopic slide were taken with a Nikon DXM 1200 camera

and software (Nikon, Tokyo, Japan). We evaluated the composition of bacteria using multicolor FISH analysis. Two group-specific probes labeled with Cy3 or Cy5 were applied simultaneously employing a universal Eub 338 FITC labeled probe. Bacteria positively hybridizing with Cy3 (yellow fluorescence) or Cy5 (red fluorescence) were quantified relative to all visible bacteria hybridizing with the Eub 338 FITC probe (green fluorescence). The methodology is reliable for semiquantitative assessment of bacterial biofilm when more than 10^5 cfu/ml are present. Quantitative assessment is possible when more than 10^7 cfu/ml are present.

Measurements of cytokines, C-reactive protein, LPS, and immunoglobulin A (IgA)-anti-LPS. The serum concentrations of TNF-alpha and interleukin (IL)-6 were measured by high-sensitivity enzyme-linked immunosorbent assay (ELISA, Quantikine HS, R&D, Minneapolis, Minnesota). The lower limits of detection were 0.12 pg/ml and 0.039 pg/ml, respectively. Plasma concentrations of high-sensitivity C-reactive protein (hs CrP) were measured by immunofluorescent assay (CRPus Kryptor, Brahms, Hennigsdorf, Germany). The lower limit of detection was 0.06 μg/ml. IgA-anti-LPS was measured by ELISA (21). Plasma LPS levels were assessed as described previously using a Limulus Amebocyte Lysate assay (Bio Whittaker, Walkerswill) (22).

Statistical analysis. Statistical analysis was performed using StatView 5.0 software (SAS Institute Inc., Cary, North Carolina). Normality of distribution was assessed using the Kolmogorov-Smirnov test. Results are reported as mean ± SEM (indicating normal distribution of data; statistical comparisons were made using the unpaired *t* test) or median [25th, 75th percentiles] (indicating non-normal distribution of data; statistical comparisons were made using the Mann-Whitney-*U* test). Frequencies were compared using the chi-square test. Relationships between parameters were assessed using simple regression. A p value <0.05 was considered significant in all analyses.

Results

There were no significant differences between control subjects and CHF patients in terms of gender, age, height, weight or body mass index (all $p > 0.05$) (Table 1). As expected, CHF patients had a lower ejection fraction and peak Vo_2.

Immunological assessments. Chronic heart failure patients showed higher plasma concentrations of TNF-alpha (3.0 ± 0.2 pg/ml vs. 2.5 ± 0.1 pg/ml), IL-6 (3.0 [1.6, 3.4] vs. 1.0 [0.8, 1.7] pg/ml), and higher blood leucocyte concentration compared with control subjects (all $p < 0.05$). C-reactive protein did not significantly differ in patients and control subjects (2.1 [0.8, 3.6] vs. 1.1 [0.5, 3.4], $p = 0.4$).

Patients compared with control subjects had higher serum concentrations of immunoglobulin A-anti-LPS (128 [96, 368] vs. 78 [41, 129] relative ELISA U/ml, $p = 0.005$). As expected, levels of free unbound endotoxin, measured in a subgroup of 10 patients and 12 control subjects, did not significantly differ (2.3 ± 0.09 vs. 2.2 ± 0.04 endotoxin U/ml, $p = 0.6$).

Functional alterations of the gut mucosa. We compared intestinal permeability and absorption in patients with CHF and control subjects to assess intestinal barrier function. The permeability index (PI) expressed as the urinary 5-h lactulose/mannitol ratio was increased in CHF patients compared with control subjects (PI: 0.023 ± 0.001 vs. 0.017 ± 0.001, $p = 0.006$) (Fig. 1A). This indicates a 35% increase in intestinal permeability in CHF patients. Furthermore, CHF patients showed a 29% decrease of D-xylose absorption compared with controls (26.7 ± 3.0% vs. 37.4 ± 1.4%, $p = 0.003$) (Fig. 1B), which reflects a diminished activity of passive carrier-mediated transport. Absorption of D-xylose in the CHF group showed no correlation with renal clearance ($r = 0.1$, $p = 0.7$). Smokers ($n = 4$) and nonsmokers showed similar permeability indexes and D-xylose recoveries in the CHF group ($p > 0.5$).

Sucralose excretion was markedly increased in CHF patients compared with control subjects (0.62 ± 0.17% vs. 0.20 ± 0.06%, $p = 0.03$). No difference was found in the gastroduodenal permeability between CHF patients and control subjects as measured by sucrose recovery (0.20 ± 0.04% vs. 0.17 ± 0.02%, $p = 0.5$).

Bowel wall thickness. Chronic heart failure patients showed increased bowel wall thickness in the terminal ileum (1.48 ± 0.16 mm vs. 1.04 ± 0.08 mm) representing the small bowel, ascending colon (2.32 ± 0.18 mm vs. 1.31 ± 0.14 mm), transverse colon (2.19 ± 0.20 mm vs. 1.27 ± 0.08 mm), descending colon (2.59 ± 0.18 mm vs. 1.43 ± 0.13 mm), and sigmoid (2.97 ± 0.27 mm vs. 1.64 ± 0.14 mm) compared with control subjects (all $p < 0.01$) (Figs. 2A and 2B).

Because diverticulosis might affect bowel wall thickness, we reanalyzed the data for the sigmoid, excluding the 5 patients with diverticulosis. In this subgroup, sigmoid wall thickness was larger in CHF patients than in control subjects (2.76 ± 0.27 mm vs. 1.41 ± 0.11 mm, $p < 0.0001$). In CHF patients, we did not find significant correlations of bowel wall thickness with age (for all intestinal segments $p > 0.3$), peak Vo_2 (all $p > 0.2$), LVEF (all $p > 0.2$), or NYHA functional class (all $p > 0.2$).

Bowel wall thickness in the ascending colon correlated with the blood concentration of leucocytes in CHF patients ($r = 0.49$, $p = 0.045$) (Fig. 3A). No such correlation was found with blood levels of hs CrP ($r = 0.27$, $p = 0.29$), TNF-alpha ($r = 0.1$, $p = 0.70$), and IL-6 ($r = 0.12$, $p = 0.66$). Bowel wall thickness in the sigmoid correlated with blood levels of hs CrP ($r = 0.57$, $p = 0.005$) (Fig. 3B), but not with blood levels of TNF-alpha and IL-6 ($r = 0.34$ and 0.27, $p = 0.12$ and 0.22, respectively).

There was a trend toward a higher thickness of the ascending colon in patients with a higher permeability in the large bowel, assessed by excretion of sucralose ($r = 0.50$, $p = 0.051$) (Fig. 3C).

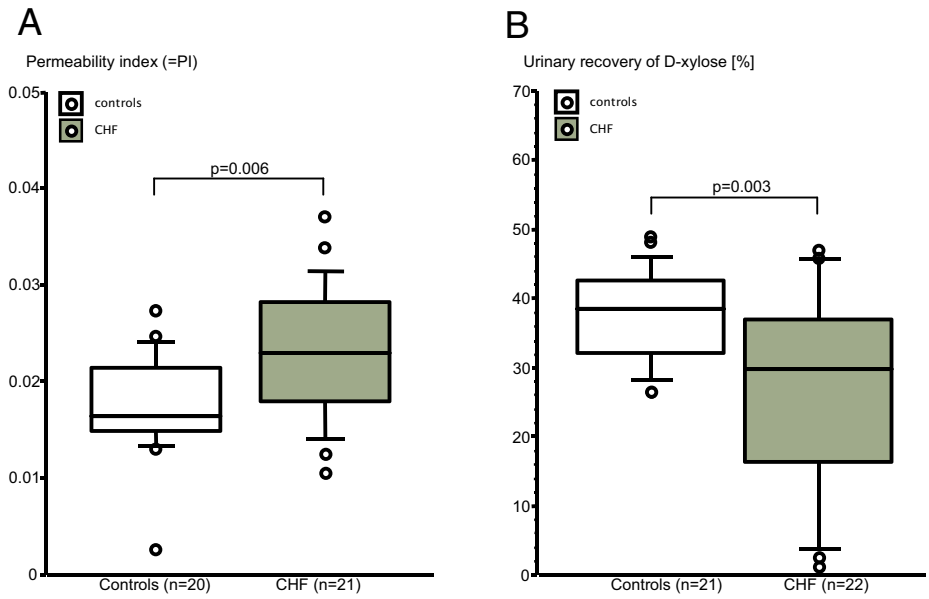

Figure 1 **Assessment of Small Intestinal Paracellular Permeability and Passive Carrier-Mediated Transport**

(A) Urinary lactulose/mannitol-index in chronic heart failure (CHF) patients (n = 21) and control subjects (n = 20). Assessments were made from 5-h urine collection after ingestion of the test sugar drink. The results reflect increased small intestinal permeability of the gut mucosa in CHF patients compared with control subjects. **(B)** Urinary recovery of orally administered D-xylose in CHF patients (n = 22) compared with control subjects (n = 21). Assessments were made from 5-h urine collection after ingestion of the test sugar drink. The results reflect reduced activity of carrier-mediated transport of D-xylose.

Bacterial biofilm. Mean density of bacteria within mucus was higher in CHF patients than in controls (10.4×10^8/ml [0.3×10^8/ml, $2{,}150 \times 10^8$/ml] vs. 0.01×10^8/ml [0.001×10^8/ml, 5×10^8/ml], $p = 0.007$) (Figs. 4A to 4C). Bacteria in CHF patients were more often adherent to the mucosa (in 70% vs. 36%, $p = 0.03$) and bacterial biofilm ranged over a higher mean area of the biopsy (35.5 ± 8.2% in CHF vs. 10.2 ± 3.7% in control subjects, $p = 0.006$).

As subjects with diverticulosis are known to be at higher risk for bacterial overgrowth and inflammatory processes of the sigmoid wall, we confirmed the findings excluding 5 CHF patients and 6 control subjects with evidence of different degrees of diverticulosis on endoscopy: in CHF patients without diverticulosis the mean concentration of bacteria was higher (29.0×10^8/ml [0.3×10^8/ml, $3{,}152 \times 10^8$/ml]) than in controls (0.001×10^8/ml [0.001×10^8/ml, 3.4×10^8/ml], $p = 0.0028$). Bacterial adherence and biofilm were also increased in CHF patients (adherence: 63% vs. 25%, $p = 0.03$; biofilm-covered area: 33.1 ± 9.3% vs. 3.4 ± 2.0%, $p = 0.004$).

In control subjects, bacteria of 16 different strains were detected, whereas in CHF patients bacteria of 20 strains were found in varying frequencies. There was a trend for a higher mean diversity of bacterial strains in patients compared with controls (6 [4, 8.5] vs. 3.5 [0, 6] strains; $p = 0.055$), but we could not identify a significant pattern.

The most frequent strains were *Bacteroides/Prevotella* in 19 of 20 patients and in 13 of 22 control subjects, *Eubacterium rectale* group in 18 of 20 patients and in 13 of 22 control subjects, and *Fusobacterium prausnitzii* (Fprau) in 18 of 20 patients and in 12 of 22 control subjects, all representing standing intestinal flora (all $p < 0.05$). In the subgroup of 15 CHF patients and 9 control subjects with a mucosal biofilm of $>10^7$ cfu/ml, the major strain-specific components of bacterial mucosal biofilm could be quantified. We found a greater biofilm portion of Fprau in this group of patients compared with control subjects (15% [3, 20] vs. 25% [19, 39]; $p = 0.04$). We found no significant difference in the proportion or absolute concentrations of the other bacteria detected in this subgroup (all $p > 0.06$).

Discussion

We report morphological and functional alterations of the gut in CHF patients. All parts of the large bowel in CHF patients display thickened walls compared with control subjects of similar age. Gut mucosa in CHF patients is functionally altered. Permeability is increased in both the small and large intestine for lactulose/mannitol and sucralose, respectively, and passive carrier-mediated transport for D-xylose in CHF patients is diminished. The concentration of bacteria in the sigmoidal mucosal biofilm and the extent of the adherence are higher in CHF patients than in control subjects.

Altered bowel structure and function and impaired mucosal barrier function have not previously been described in CHF patients. An increased bowel wall thickness is a frequent finding in various conditions, such as acute isch-

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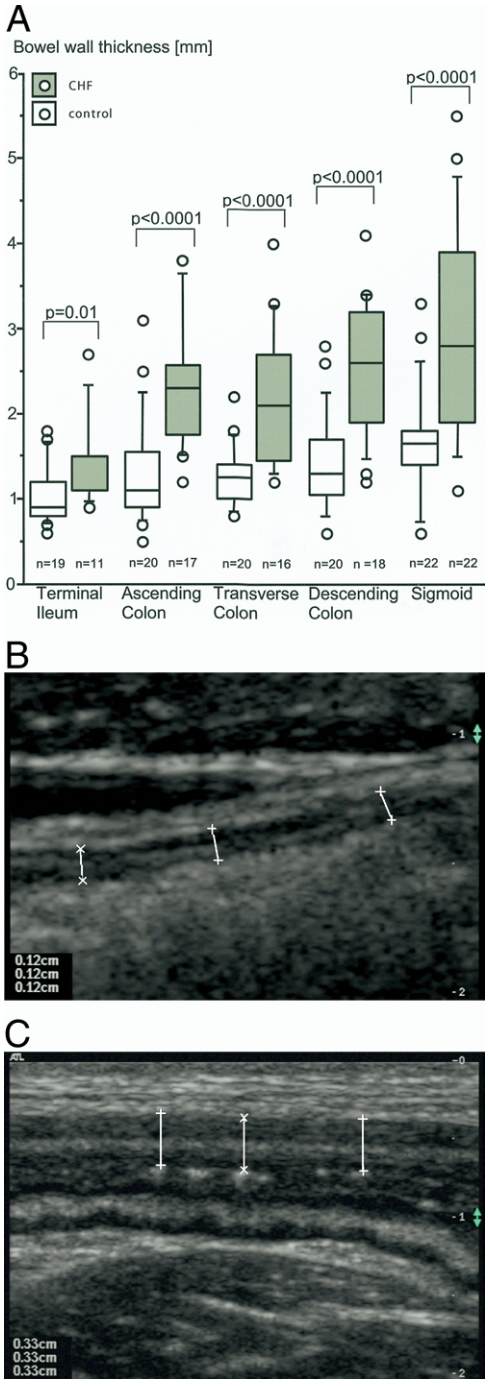

Figure 2 **Bowel Wall Thickness in CHF Patients Compared With Control Subjects**

(A) Bowel wall thickness (assessed using transcutaneous abdominal sonography, 12 MHz linear-array transducer) of terminal ileum, ascending colon, transverse colon, descending colon and sigmoid in chronic heart failure (CHF) patients compared with control subjects. **Box plots** indicate medians as well as 25th and 75th percentiles. **(B, C)** Measurement of bowel wall thickness in a healthy control subject **(B)** and a patient with CHF **(C)**. Within the bowel wall, 3 layers can be differentiated. The hypoechoic layer next to the lumen corresponds to the mucosa and the second echogenic layer corresponds to the submucosa. The third layer is again hypoechoic and corresponds to the muscularis propria. Overall thickness of the bowel wall was measured from the first mucosal interface echo to the first serosal echo.

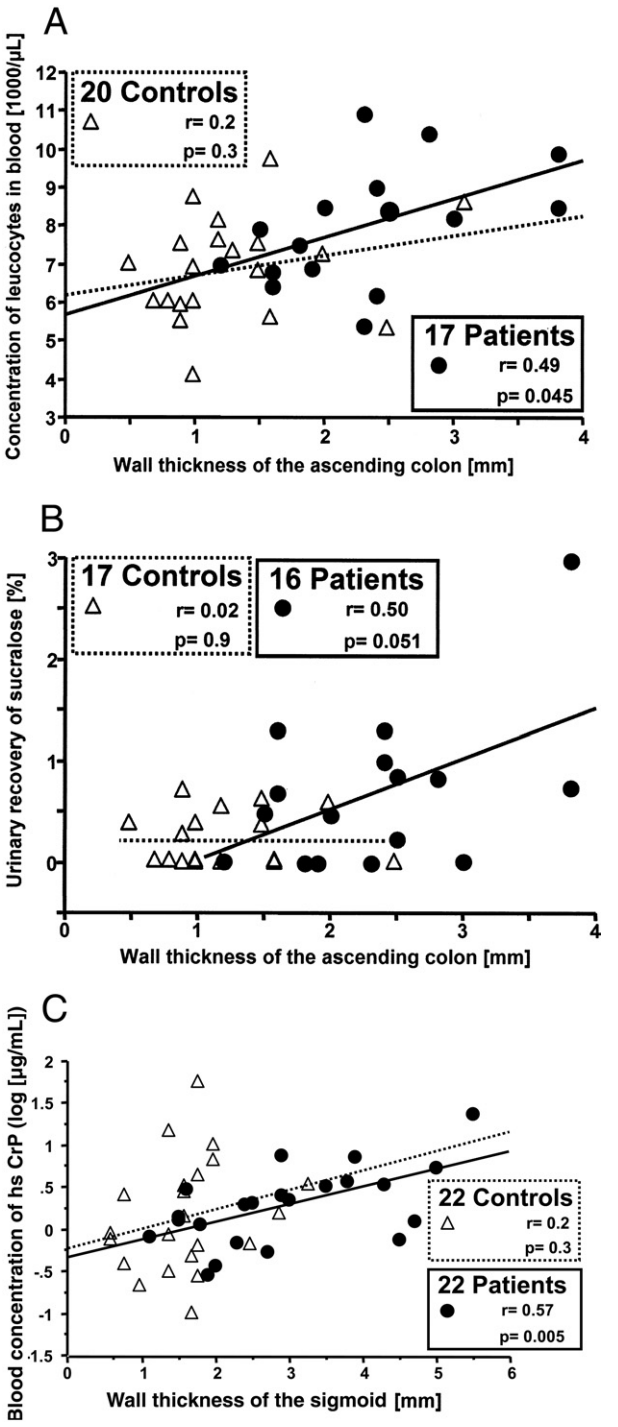

Figure 3 **Correlation Analysis**

(A) Correlation of bowel wall thickness of the ascending colon with the concentration of leucocytes in blood in chronic heart failure (CHF) patients compared with control subjects. **(B)** Correlation of bowel wall thickness of the ascending colon with the urinary recovery of sucralose in CHF patients compared with control subjects. **(C)** Correlation of bowel wall thickness of the sigmoid with log-transformed concentration of high-sensitivity C-reactive protein (hs CrP) in blood in CHF patients compared with control subjects.

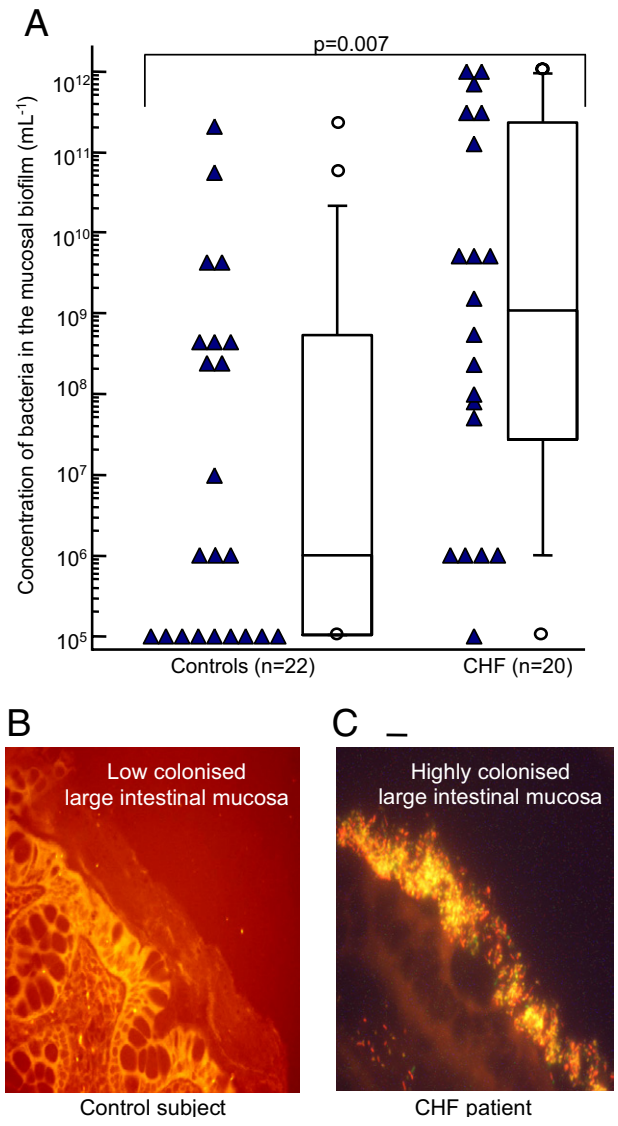

Figure 4 **Sigmoidal Mucosal Bacterial Biofilm in CHF Patients Compared With Control Subjects**

(A) Concentration of bacteria (**bars** represent median as well as 25th and 75th percentiles) in the mucosal biofilm of biopsies taken during sigmoidoscopy. Samples of chronic heart failure (CHF) patients (n = 20) and control subjects (n = 22) were evaluated by fluorescence in situ hybridization (FISH). **(B, C)** Digital fluorescence pictures showing a higher concentration of bacteria in the mucosal biofilm of the sigmoid as assessed by FISH in 1 representative CHF patient **(C)** and a representative control subject **(B).**

emic colitis (23), inflammatory bowel disease (24), and food hypersensitivity (25). A greater bowel wall thickness in CHF may suggest the presence of bowel wall edema. That there was a higher permeability of the colonic mucosa for sucralose and a trend toward a positive correlation between sucralose excretion and ascending colon wall thickness suggests that the ascending colon is relevant to the pathophysiology of CHF. We cannot completely rule out influences of the patients' long-term medication leading to increases in permeability across the gut wall. On the other hand, we believe that the use of in vivo sugar permeability tests presents a diagnostic avenue that reflects the clinical situation in CHF patients receiving optimal conventional medication.

The conditions that make the bowel wall less resistant to translocation are many. Intramucosal acidosis, which occurs in about 50% of patients with circulatory failure (6,26,27), points to an inadequate oxygen supply and intestinal ischemia (28). An increase in gastric intramucosal carbon dioxide pressure occurs in recompensated CHF patients even at low levels of exercise (5). Diminished gut circulation and disturbed microcirculation may contribute to local edema of the bowel wall and to malabsorption and barrier dysfunction of the mucosa. Diminished passive carrier-mediated transport of D-xylose, as found in this study, indicates a dysfunction of transport proteins in CHF and is a surrogate marker of intestinal ischemia (29). Similar dysfunctions may contribute to nutritional perturbations that could promote the development of cardiac cachexia (30,31).

Inadequate mucosal perfusion is known to increase intestinal mucosal permeability (6). We detected a higher lactulose/mannitol ratio in CHF patients, reflecting increased permeability of the epithelial layer of the gut. This permeability index has been shown to be increased in burn injuries, in patients undergoing cardiopulmonary bypass surgery, and in patients who developed multiorgan failure, and is thought to reflect transient intestinal hypoperfusion (7,9,32,33). Lactulose is a nonmetabolizable disaccharide that crosses the small intestinal epithelium by passive diffusion, mainly through paracellular routes. Lactulose is a comparatively big molecule (molecular weight 342.3 Dalton) that permeates across paracellular pathways that are normally limited, whereas mannitol (molecular weight 182.2 Dalton) diffuses readily through cell membranes and paracellular routes involving high-incidence small aqueous mucosal pores (34). This test thereby assesses small intestinal paracellular integrity.

The morphological equivalent of the mucosal barrier function is the epithelial apical junctional complex, which consists of tight junctions and adherence junctions. It is prone to several influencing factors, such as hypoxia and proinflammatory cytokines. Proinflammatory cytokines such as interferon gamma and TNF-alpha have been shown to disrupt the epithelial barrier function, consequently inducing a state of hyperpermeability of the gut epithelium. This is associated with internalization of apical junctional complex transmembrane proteins, as shown in studies of colonic epithelial cell lines (35). We could not document a relationship between markers of clinical status or inflammation and measures of intestinal permeability. Further studies are needed to better understand the regulation of intestinal permeability in CHF. We did find a positive correlation between thickness of the ascending colon and leucocyte count in CHF patients. This may be concordant with the prognostic value of an elevated white blood count for a higher cardiovascular mortality and worse prognosis in CHF (36–40).

The presence of mucosal barrier dysfunction that we detected in CHF patients is in keeping with the hypothesis of endotoxin translocation (4). In contrast to earlier reports, we have directly investigated transmembrane permeability of the gut mucosa. The findings of increased bowel wall thickness, increased permeability index, lower passive carrier-mediated transport of D-xylose, higher colonic permeability for sucralose, and higher concentrations of a mostly adherent bacterial biofilm in these patients, provide clinical evidence that the intestine is pathologically altered in CHF patients. These morphological and functional changes may result in decreased host defense against adherent bacteria. When the liver is not capable of clearing portal blood of LPS, then, as seen in CHF patients with edematous decompensation, plasma levels of LPS are increased (11). One would not expect elevated systemic blood levels of endotoxin in our study for 2 reasons. First, patients were not decompensated (11) and liver enzymes were not significantly elevated. Second, LPS is usually bound to lipopolysaccharide-binding proteins (41) and blood lipids (42) in an individual manner. These are the reasons that even patients with severe ulcerative colitis can display a normal amount of free LPS in systemic blood (43). However, absolute LPS blood load is difficult to assess, and immunoglobulin A–anti-LPS, which was higher in patients compared with control subjects, provides an alternative for measuring endotoxin bioactivity and interaction in the patient.

The finding of an increased intestinal concentration of mostly adherent bacteria in CHF patients shows similarities with the pathology found in patients with inflammatory bowel disease where mucosal bacteria were found at concentrations $>10^9$/ml in 90% to 95% of patients and in only 35% of healthy controls (20). In these studies, adherent-invasive *Escherichia coli* were identified (44).

Most of the enteral bacteria are facultative pathogens. Since the phenotypic properties of bacteria represented by *Enterobacteriacae* and other bacteria were not studied long-term, we cannot exclude the possibility that some of the bacteria found attached to the mucosa could be pathogenic. However, the high diversity of mucosal bacteria and the individual character of their composition in each patient do not support that idea. More likely, the abnormal mucus barrier allows intestinal bacteria to penetrate mucus and contact mucosa.

There are 2 ways by which adherent bacteria could contribute to chronic inflammation seen in CHF. First, the mere adherence of a microbe to the intestinal epithelium without invasion or translocation can induce mucosal cytokine release and disrupt epithelial barrier function (45). Luminal hypoxia, hypercarbia, changes in local pH, and redox state, as well as norepinephrine, are all known to be potent activators of bacterial virulence in adherent bacteria (46). Second, microbial products of adherent bacteria could enter the systemic circulation through the disrupted intestinal epithelial barrier. Recent studies on selective decontamination of the gut in CHF patients have resulted in a decrease in some inflammatory markers underscoring the potential importance of gut bacteria as one source of inflammation in CHF (47).

Conclusions

We have found significant morphological and functional alterations of the intestine in CHF patients. These findings are consistent with restricted intestinal perfusion and consequent mucosal edema, a higher intestinal permeability, and a lack of immunological defense with an augmented bacterial biofilm. Altered mucosal permeability and function of the gut in CHF could contribute to chronic inflammation. Chronic heart failure is a multisystemic disorder associated with alterations of intestinal function.

Reprint requests and correspondence: Prof. Stefan D. Anker, Division of Applied Cachexia Research, Department of Cardiology, Charité, Campus Virchow-Klinikum, Augustenburger Platz 1, D-13353 Berlin, Germany. E-mail: s.anker@cachexia.de.

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