Detection of Intestinal Tissue Perfusion by Real-Time Breath Methane Analysis in Rat and Pig Models of Mesenteric Circulatory Distress

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Objectives: Methane (CH_4) breath test is an established diagnostic method for gastrointestinal functional disorders. Our aim was to explore the possible link between splanchnic circulatory changes and exhaled CH_4 in an attempt to recognize intestinal perfusion failure.

Design: Randomized, controlled in vivo animal study.

Setting: University research laboratory.

Subjects: Anesthetized, ventilated Sprague-Dawley rats (280 ± 30 g) and Vietnamese minipigs (31 ± 7 kg).

Interventions: In the first series, CH_4 was administered intraluminally into the ileum before 45 minutes mesenteric ischemia or before reperfusion in non-CH_4 producer rats to test the appearance of the gas in the exhaled air. In the porcine experiments, the superior mesenteric artery was gradually obstructed during consecutive, 30-minute flow reductions and 30-minute reperfusions achieving complete occlusion after four cycles ($n = 6$), or nonocclusive mesenteric ischemia was induced by pericardial tamponade ($n = 12$), which decreased superior mesenteric artery flow from 351 ± 55 to 182 ± 67 mL/min and mean arterial pressure from 96.7 ± 18.2 to 41.5 ± 4.6 mm Hg for 60 minutes.

Measurements and Main Results: Macrohemodynamics were monitored continuously; RBC velocity of the ileal serosa or mucosa was recorded by intravital videomicroscopy. The concentration of exhaled CH4 was measured online simultaneously with high-sensitivity photoacoustic spectroscopy. The intestinal flow changes during the occlusion-reperfusion phases were accompanied by parallel changes in breath CH_4 output. Also in cardiac tamponade-induced nonocclusive intestinal ischemia, the superior mesenteric artery flow and RBC velocity correlated significantly with parallel changes in CH_4 concentration in the exhaled air (Pearson's $r = 0.669$ or $r = 0.632$, respectively).

Conclusions: we report a combination of in vivo experimental data on a close association of an exhaled endogenous gas with acute mesenteric macro- and microvascular flow changes. Breath CH_4 analysis may offer a noninvasive approach to follow the status of the splanchnic circulation. (*Crit Care Med* 2019; 47:e403–e411)

Key Words: intestinal ischemia; methane; microcirculation; photoacoustic spectrometry

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Visceral ischemic syndromes are potentially life-threatening conditions, where the detection is of critical importance (1). The gold standard for diagnosing mesenteric ischemic events is CT angiography and selective angiography of the superior mesenteric artery (SMA) (2, 3). These invasive, rather expensive, and relatively inaccessible methods require experienced professionals to establish the proper diagnosis, especially in the case of nonocclusive ischemic forms (4), and the unstable patients need transportation to the radiology unit. Timely and specific recognition, however, is critical

because hypoperfusion of the splanchnic vascular bed disrupts the integrity of the mucosal barrier, which quickly becomes permeable to luminal foreign materials (5–7). Indeed, the mortality rate for acute and nonocclusive mesenteric ischemic forms is still very high (8, 9).

In addition to dietary wastes, microbes and bacterial products, the gastrointestinal lumen contains various gases, such as oxygen, CO_2, hydrogen, and ammonia (10). Methane (CH4) is also present in the gaseous mixture and measurable in the exhaled breath in approximately 30–60% of healthy adults (11). Furthermore, a recent human study using laser absorption spectroscopy and stable carbon isotopes has provided conclusive evidence that all individuals may produce endogenous CH_4, which, however, cannot be detected with conventional analytical tools (12).

In this context, we hypothesized that exhaled CH_4 levels will change in association with intestinal perfusion alterations, and variations in breath CH_4 output may thus be related to the flow conditions of the mesenteric microcirculation. We assumed that due to its physicochemical properties, intraluminally generated CH_4 traverses the mucosa and enters the splanchnic microcirculation freely. Thereafter, CH_4 is transported by the circulating blood, and, when reaching the lungs, it is partially released into the breath if the partial pressure is higher than that in the atmosphere, where it is normally about 1.8 parts per million by volume (13, 14). Therefore, we also hypothesized that CH_4 exhalation is influenced by the mesenteric circulation if the gas is endogenously generated or supplemented intraluminally from an external source.

Based on this background, we designed in vivo tests to investigate and characterize the diagnostic value of real-time breath CH_4 analysis for the detection of the actual status of the mesenteric perfusion. To this end, we employed a custom-built near-infrared laser technique-based photoacoustic spectroscopy (PS) system, which has been validated for dynamic measurements of CH_4 emissions in previous human and animal studies (15–18). Our specific aim was to examine the possible association between exhaled CH_4 levels and alterations in the mesenteric macro- and microvascular blood flow under strictly controlled conditions in clinically relevant animal models of occlusive and nonocclusive splanchnic ischemia-reperfusion (IR) in CH_4 producer pigs or after intraluminal CH_4 supplementation in previously nonproducer rats.

MATERIALS AND METHODS

The experiments were performed in accordance with National Institutes of Health guidelines on the handling and care of experimental animals and European Union Directive 2010/63 for the protection of animals used for scientific purposes (approval number: V/148/2013).

Rat Studies

Male Sprague-Dawley rats ($n = 50$, 280 ± 30 g), housed in plastic cages at room temperature with a 12 hours dark-light cycle and kept on standard laboratory chow with tap water ad libitum, were placed in a hermetically sealed glass cylinder to measure the whole-body CH_4 concentration after a 10-minute equilibration period. The animals were considered to be CH_4 producers if the CH_4 release exceeded the background level by greater than 1 ppm (13). We selected two groups: Group 1 ($n = 33$), without measurable CH_4 (MetNonprod) and Group 2 ($n = 17$), with detectable CH_4 emissions (MetProd group). The percentage of CH_4 producers was approximately the same as in the case of the human population (**Table S1**, Supplemental Digital Content 1, http://links.lww.com/CCM/E369). The MetNonprod rats were used thereafter to test the effects of intraluminally administered CH_4 on exhaled CH_4 levels (see below).

The animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally), the trachea was dissected free and cannulated with a silicone tube. Mechanical ventilation (Inspira Advanced Safety Ventilator 55–7058; Harvard Apparatus, Holliston, MA) was started with 8–9 mL/kg volume of room air. After a midline abdominal incision, the SMA was dissected free, and an ultrasonic flow probe (Transonic Systems, Ithaca, NY) was placed around the vessel to measure the mesenteric blood flow. Hemodynamics (mean arterial pressure [MAP], heart rate [HR] and SMA flow) were continuously monitored with a computerized data acquisition system (SPEL Advanced HAEMOSYS 1.17; Experimetria, Budapest, Hungary), and records were taken at 5 minutes before and 30 minutes after the beginning of ischemia and at 5, 30, and 60 minutes after the reestablished blood flow to register IR-induced changes. Intravital examination of the serosal microcirculation of the ileum was conducted with orthogonal polarization spectral (OPS) imaging system (Cytoscan A/R; Cytometrics, Philadelphia, PA) (**Fig. S1*A***, Supplemental Digital Content 2, http://links.lww.com/CCM/E370; **legend**, Supplemental Digital Content 13, http://links.lww.com/CCM/E381). At the end of the protocol, a whole thickness tissue sample was taken from the ileum 10 cm orally from the cecum to determine inflammatory markers (myeloperoxidase, xanthine oxidase activity, and nitrite and nitrate levels) (see **Supplemental Digital Content 3**, http://links.lww.com/CCM/E371).

In order to examine the connection between baseline mesenteric circulatory variables and breath CH_4, a subgroup ($n = 7$; MetProd+IR group) was randomly selected from CH_4-producing animals, and occlusion of the SMA was induced and maintained for 45 minutes (Fig. S1*A*, Supplemental Digital Content 2, http://links.lww.com/CCM/E370; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381). In this setup, a rat was considered MetProd if the minimum points of the PS curve registering the tracheal exhaled CH_4 levels in real time continuously exceeded the maximum points of the PS curve registering the room air CH_4 content for 10 minutes (**Fig. S2**, Supplemental Digital Content 4, http://links.lww.com/CCM/E372; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381).

In a further step, the effects of exogenous CH_4 were examined in MetNonprod rats to model the nonproducer part of the human population or a situation when the baseline values of exhaled CH_4 are unknown. The animals were randomly divided into subgroups, and SMA ischemia was induced

(Table S1, Supplemental Digital Content 1, http://links.lww.com/CCM/E369; Fig. S1*A*, Supplemental Digital Content 2, http://links.lww.com/CCM/E370—legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381). The MetNonprod+Met+IR group received 10 mL/kg normoxic artificial air containing 2.18% CH_4 (at atmospheric pressure and room temperature) intraluminally via a 25 G needle attached to a gas-tight syringe in the proximal jejunum before the start of SMA ischemia ($n = 6$), whereas the MetNonprod+IR+Met animals received intraluminal CH_4 in the same way after the start of mesenteric ischemia ($n = 7$). The control group (MetNonprod+Air) received 10 mL/kg synthetic air without inducing IR ($n = 6$), and the effects of IR alone were also investigated in MetNonprod+IR animals ($n = 6$).

Pig Studies

Graded SMA Occlusion. Outbred Vietnamese minipigs ($n = 6$; 31 ± 7 kg) were used, and the animals were fasted for 12 hours before the procedures with access to tap water ad libitum. All members of the population were CH_4 producers.

Anesthesia was induced with a mixture of ketamine (20 mg/kg intramuscular) and xylazine (2 mg/kg intramuscular) and was maintained with a continuous infusion of propofol (6 mg/kg/hr IV; Fresenius Kabi, Bad Homburg, Germany), midazolam (1.2 mg/kg/hr; Torrex Chiesi Pharma, Vienna, Austria), and fentanyl (0.02 mg/kg/hr; Richter Gedeon, Budapest, Hungary). After endotracheal intubation, mechanical ventilation was started with a tidal volume of 10 mL/kg. The left jugular vein was cannulated for fluid and drug administration as was the left femoral artery for the measurement of MAP, HR, and cardiac output (CO) by transpulmonary thermodilution (PICCO Plus; PULSION Medical Systems, Feldkirchen, Germany). Ringer's lactate infusion was administered at a rate of 10 mL/kg/hr. After a median laparotomy, the SMA was dissected free, and a tourniquet was positioned proximal to the flow probe placed around the SMA. After baseline measurements, graded mesenteric arterial occlusion was started by gradually tightening the tourniquet, with each cycle divided into 30 minutes hypoperfusion and 30 minutes reperfusion periods. Complete occlusion with no SMA flow was achieved after four cycles (see details in Table S1, Supplemental Digital Content 1, http://links.lww.com/CCM/E369; **Fig. S1*B***, Supplemental Digital Content 2, http://links.lww.com/CCM/E370; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381).

Pericardial Tamponade. Outbred Vietnamese minipigs from the same breed as the study above ($n = 12$, weighing 24 ± 3 kg) were used; anesthesia was induced and maintained as described previously. Ventilation, hemodynamic measurements (MAP, HR, CO, and SMA flow), blood gas monitoring, and fluid replacement were carried out according to the methods used in the previous study. In addition, an intravital examination of the mucosal microcirculation was conducted with OPS imaging system; after the end of the microscopic recordings, the intestinal wall was closed with seromuscular sutures.

Group 1 ($n = 6$) served as a sham-operated control, with the same surgical interventions, time frame, and sampling as in Group 2 ($n = 6$) but without the induction of cardiac tamponade. In both groups, the diaphragm was accessed through a median laparotomy; a 4-cm incision was made at the sternal part, and a cannula was fixed into the pericardial cavity with a pledgeted purse string suture. In Group 2, cardiac tamponade was induced for 60 minutes by intrapericardial administration of heparinized own blood, and the MAP was maintained between 40 and 45 mm Hg. After this period, the fluid was removed from the pericardial sac, and the animals were monitored for 180 minutes in the posttamponade phase. The microcirculation of the ileal mucosa was recorded at baseline, 30 minutes after the relief of tamponade (90 min) and at the end of the experiments (240 min) (see details in Table S1, Supplemental Digital Content 1, http://links.lww.com/CCM/E369; **Fig. S1*C***, Supplemental Digital Content 2, http://links.lww.com/CCM/E370—legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381).

Exhaled CH_4 Analysis

We employed a near-infrared laser technique–based PS apparatus (16). Briefly, PS is a subclass of optical absorption spectroscopy that measures optical absorption indirectly via the conversion of absorbed light energy into acoustic waves due to the thermal expansion of absorbing gas samples. The amplitude of the generated sound is directly proportional to the concentration of the absorbing gas component. The gas sample is passed through the photoacoustic cell, in which signal generation takes place, and the photoacoustic signal produced is then detected by microphone (16).

The gas samples were taken continuously from the exhalation outlet of the ventilator at a 25–30 mL/min rate in rats and at a 150 mL/min rate in pigs during the experiments. In case of pig studies, the baseline exhaled CH_4 values were determined, and the values were thereafter subtracted from the test values.

For the microcirculatory analyses, 30 seconds periods were recorded three times, at three nonoverlapping fields. For practical reasons, the corresponding macrohemodynamics were also recorded for 90 seconds, and the mean values were used for data comparisons. In parallel, CH_4 values detected online were also averaged for identical 90 seconds periods.

Intravital Videomicroscopy

Microscopic OPS images were recorded with an S-VHS video recorder 1 (Panasonic AG-TL 700; Matsushita Electric Ind. Co. Ltd., Osaka, Japan). Quantitative assessment of the microcirculatory variables was performed offline with a frame-to-frame analysis of the videotaped images. RBC velocity (RBCV, μms^{-1}) in the postcapillary venules was determined in three separate fields by means of a computer-assisted image analysis system (IVM Pictron, Budapest, Hungary).

Statistical Analysis

Data analysis was performed with a statistical software package (SigmaStat for Windows; Jandel Scientific, Erkrath, Germany).

Normality of data distribution was analyzed with the Shapiro-Wilk test. The Friedman analysis of variance on ranks was applied within groups. Time-dependent differences from the baseline for each group were assessed with Dunn's method. In the rat study, differences between groups were analyzed with the Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn's method. In the pig study, Mann-Whitney *U* test was applied to analyze differences between groups, followed by Dunn's method. Median values and 75th and 25th percentiles are provided in the figures; *p* values of less than 0.05 were considered significant. Correlations between two variables were examined using Pearson's correlation coefficient (*r*); in the figures, regression lines and 95% CIs are given.

RESULTS

Rat Experiments

The breath CH_4 concentration in MetProd+IR animals decreased immediately after SMA occlusion, did not exceed the background values until the end of the ischemic period, and started to increase immediately after the reestablished perfusion (**Fig. 1**).

In this setup, we ascertained that intraluminal administered CH_4 can be detected in the exhaled air in previously MetNonprod rats (Fig. 1). Specifically, the data demonstrated the need for intraluminal CH_4 levels to achieve diagnostic significance in MetNonprod animals. When the splanchnic arterial circulation was blocked, the surplus CH_4 appeared in the breath only after the restoration of the mesenteric blood flow.

The exhaled CH_4 levels in MetNonprod+IR and MetNonprod+Air groups were stable, and the values did not change during the observation period. In the MetNonprod+Met+IR group, the CH_4 concentration increased immediately after the introduction of the gas mixture into the gastrointestinal lumen and then dropped to the background level following the induction of ischemia, whereas exhaled CH_4 concentrations started to increase again during the onset of reperfusion. In the MetNonprod+IR+Met group, the intraluminal CH_4 during the ischemic phase did not change the breath CH_4 output, but there was another increase in the exhaled CH_4 concentration after the start of reperfusion (Fig. 1). The continuous data registration revealed a steep and simultaneous elevation in SMA flow and exhaled CH_4 concentrations with an approximately 35 seconds lag. After this delay, both peaks and curves changed in parallel during reperfusion and decreased until reaching steady states (**Fig. 2*A***).

SMA flow and breath CH_4 level changes correlated significantly in the MetNonprod+IR+Met group (**Fig. 2*B***), whereas this correlation was not present in the MetNonprod+Met+IR group (**Fig. S3*A***, Supplemental Digital Content 5, http://links.lww.com/CCM/E373; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381). Similarly, the mean RBCV correlated with the exhaled CH_4 levels in the MetNonprod+IR+Met group (**Fig. 2*C***), and this association was not present in the MetNonprod+Met+IR group (**Fig. S3*B***, Supplemental Digital Content 5, http://links.lww.com/CCM/E373; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381). See the details of hemodynamics and biochemical data in **Tables S2–S4** (Supplemental Digital Content 6, http://links.lww.com/CCM/E374).

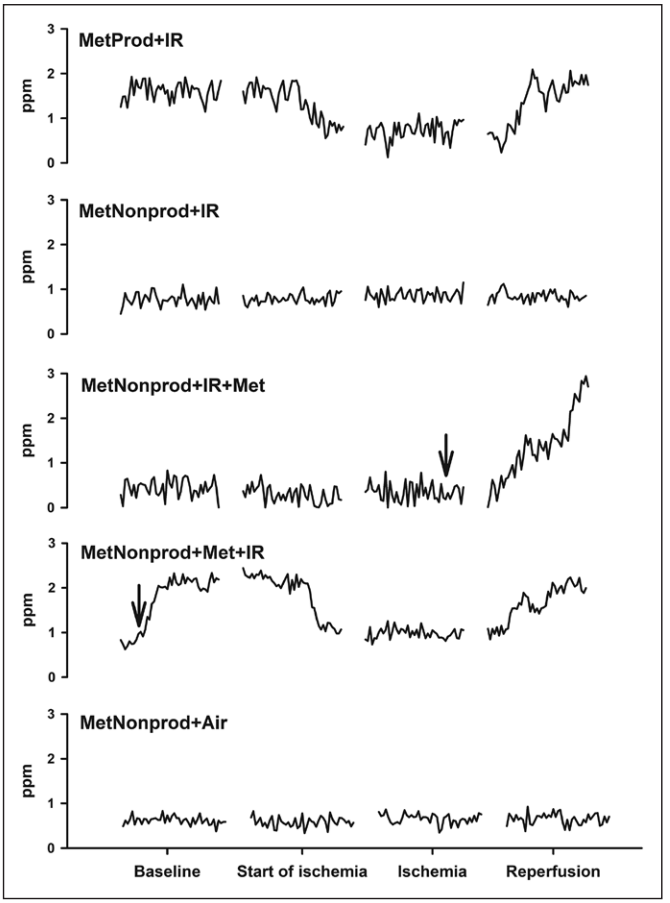

Figure 1. Original tracings representing the changes in exhaled methane levels in each experimental group of rats at four time points. Introduction of methane gas mixture is indicated (*arrow*). IR = ischemia-reperfusion, Met = intraluminally administered 10 mL/kg normoxic artificial air containing 2.18% CH_4 (at atmospheric pressure and room temperature), MetNonprod = without measurable CH_4, MetProd = with detectable CH_4 emissions.

Pigs With Graded Mesenteric Occlusion

At baseline, the exhaled CH_4 concentration was in the range of 12.1–90.5 ppm, approximately corresponding to the values usually present in CH_4-producing humans (9). The stepwise decrease in SMA flow (**Fig. 3*A***) was rigorously followed by parallel changes in breath CH_4 output (**Fig. 3*B***). There was no significant correlation between HR, MAP, and exhaled CH_4 levels (**Table S5**, Supplemental Digital Content 6, http://links.lww.com/CCM/E374), whereas the SMA flow and the breath CH_4 concentration changes correlated significantly ($r = 0.839$) **Fig. 3*C***).

Cardiac Tamponade in Pigs

Next, we employed a clinically relevant model of nonocclusive mesenteric ischemia. As an endpoint of tamponade, MAP was maintained for 60 minutes in the 40–45 mm Hg interval which was accompanied by a significant decrease

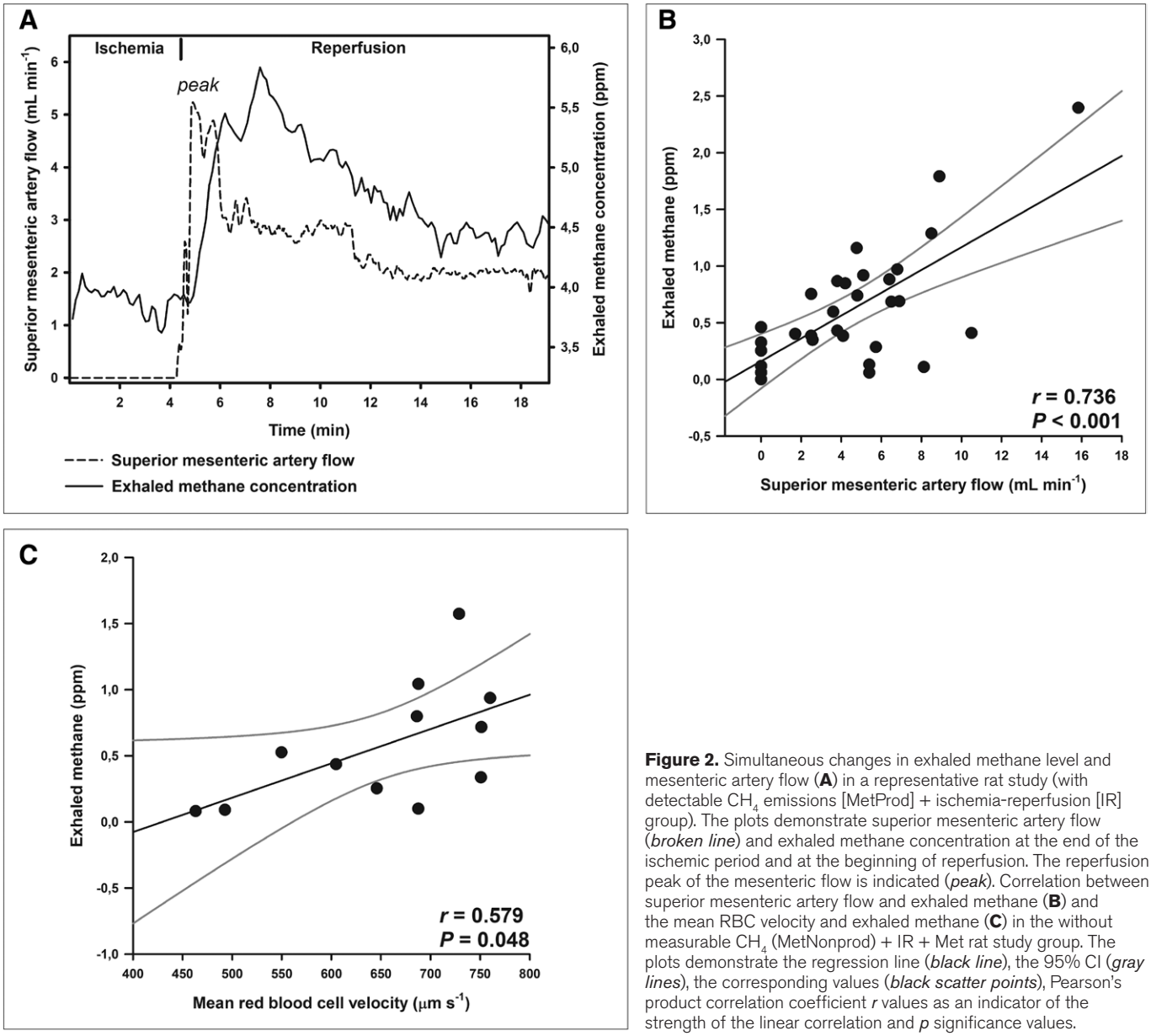

Figure 2. Simultaneous changes in exhaled methane level and mesenteric artery flow (**A**) in a representative rat study (with detectable CH_4 emissions [MetProd] + ischemia-reperfusion [IR] group). The plots demonstrate superior mesenteric artery flow (*broken line*) and exhaled methane concentration at the end of the ischemic period and at the beginning of reperfusion. The reperfusion peak of the mesenteric flow is indicated (*peak*). Correlation between superior mesenteric artery flow and exhaled methane (**B**) and the mean RBC velocity and exhaled methane (**C**) in the without measurable CH_4 (MetNonprod) + IR + Met rat study group. The plots demonstrate the regression line (*black line*), the 95% CI (*gray lines*), the corresponding values (*black scatter points*), Pearson's product correlation coefficient *r* values as an indicator of the strength of the linear correlation and *p* significance values.

in CO (**Table 1**), and a significantly decreased SMA flow pointed to the redistribution of organ perfusion (**Fig. S4*A***, Supplemental Digital Content 7, http://links.lww.com/CCM/E375). After the removal of the pericardial fluid, the MAP remained significantly lower compared with the sham-operated group (Table 1). The SMA flow returned to the control values; however, it remained significantly depressed compared with the sham-operated group at the end of the observation period.

The breath CH_4 concentration decreased significantly during the tamponade period. After the relief of tamponade, the exhaled CH_4 levels increased immediately, and there were no significant differences in the breath CH_4 output variables compared with the sham-operated group or the baseline values (**Fig. 4*A***; and **Fig. S5**, Supplemental Digital Content 8, http://links.lww.com/CCM/E376—legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381).

The mucosal RBCV was gradually reduced during the tamponade and remained significantly lower by the end of the observation period (**Fig. S4*B***, Supplemental Digital Content 7, http://links.lww.com/CCM/E375; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381). A statistically significant link was present between exhaled CH_4 concentration and SMA flow ($r = 0.669$) (**Fig. 4*B***). Furthermore, a significant correlation was found between mucosal RBCV and breath CH_4 changes ($r = 0.632$) (**Fig. 4*C***) as well, the correlation between these variables was similar to those observed in the rat study (**Fig. S6**, Supplemental Digital Content 9, http://links.lww.com/CCM/E377; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381). Correlations between SMA flow and exhaled CH_4 levels in individual animals are shown in **Figure S7** (Supplemental Digital Content 10, http://links.lww.com/CCM/E378; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381), **Figure S8** (Supplemental Digital Content 11, http://links.lww.com/CCM/E379; legend,

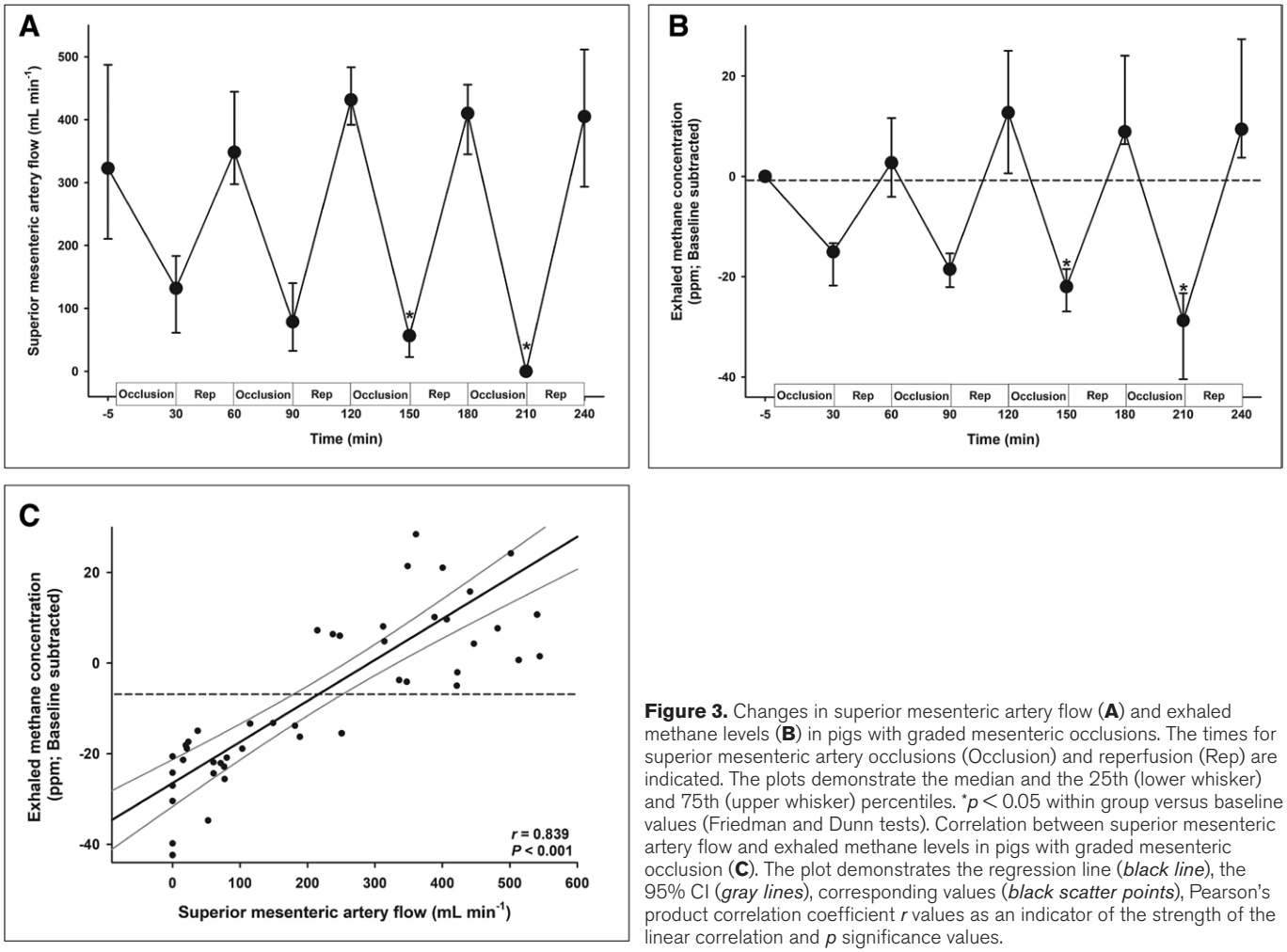

Figure 3. Changes in superior mesenteric artery flow (**A**) and exhaled methane levels (**B**) in pigs with graded mesenteric occlusions. The times for superior mesenteric artery occlusions (Occlusion) and reperfusion (Rep) are indicated. The plots demonstrate the median and the 25th (lower whisker) and 75th (upper whisker) percentiles. *$p < 0.05$ within group versus baseline values (Friedman and Dunn tests). Correlation between superior mesenteric artery flow and exhaled methane levels in pigs with graded mesenteric occlusion (**C**). The plot demonstrates the regression line (*black line*), the 95% CI (*gray lines*), corresponding values (*black scatter points*), Pearson's product correlation coefficient *r* values as an indicator of the strength of the linear correlation and *p* significance values.

TABLE 1. Cardiac Tamponade in Pigs

Groups		Baseline	30 min	60 min	120 min	180 min	240 min
Mean arterial pressure (mm Hg)							
Sham-operated	Median	104.5	99	105	101.5	111.5	98.5
	25–75th percentile	84.3–113.8	87.3–115.3	90.8–110.5	90–117.3	93.8–120.3	84.3–115.8
Tamponade	Median	92.5	41.0[a,b]	40.5[a,b]	65.0[b]	65.5[b]	67.5[b]
	25–75th percentile	80.5–117.8	36.0–42.5	38.3–45.3	51.5–88.8	54.3–78.8	57.3–85.3
Heart rate (beats/min)							
Sham-operated	Median	104	94	87	85	84	91
	25–75th percentile	78–123	78–116	77–109	73–110	66–109	68–107
Tamponade	Median	113	135[a,b]	128[a,b]	120[b]	120[b]	135[a,b]
	25–75th percentile	105–117	120–153	119–151	103–127	105–130	127–141
Cardiac index (L/min/m^2)							
Sham-operated	Median	2.59	2.60	2.45	2.30	2.16	2.10
	25–75th percentile	2.35–2.77	2.40–2.79	2.16–2.84	2.08–2.92	1.95–2.58	1.99–2.54
Tamponade	Median	2.54	1.18[a]	1.33[a]	2.27	2.61	2.39
	25–75th percentile	2.37–2.85	0.57–1.29	1.07–1.43	2.03–2.82	2.09–2.79	2.18–2.91

[a]$p < 0.05$ within groups vs baseline values.
[b]$p < 0.05$ between groups vs sham-operated values.

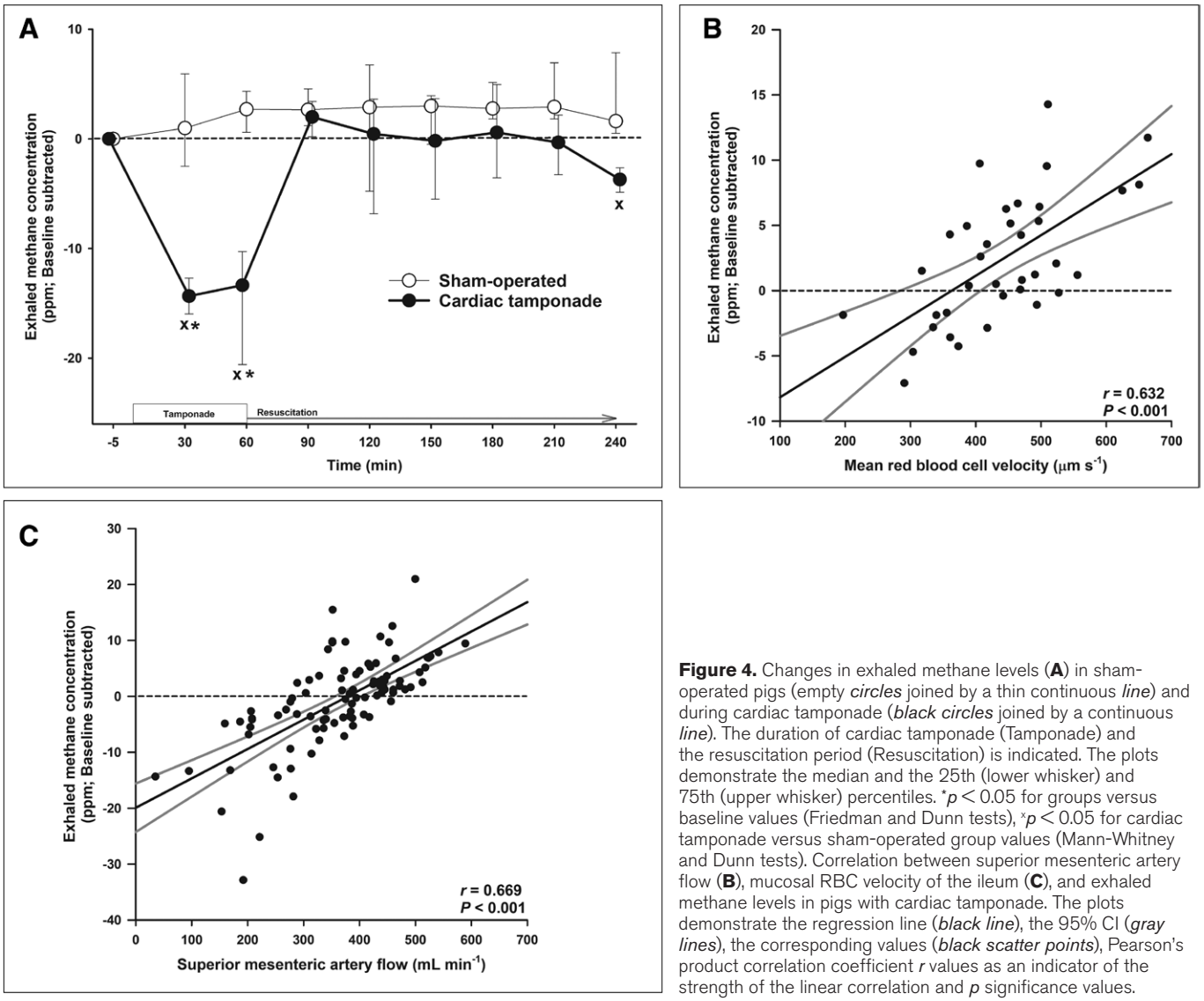

Figure 4. Changes in exhaled methane levels (**A**) in sham-operated pigs (empty *circles* joined by a thin continuous *line*) and during cardiac tamponade (*black circles* joined by a continuous *line*). The duration of cardiac tamponade (Tamponade) and the resuscitation period (Resuscitation) is indicated. The plots demonstrate the median and the 25th (lower whisker) and 75th (upper whisker) percentiles. $^{*}p < 0.05$ for groups versus baseline values (Friedman and Dunn tests), $^{x}p < 0.05$ for cardiac tamponade versus sham-operated group values (Mann-Whitney and Dunn tests). Correlation between superior mesenteric artery flow (**B**), mucosal RBC velocity of the ileum (**C**), and exhaled methane levels in pigs with cardiac tamponade. The plots demonstrate the regression line (*black line*), the 95% CI (*gray lines*), the corresponding values (*black scatter points*), Pearson's product correlation coefficient *r* values as an indicator of the strength of the linear correlation and *p* significance values.

Supplemental Digital Content 13, http://links.lww.com/CCM/E381), and **Figure S9** (Supplemental Digital Content 12, http://links.lww.com/CCM/E380; legend, Supplemental Digital Content 13, http://links.lww.com/CCM/E381).

DISCUSSION

Both recognition and follow-up of mesenteric ischemic syndromes are difficult, and there is a clear need to add new approaches and tools to the current diagnostic repertoire (22). Our study explored the alternative use of the breath CH_4 test in evaluating the status of the mesenteric perfusion, and we found a robust, dynamic correlation between the microcirculatory component of the mesenteric circulation and parallel changes in breath CH_4 output. The decreases in mesenteric macro- and microcirculatory flow resulted in a parallel reduction in exhaled CH_4 concentration regardless of occlusive or nonocclusive forms of ischemia. Specifically, the level of exhaled CH_4 did not change as long as ischemia persisted and increased rapidly as the mesenteric flow was restored. The amount of CH_4 required for diagnosis can be endogenous or exogenous, deriving from an intraluminal source; intraintestinally administered CH_4 becomes quickly detectable in the exhaled air in previously non-CH_4 producer rats. In any case, surplus CH_4 only appears in the breath and thus in the outside air after the restoration of mesenteric blood flow.

At present, the most reliable methods for the diagnosis of mesenteric circulatory disturbances are multidetector row CT angiography or digital subtraction angiography; however, once performed, they provide a snapshot view and continuous monitoring is not possible after the procedure. Noninvasive methods, such as indirect tonometry and sublingual intravital microscopy using OPS imaging or sidestream darkfield techniques, have severe limitations (19).

Today, analysis of CH_4 in the exhaled air used for the diagnostics of functional gastrointestinal pathologies, like constipation-predominant forms of irritable bowel syndrome or lactose intolerance, where the detection of CH_4 concentration changes relative to other gaseous compounds, can facilitate the diagnosis (20–22). Nevertheless, the intra- and intersubject

variabilities of the measurements are usually very large, CH_4 producers may stop excreting, nonproducers may start to excrete CH_4, and, occasionally, CH_4-producing status does not change after antibiotic treatments targeting the intestinal methanogenic flora. Indeed, it has recently been suggested that the clinical implications of breath CH_4 analyses should undergo an in-depth revision (22). It should be added that in clinical laboratory practice breath CH_4 levels are usually analyzed by means of gas chromatography (GC) and GC-mass spectrometry, and the sampling frequency of these traditional, discontinuous methods is limited (23, 24). Here, we have used a gas-measuring device enabled for the continuous real-time determination of exhaled CH_4 output. The PS system was tested for whole-body and single-breath CH_4 analyses in previous surveys, and CH_4 changes have proved to be reproducible and highly specific for CH_4 in a wide dynamic range (16).

We used three different models of mesenteric circulatory distress and different species to investigate the possible association between breath CH_4 output and mesenteric hemodynamics. Rats produce CH_4 approximately in the same ratio as humans, at least in our facility. Therefore, the non-CH_4 producer rat population was used to test the changes in exhaled CH_4 and alterations in the mesenteric circulation in a nonproducer scenario and also to test the breath effects and consequences when CH_4 was supplied intraluminally from an external source. The size and the digestive system of the pig are comparable with those of humans, but in contrast to humans, pigs are always CH_4 producers (25, 26). Therefore, this animal model was chosen to investigate the possible connections between exhaled CH_4 levels and hemodynamic changes in less controlled, but clinically more relevant circumstances.

Our data may clearly expand the scope of traditional breath CH_4-based diagnostics, but the present study is primarily a proof-of-concept investigation and does have some limitations. First, the sensitivity of the PS system for the diagnosis of mesenteric ischemia with different degrees and durations should be determined in additional, prospective studies. Furthermore, it would be important to know the association between artificially depressed CH_4 production (e.g., administration of inhibitors of the prokaryote methanogenesis) and the mucosal microcirculatory responses. Besides, our data demonstrate that CH_4 levels may allow the detection of mesenteric perfusion changes if the baseline exhaled CH_4 concentration is known and exceeds the CH_4 level of the surrounding air. The current setup may be well used for noninvasive monitoring of mesenteric mucosal perfusion during planned interventions, but the CH_4 nonproducer ratio of the human population is an important issue to consider. In experimental settings, there are many possibilities to manipulate CH_4 content in the gastrointestinal tract. Mammalian CH_4 production can be increased with the availability of substrates through carbohydrate ingestion (e.g., lactulose and glucose) or with the administration of synthetic phenazine neutral red dye, which induces the transient growth of methanogenic strains (27), but clinically more feasible methods, such as the application of CH_4-enriched fluids (28), might also be possible approaches for exogenous CH_4 delivery. Comparative analysis with additional diagnostics, such as detection of lactate or intestinal fatty acid–binding protein levels, should also be performed to obtain a broader view of the accuracy and predictability of the method.

CONCLUSIONS

The results provided in vivo evidence for a significant correlation between exhaled CH_4 and intestinal microcirculatory changes in CH_4-producing conditions when the range of baseline endogenous CH_4 output exceeds the range of atmospheric CH_4 concentration. The real-time analysis of exhaled CH_4 levels merits attention toward the development of a new diagnostic concept for noninvasive detection of mesenteric ischemia.

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