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BRIEF ARTICLE

Non-invasive panel tests for gastrointestinal motility monitoring within the MARS-500 Project

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Abstract

AIM: To develop an integrated approach for monitoring gastrointestinal motility and inflammation state suitable for application in long-term spaceflights.

METHODS: Breath tests based on the oral administration of ^{13}C-labeled or hydrogen-producing substrates followed by the detection of their metabolites ($^{13}CO_2$ or H_2) in breath were used to measure gastrointestinal motility parameters during the 520-d spaceflight ground simulation within the MARS-500 Project. In particular, the gastric emptying rates of solid and liquid contents were evaluated by ^{13}C-octanoic acid and ^{13}C-acetate breath tests, respectively, whereas the orocecal transit time was assessed by an inulin H_2-breath test, which was performed simultaneously with the ^{13}C-octanoic acid breath test. A ready-to-eat, standardized pre-packaged muffin containing 100 mg of ^{13}C-octanoic acid was used in the ^{13}C-octanoic acid breath test to avoid the extemporaneous preparation of solid meals. In addition, a cassette-type lateral flow immunoassay was employed to detect fecal calprotectin, a biomarker of intestinal inflammation. Because no items could be introduced into the simulator during the experiment, all materials and instrumentation required for test performance during the entire mission simulation had to be provided at the beginning of the experiment.

RESULTS: The experiments planned during the simulation of a manned flight to Mars could be successfully performed by the crewmembers without any external assistance. No evident alterations (*i.e.*, increasing or decreasing trends) in the gastric emptying rates were detected using the ^{13}C-breath tests during the mission simulation, as the gastric emptying half-times were in the range of those reported for healthy subjects. In contrast to the ^{13}C-breath tests, the results of the inulin H_2-breath test were difficult to interpret because of the high variability of the H_2 concentration in the breath samples, even within the same subject. This variability suggested that the H_2-breath test was strongly affected by external factors, which may have been related to the diet of the crewmembers or to environmental conditions (*e.g.*, the accumulation of hydrogen in the simulator microenvironment). At least in closed microenvironments such as the MARS-500 simulator, ^{13}C-breath tests should therefore be preferred to H_2-breath tests. Finally, the fecal calprotectin test showed significant alterations during the mission simulation: all of the crewmembers were negative for the test at the beginning of the simulation but showed various degrees of positivity in at least one of the subsequent tests, thus indicating the onset of an intestinal inflammation.

CONCLUSION: Breath tests, especially those ^{13}C-based, proved suitable for monitoring gastrointestinal motility in the 520-d isolation experiment within

MARS-500 project and can be applied in long-term spaceflights.

Key words: Breath test; Gastrointestinal inflammation; Gastrointestinal motility; Spaceflight; Stress

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INTRODUCTION

A manned mission to Mars is currently starting to garner a consistent level of support, as exploration roadmaps are under study by various space agencies. Nevertheless, several issues related to the health of humans during such a long space mission still must be solved.

Extended-duration space missions expose the crewmembers to microgravity, radiation and a stressful environment due to mission-related factors (*e.g.*, confinement, isolation, anxiety, physiologic stress, sleep deprivation and modifications of their nutrition regimes, circadian rhythms and microbial environments) that affect their physiological status[1]. To properly monitor the crewmembers' health status during a real space mission, a suitable panel of biochemical tests and related analytical instrumentation should be developed, implemented in the space module and validated for its clinical utility and applicability in spaceflight. These tests should be easily performed onboard by the crewmembers on non-invasively collectable biological samples (*e.g.*, saliva, breath expatriate, urine, or stool) and employing compact devices in a point-of-care format.

Among the alterations that might occur in long-term spaceflights, changes in the gastrointestinal (GI) motility and related gut inflammatory states are of particular relevance. The main factors affecting GI motility are the physical properties of the solid and liquid contents of the stomach and intestine and the functional, hormonal and enzymatic changes in those organs. Spaceflight-related changes in GI function, such as fluid shifts, combined with reduced fluid intake, would tend to decrease GI motility. Although GI motility has not been systematically studied in spaceflight, a significant increase in the mouth-to-caecum transit time has been demonstrated in ground simulations (10 d of -6° head-down bed resting[2,3] and water immersion[4]).

Previous studies have demonstrated that adequate nutritional status is critical to maintaining crew health during extended-duration spaceflight[5-8], and a common cause of reduced dietary intake, especially during the first d of a mission, is space motion sickness[9]. The impact of psychological, physical, and immunological stressors on GI motility, duodenal and biliary secretion, epithelial permeability, and inflammation is currently thoroughly documented, and stress has a major influence on digestive diseases. Gastrointestinal motor dysfunctions, mainly caused by stress conditions, alteration of circadian rhythms and nutritional regimen, may also represent themselves as additional stress factors[10,11]. Decreased GI motility will, in turn, result in delayed intestinal absorption, alterations in the intestinal microflora and decreased bioavailability of orally administered drugs[12]. Such possible alterations must be expeditiously and continuously detected to guide the adoption of the actions necessary to avoid negative consequences to the crewmembers' health and, more generally, wellness (and thus to the crew's efficiency).

In this work, we present an integrated approach to the non-invasive monitoring of GI motility and inflammation state that was optimized in the frame of the MARS-500 project. This project was realized by the State Scientific Center of the Russian Federation-Institute of Biomedical Problems of the Russian Academy of Sciences (IBMP), under the auspices of Roscosmos and the Russian Academy of Sciences and in collaboration with the European Space Agency and other space agencies and institutions from all over the world. The project consisted of several isolation experiments, including a final 520-d isolation (the longest spaceflight ground simulation ever conducted) designed to simulate a round-trip manned mission to Mars. The project aimed at obtaining useful information about physical and psychological problems that astronauts might face during a long stay onboard an interplanetary space vehicle and to set up technologies for monitoring their health status with possible application in real space missions.

The integrated approach herein described employed breath tests (BTs) for the evaluation of GI motility. Indeed, ^{13}C- and H_2-BTs based on the oral administration of ^{13}C-labeled or hydrogen-producing substrates followed by the detection of the metabolites of these substrates ($^{13}CO_2$ or H_2, respectively) in the breath represent a convenient, non-invasive and efficient procedure for obtaining information on motor and organ functions of the GI system. Such tests are routinely used for the detection of alterations in GI motility, bacterial overgrowth, and lactose intolerance, among other issues, and for the diagnosis of infection with *Helicobacter pylori*[13-15]. We evaluated the gastric emptying rates of solid and liquid content by ^{13}C-octanoic acid and ^{13}C-acetate BT, respectively, whereas the orocecal transit time was assessed by an H_2-BT that used inulin as the hydrogen-producing substrate (the latter BT was performed simultaneously with the ^{13}C-octanoic acid BT for the measurement of the gastric emptying rate of solids). In addition, a cassette-type lateral flow immunoassay was employed for detecting fecal calprotectin, a biomarker of intestinal inflammation.

Because they are non-invasive and easily self-performed, BTs are potentially transferrable to the space environment, provided protocol standardization and the

development of compact on-board instrumentation. Miniaturized instrumentation based on electrochemical gas sensors is available for H_2-BT, whereas compact instrumentation based on non-dispersive infrared spectroscopy (NDIRS) has been developed as an alternative to isotope ratio mass spectrometry (IRMS) for the measurement of $^{13}CO_2$ in breath[16]. In perspective, miniaturized dedicated analytical instrumentation suitable for on-board operation by the crewmembers will make this integrated approach applicable in real space missions, thus providing a useful tool for the early detection of dysfunctions of the GI system and the adoption of suitable countermeasures, such as diet adjustments or pharmacological interventions.

MATERIALS AND METHODS

Subjects

The crew was composed of six male subjects, who at the beginning of the experiment had a median age of 31 years (range 27-38 years), median body weight of 81 kg (range 74-100 kg), and median body mass index of 26.3 kg/m^2 (range 23.6-32.3 kg/m^2). During the mission simulation, all of the crewmembers received the same diet, the composition of which was almost identical to that of the diet used in the International Space Station[17].

Ethics

All of the scientific investigations performed in the frame of the MARS-500 experiments were reviewed and approved by the IBMP Committee on Bioethics, and all of the volunteers signed the written informed consent for participation in the experiment.

Materials employed for diagnostic tests

A standard muffin meal (EXPIROGer®, manufactured and packaged by Sofar SpA, Milan, Italy) containing 100 mg of ^{13}C-octanoic acid was employed in the ^{13}C-BT for the measurement of the gastric emptying rate of solid meals. The muffin (weight 100 g) had a 378 kcal (1589 kJ) calorie content and the following composition: 5.5 g of proteins, 57.5 g of carbohydrates, 14.0 g of fats (corresponding to 5.8%, 60.8%, and 33.3% of the total calories, respectively), 1.1 g of dietary fiber and 16.7% moisture. Stable ^{13}C-isotope-labeled sodium acetate (99% isotope purity) was purchased from Cambridge Isotope Laboratories (Andover, MA). Inulin (Beneo™ HP-Gel) with a degree of polymerization of 5-60 was obtained from Orafti (Oreye, Belgium). The enteral nutrition solution Nutrizon standard was manufactured by Otsuka Pharmaceutical (Tokyo, Japan) and had (for 100 mL) a 110 kcal (420 kJ) calorie content, 15% of which were from proteins and 55% from carbohydrates. The semiquantitative rapid immunochromatographic test for the detection of calprotectin in feces (PreventID® Cal Detect®) was produced by Preventis GmbH, Wiesenstr, Germany. The test allowed an easy visual evaluation of fecal calprotectin, providing three degrees of positivity: low (< 15 μg/g), medium (15-60 μg/g), and high (> 60 μg/g).

Assay protocols

Breath tests were performed during the Baseline Data Collection period (BDC; before the start of the simulation) and in three separate experimental sessions at approximately d 100, 240 and 475 of the mission simulation. During each experimental session, different ^{13}C-BTs performed on the same subject were staggered by at least 3 d to allow the washout of the administered substrates and the recovery of basal ^{13}C levels.

The combined ^{13}C- and H_2-BT for the measurement of the gastric emptying rate of solids and the orocecal transit time consisted of the simultaneous administration of the EXPIROGer® standard meal and inulin, followed by the measurement of the kinetics of the appearance of $^{13}CO_2$ and H_2 in the breath. In preparation for the test, the crewmembers were requested to refrain from fatty meals or a high intake of dietary fiber the day before the test. Antibiotics, fermented milk products and laxatives were also avoided during the 10-d period preceding the test. After an overnight fast, breath samples were collected to measure the basal levels of $^{13}CO_2$ and H_2. Subsequently, the subjects received the EXPIROGer® standard meal and 5.0 g of inulin dissolved in 200 mL of water. Breath samples for $^{13}CO_2$ analysis were collected up to 240 min after substrate ingestion in 12-mL glass tubes, which were then transferred outside the simulator for analysis. Samples for the evaluation of breath H_2 content were collected in plastic bags up to 440 min after substrate ingestion, and the concentration of H_2 was measured on-board immediately after each breath sample had been collected. During the test, the subjects were allowed to drink water and, after 4 h, to resume their usual dietary regimens.

The ^{13}C-BT for the measurement of the gastric emptying rate of liquids consisted of the administration of sodium ^{13}C-acetate followed by the measurement of the kinetics of the appearance of $^{13}CO_2$ in the breath. After an overnight fast, breath samples were collected to measure the basal level of $^{13}CO_2$. Subsequently, the subjects orally received 150 mg of sodium ^{13}C-acetate dissolved in 500 mL of Nutrizon enteral nutrition solution, and breath samples were collected up to 240 min after substrate ingestion in 12-mL glass tubes, which were then transferred outside the simulator for analysis. After assuming the substrate, the subjects were requested not to ingest any additional food or drink until the end of the test.

The fecal calprotectin test was performed directly by the crewmembers during the BDC and on day 130, 220, and 475 of the mission simulation following the instructions provided by the manufacturer (the test was repeated twice in each experimental session).

Sample analysis

For the measurement of the $^{13}CO_2/^{12}CO_2$ ratio, breath samples were analyzed using a BreathMAT IRMS (Ther-

Table 1 Dynamics of the body mass (kg) of the crewmembers

	Crewmember					
	A	B	C	D	E	F
BDC	81.5	99.5	76.6	86.9	82.5	73.5
Exp. session 1	+3.5	-2.0	+4.3	-1.0	+0.1	+0.2
Exp. session 2	+3.3	-8.2	+4.0	-4.6	-2.0	-4.7
Exp. session 3	+1.5	-20.4	-3.8	-6.9	-1.2	-6.8
End of mission simulation	-1.1	-22.6	-5.4	-9.7	-4.0	-7.2

BDC: Baseline Data Collection.

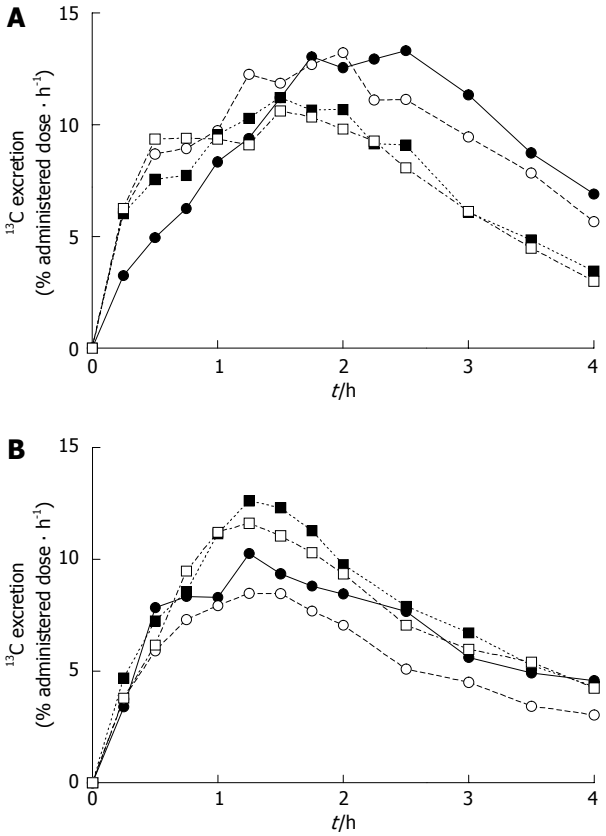

Figure 1 ^{13}C-breath test for the evaluation of gastric emptying rates. Representative $^{13}CO_2$ excretion kinetic profiles obtained in the ^{13}C-breath test for the evaluation of the gastric emptying rates of (A) solids and (B) liquids performed during the Baseline Data Collection period (•) and during the mission simulation (experimental session 1: ◦; experimental session 2: ▪; experimental session 3: ▫).

mo Finnigan MAT GmbH, Bremen, Germany). The measurement of breath H_2 levels was performed onboard by the crewmembers using a portable H_2 analyzer equipped with a miniaturized electrochemical cell (Lactotest 102, Medical Electronic Construction R&D sprl, Brussels, Belgium).

Statistical analysis

The ^{13}C-BT results, given as the $^{13}CO_2$ content of the exhaled CO_2 expressed in δ‰ PDB units (zero δ‰ PDB corresponds to 1.12372% ^{13}C atoms), were processed to evaluate the rate of excretion of $^{13}CO_2$ produced by the metabolism of the ^{13}C-labeled substrate, which was expressed as a percentage of the administered dose per hour. To this purpose, the total expiratory CO_2 production of each subject was assumed to be 300 mmol/m^2 of body surface/h[18], and the body surface was computed as described by Haycock *et al*[19]. For the evaluation of the relevant gastric emptying parameters, the excretion kinetics were analyzed by a least-square fitting procedure using a suitable equation[18], and the gastric emptying half-times were calculated from the coefficients of the equation[20].

The H_2-BT results, given as the H_2 breath concentrations in ppm, were processed for evaluating the enrichment of H_2 in the breath over the basal value due to the fermentation of inulin by the intestinal microflora and then plotted as a function of time; the orocecal transit time was assessed as the time at which the breath hydrogen content rose 10 ppm above the basal value[21].

To assess alterations in GI motility, the results of the breath tests performed during the BDC and during the mission simulation were compared by one-way ANOVA for matched data with Dunnett's post-test using GraphPad Prism version 5.03 (GraphPad Software, San Diego, CA). Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Crew health status

The periodic blood biochemical function tests and clinical examinations during the mission simulation did not show any significant pathology or physiological alteration. Comparison of the body weights of the crewmembers during the BDC and at the end of the mission simulation indicated that one subject (B) displayed a significant reduction in weight (-21%), whereas for the other subjects, the reduction was lower (C, D, E and F) or negligible (A). Although no net increases in body weight were observed, subjects A and C experienced a rise in body mass during the first part of the experiment (Table 1).

^{13}C-BT for gastric emptying rate

Figure 1 shows representative $^{13}CO_2$ excretion kinetic profiles obtained in the ^{13}C-BT for the evaluation of the gastric emptying rates of solids and liquids performed during BDC and in the different experimental sessions during the mission simulation. The gastric emptying half-times obtained for the six crewmembers by analyzing the $^{13}CO_2$ excretion kinetic profiles using the procedure described in the Statistical Analysis section are reported in Table 2.

It can be observed that at the beginning of the simulation (BDC), certain subjects (*i.e.*, A and D) had long gastric emptying half-times of solids (*e.g.*, 4.4 and 5 h for A and D, respectively) and that this behavior was maintained in most of the experimental sessions performed during the mission simulation. As a general rule, long gastric emptying half-times of solids were paralleled (albeit to a lesser extent) by relatively long gastric emptying half-times of liquids, although a large variability in the differences between the two times was observed. Nevertheless,

Table 2 Gastric emptying half-times (h) evaluated by ^{13}C-breath test

Experimental session	Crewmember A	B	C	D	E	F	mean ± SD
Solids							
BDC	4.4	2.8	3.3	5.0	3.2	2.8	3.5 ± 1.0
Exp. session 1	2.7	2.7	2.2	6.2	2.9	2.9	3.2 ± 1.5
Exp. session 2	3.7	2.2	2.9	3.5	2.8	2.5	2.8 ± 0.5
Exp. session 3	4.9	2.2	2.7	4.8	3.4	2.6	3.3 ± 1.2
Liquids							
BDC	2.6	2.3	2.4	3.0	2.8	1.9	2.5 ± 0.4
Exp. session 1	2.6	2.0	2.2	2.8	2.6	2.5	2.5 ± 0.3
Exp. session 2	2.6	2.1	2.7	2.6	2.6	2.5	2.5 ± 0.2
Exp. session 3	2.9	2.0	2.6	4.0	2.8	2.6	2.8 ± 0.7

BDC: Baseline Data Collection.

no evident increasing or decreasing trend in the gastric emptying half-times was detected for any crewmember during the mission simulation; most of the measured gastric emptying half-times were in the range of those reported for healthy subjects[18,22], although in certain cases, rather high values were obtained.

H_2-BT for orocecal transit time

Figure 2 shows the H_2 excretion kinetic profiles obtained in the H_2-BT for the evaluation of the orocecal transit time. The H_2 breath concentrations showed a large variability, sometimes decreasing below the basal level, which increased the difficulty of identifying the H_2 excretion kinetic profiles and evaluating the orocecal transit time by applying the standard criteria reported in the literature (*i.e.*, by identifying the first time at which the breath hydrogen concentration increased by at least 10 ppm above the baseline value).

Although, in several cases, acceptable H_2 excretion profiles were obtained (for example, crewmember D showed high H_2 breath concentrations at long times after substrate ingestion, which were paralleled by a delayed gastric emptying of solids), the overall results suggested that the inulin H_2-BT was negatively affected by external factors, which may have been related to the simulation environment, such as the closed chamber simulating the space station.

Fecal calprotectin test

Table 3 summarizes the results of the fecal calprotectin test for the evaluation of intestinal inflammation performed during the BDC and during the mission simulation. The results are given as scores according to the semi-quantitative evaluation of calprotectin concentration in fecal samples that was performed with the test. Notably, the crewmembers were negative for the fecal calprotectin test during the BDC, but for all of them positive results were obtained in at least one of the tests performed during the mission simulation. The observed degrees of intestinal inflammation varied from low (in two subjects) to high (in four subjects).

Table 3 Results of the fecal calprotectin test[1]

Experimental session	Crewmember A	B	C	D	E	F
BDC	-	-	-	-	-	-
Day 130	-	+++	+	+	-	+++
Day 220	-	-/+[2]	-	-	+++	-/+[2]
Day 475	+++	-/+++[2]	+	-	-/+[2]	+++

[1]Legend: (-) negative, (+) low positivity (< 15 μg/g), (++) medium positivity (15-60 μg/g), (+++) high positivity (> 60 μg/g); [2]The repeated tests gave different results. BDC: Baseline Data Collection.

DISCUSSION

Continuous and non-invasive monitoring of the health status of the crewmembers during space missions requires the development of cutting-edge technologies; their requirements (simple analytical procedures, possibility of self-administration, use of portable point-of-care instrumentation, long shelf-life of reagents) are similar to those faced in critical medicine (*e.g.*, clinical medicine in emergency situations, remote field locations or third-world countries). Thus, new technological solutions that are suitable for the space environment will benefit medical diagnostics for all of us.

In this work, ^{13}C- and H_2-BT were employed for the non-invasive monitoring of GI motility during the MARS-500 project. The accuracy of ^{13}C- and H_2-BT for the measurement of motor functions of the GI system has been demonstrated by several studies[21,23-25]. However, the application of BT in the space environment still requires certain improvements. For example, the ^{13}C-octanoic acid BT is typically performed using extemporaneously prepared meals (*e.g.*, ^{13}C-octanoic acid is incorporated into egg yolk, which is then pan-cooked and consumed with bread and butter), which makes meal standardization difficult and limits test reproducibility. To overcome this drawback, we employed a ready-to-eat test meal (a muffin containing 100 mg of ^{13}C-octanoic acid) with carbohydrate, lipids, proteins and calorie content optimized for the BT performance. The long-term stability of this test meal and its suitability for the measurement of the gastric emptying rate of solids have been evaluated in a multicenter study[26]. Moreover, the muffin is designed for diagnostics; thus, it is gluten-, lactose- and glucose-free to enable its administration to subjects who are affected by celiac disease, lactose intolerance or diabetes, and the unpleasant taste and odor that are characteristic of short-chain fatty acids are efficiently masked. We also combined the ^{13}C-octanoic acid BT for measuring the gastric emptying rate of a solid meal and the inulin H_2-BT for measuring the orocecal transit time into a single test to reduce the number of experimental sessions in the mission simulation and to allow the direct comparison of two different indexes of GI motility, avoiding subject day-to-day variability.

Regarding the instrumentation employed for the an-

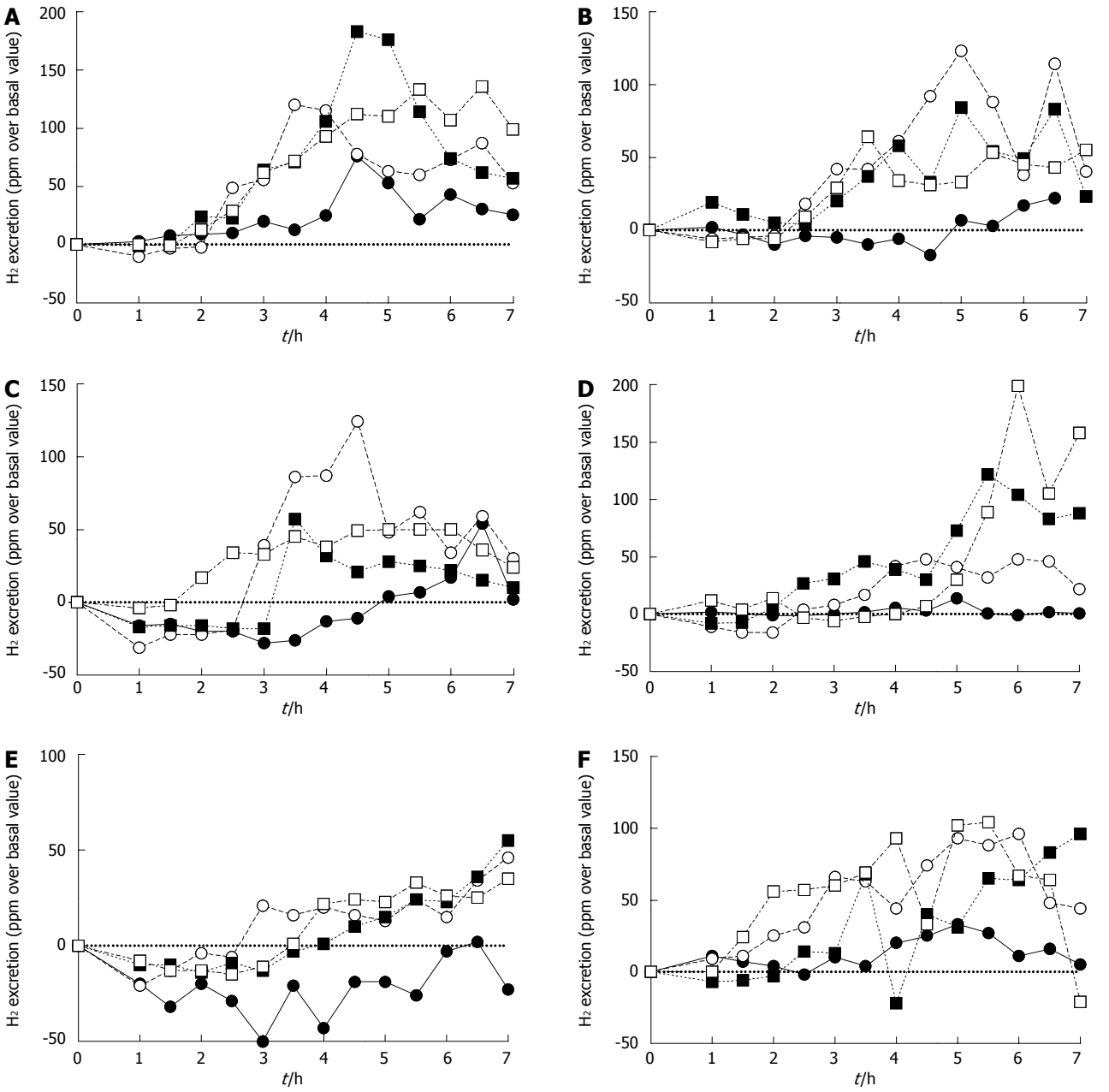

Figure 2 H_2-breath test for the evaluation of orocecal transit time. Hydrogen excretion kinetic profiles obtained in the H_2-breath test (BT) for the evaluation of the orocecal transit time performed during the Baseline Data Collection period (•) and during the mission simulation (experimental session 1: ◦; experimental session 2: ▪; experimental session 3: ▫). This BT was performed simultaneously with the 13C-octanoic acid BT for the evaluation of the gastric emptying rate of solids (A-F).

alysis of the breath samples, the measurement of the $^{13}CO_2/^{12}CO_2$ ratio was performed by IRMS in an external laboratory. However, NDIRS, which is more amenable to miniaturization, could also be used [17,20]. Work is in progress to develop a miniaturized hybrid analytical device combining the NDIRS technology for $^{13}CO_2$ measurement with the fuel cell technology for H_2 measurement employed in the Lactotest 102 H_2 breath analyzer. Such a device will allow the simultaneous onboard measurement of the $^{13}CO_2/^{12}CO_2$ ratio and H_2 concentration in a single breath sample, thus avoiding the need for separate breath sample collection in dual BT.

The results obtained during the MARS-500 experiments did not show significant alterations in the gastric emptying rates of solids and liquids (researchers are currently increasingly inclined to use only gastric emptying half-times when reporting the results of the ^{13}C-octanoic acid BT; therefore, we do not discuss other gastric emptying parameters, such as the lag time). Subjects A and D presented long gastric emptying half-times of solids with high variability, but no unambiguous trends were observed. Moreover, it should be taken into account that Choi *et al*[27,28] suggested that the truncation of the observation period of ^{13}C-octanoic acid BT to four hours could lead to an overestimation of gastric emptying half-times. Therefore, the long half-times measured for subjects A and D could be at least in part ascribed to this factor (indeed, these gastric emptying half-times were

close to or even longer than the observation period).

In contrast, the results of the H_2-BT for the orocecal transit time, performed simultaneously with the ^{13}C-octanoic acid BT, were difficult to interpret because the high variability of the H_2 concentration in the breath samples did not allow a reliable evaluation of the orocecal transit times. Nevertheless, certain results suggested, as expected, a positive correlation with gastric emptying half-times. For example, in subject D, who showed the longest gastric emptying half-times for solids, the highest concentrations of H_2 in the breath were often detected at longer times in comparison with the other subjects. These results suggested that the H_2-BT was strongly affected by external factors, such as the diet of the crewmembers (hydrogen can be produced by the fermentation of other food sugars and related substances, such as dietary fiber) and the environmental conditions (*e.g.*, the possible accumulation of hydrogen in the simulator microenvironment). Indeed, hydrogen concentrations up to 30-40 ppm were recorded inside the simulator, whereas external values remained below 1.0 ppm. Moreover, the portable H_2 analyzer employed in this experiment required manual injection of the breath sample; thus, the reproducibility of the measurement could be improved by implementing automated sample management procedures. Nevertheless, in the absence of further information, it might be concluded that in closed microenvironments, such as the MARS-500 simulator, ^{13}C-BTs should be preferred to H_2-based tests. In particular, the lactose ^{13}C-ureide BT, which has been established as a reliable test for the assessment of orocecal transit time[29,30], could represent an alternative to the inulin H_2-BT.

In contrast to ^{13}C-BTs, the fecal calprotectin test detected significant alterations during the mission simulation: all of the crewmembers were negative for the test during the BDC but showed various degrees of positivity (from low for subjects C and D to high for subjects A, B, E, and F) in at least one of the tests performed during the mission simulation. Calprotectin is a sensitive fecal marker of intestinal inflammation that is used to differentiate between organic intestinal diseases (*e.g.*, chronic inflammatory diseases, infectious diseases, or colon cancer) and functional intestinal diseases (*e.g.*, irritable bowel syndrome)[31,32]. Application of calprotectin test for screening asymptomatic subjects has also been reported[33,34]. Fecal calprotectin can be determined with high specificity and sensitivity using the CalDetect® lateral flow immunoassay[35]. Because it has been already demonstrated in animal models and humans that stress influences the inflammatory response[36,37], the stress conditions experienced by the crewmembers could be responsible for the observed intestinal inflammation, although external factors related to diet and environment, as well as possible alterations in the intestinal microflora, cannot be excluded.

In conclusion, the results obtained in the MARS-500 mission simulation suggested that the stress level experienced by crewmembers during the mission simulation had no significant impact on the GI motility. Because previous experiments performed in microgravity conditions showed alterations in the GI motility[38,39], it could be concluded that microgravity should have a major impact on GI motor functions, whereas stress-related factors might contribute to the onset of motility alterations but are not the primary cause. Nevertheless, useful information on the possible application of BTs in future isolation experiments or real space missions has been obtained. Due to their simplicity of performance, ability to be performed repeatedly, safety, and non-invasiveness, ^{13}C-BTs represent a promising approach for the monitoring of alterations of motor and/or organ functions of the GI system, thus moving space medicine closer to clinical observation systems used on Earth. In the MARS-500 experiments, $^{13}CO_2$ analysis in breath samples was performed by IRMS in an external analysis facility, but portable analytical instruments for $^{13}CO_2$ breath analysis (for example, based on the NDIRS technology) integrated within an informatics framework for data acquisition, analysis, and remote transmission will allow crewmembers to perform such tests autonomously. Regarding H_2-BT, suitable portable H_2 breath analyzers are already available, but the results suggested that the performance of this BT is strongly affected by external factors; thus, it could be concluded that in this type of application, ^{13}C-BTs should be preferred to H_2-based tests. In addition, the measurement of fecal calprotectin by a cassette-type lateral flow immunoassay evidenced a significant degree of intestinal inflammation in all the crewmembers. Although no clinical symptoms associated with intestinal inflammation were reported during the mission simulation, the possibility that a combination of isolation, stress and dietary factors (*i.e.*, prolonged nutrition with canned and preserved foods) could favor the onset of this pathological status should be considered in future mission simulations or real space flights.

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COMMENTS

Background

Extended-duration space missions expose the crewmembers to microgravity, radiation, stress and other factors that can affect their physiological status. For instance, changes in gastrointestinal motility may result in the reduced intestinal

absorption of nutrients, alterations in the intestinal microflora and decreased bioavailability of orally administered drugs. Such possible alterations must be detected expeditiously to avoid negative consequences to the crewmembers' health and, more generally, wellness.

Research frontiers

The evaluation of the gastrointestinal motility during a real space mission requires biochemical tests that can be easily performed onboard by the crewmembers. Biological samples should be easily collectable in a microgravity environment (*e.g.*, saliva or breath expatriate) and analyzed using compact devices in a point-of-care format. Tests and related analytical instrumentation are to be implemented in the space module and validated for its clinical utility and applicability in spaceflight.

Innovations and breakthroughs

In this study, ^{13}C- and H_2-breath tests for the monitoring of gastrointestinal motility have been designed to be self-performed without any external assistance by the subjects participating in the final 520-d isolation experiment in the frame of the MARS-500 project. The reagents for breath test performance have been optimized for long-term storage (no materials could be introduced into the simulator during the isolation period) and minimum preparation required before use; a portable H_2 analyzer equipped with a miniaturized electrochemical cell has been provided to allow the onboard measurement of breath H_2 levels by the crewmembers. A commercially available cassette-type lateral flow immunoassay was also employed for detecting fecal calprotectin, a biomarker of intestinal inflammation.

Applications

The study suggested that breath tests, especially those based on ^{13}C, could be employed for the monitoring of alterations of motor and/or organ functions of the gastrointestinal system in future isolation experiments or real space missions.

Peer review

The authors present an interesting application of non-invasive gastrointestinal (GI) motility and lower intestinal inflammation tests in a closed-chamber space simulation. Although the results overall reveal no significant change in gastric emptying and require additional confirmation, this study represents an interesting demonstration of how GI monitoring may be achieved with very limited resources. This battery of tests could find application not only in outer space but also in bedside testing in a variety of clinical environments, both inpatient and outpatient.

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