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Tolle, G., Serreli, G., Deiana, M. *et al.* Lipidomics Changes Observed when Caco-2 Are co-cultured with the Enterohemorrhagic *E. Coli* Under Simulated Microgravity. *Microgravity Sci. Technol.* **38**, 5 (2026).

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The publisher's version is available at:
https://doi.org/10.1007/s12217-025-10227-y

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Lipidomics changes observed when Caco-2 are co-cultured with the enterohemorrhagic *E. coli* under simulated microgravity

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Abstract

The influence of simulated microgravity on cellular processes has attracted growing interest due to its relevance in space biology and medical research. This study investigates the impact of simulated microgravity on Caco-2 cells, a widely used human epithelial colorectal adenocarcinoma cell line, co-cultured with the pathogenic enterohemorrhagic *E. coli* O157:H7 strain. The experiment explored cells lipids alterations and metabolic changes under microgravity conditions compared to terrestrial gravity. Using a lipidomics approach under simulated microgravity we observed an upregulation of lysophosphatidylcholines LysoPC 14:0, LysoPC 16:0, LysoPC 16:1, LysoPC 17:0, LysoPC 18:0, LysoPC 18:1, LysoPC 20:1; phosphatidylcholines PC 14:0/14:0, PC 14:0/16:1, PC 16:0/16:1 and PC 18:0/18:1 as well as ceramides Cer 18:1;O_2/16:0, Cer 18:1;O_2/24:0, Cer 18:2;O_2/24:0. Conversely, we detected a downregulation of sphingomyelins SM 18:1;O_2/16:0, SM 18:1;O_2/24:0, and SM 18:2;O_2/24:0, and the betaine lipids of microbial origin diacylglyceryl-trimethylhomoserine DGTS 16:0/19:1 and DGTS 19:1/19:1.

These findings provide insights into the molecular adaptations of human epithelial cells in response to microgravity and offer a foundation for future studies on microbiome-host dynamics in spaceflight environments.

Keywords: enterocytes, spaceflights, lysophosphatidylcholine, phosphatidylcholine, ceramides, sphingomyelins.

1. Introduction

Microgravity is a unique condition encountered by astronauts during spaceflights that profoundly affects biological systems at the molecular and cellular levels (Manna, O.M. et al., 2024). At cellular level microgravity disrupts key processes, such as proliferation, differentiation, and metabolism, with significant implications for human health especially during long-term space missions (Tomsia, M. et al., 2024). Among the cellular changes, alterations in lipid metabolism have emerged as critical areas of investigation due to the role of lipids in maintaining cellular structure, signaling, and energy homeostasis. Understanding these effects is essential to address the challenges posed by space travels and to advance fundamental knowledge in space biology.

Caco-2 cells, derived from human epithelial colorectal adenocarcinoma, are a widely used model system for studying intestinal barrier function, nutrient absorption, and epithelial-microbe interactions (Ramos, P. et al., 2023; Sun, H. et al., 2008). These cells are particularly relevant for simulating the human intestinal environment *in vitro*, as they differentiate into polarized epithelial layers resembling the intestinal lining under specific culture conditions. Due to their physiological relevance, Caco-2 cells are used to study the effects of microgravity on epithelial cell function (Alvarez, R. et al., 2019). We recently reported that when Caco-2 cells were exposed to simulated microgravity conditions alteration of lipids such as ceramides, phosphatidylcholines and sphingomyelins. These lipids are essential for maintaining cell structure, signaling, and function, and their roles in microgravity could affect various biological processes, such as cellular adaptation to space, and stress response (Tolle, G. et al., 2024).

Microgravity also influences microbial behavior, often enhancing bacterial virulence, growth, and metabolic activity (Rosenzweig et al., 2010). This has been demonstrated in various bacterial species, including *E. coli*, a pathogenic commensal member of the human gut microbiota and a model organism for studying host-pathogen dynamics (Richter, T.K. et al., 2018). *E. coli* is particularly valuable in co-culture systems for its ability to interact with epithelial cells and

modulate cellular processes, including immune responses and metabolism (He, X. et al., 2013). However, little is known about how microgravity shapes the interactions between host cells and bacteria, especially at the molecular level. Co-culture systems, where host cells and microbes are grown together, provide a powerful tool for studying these interactions in a controlled environment. Co-cultures mimic the complexity of the intestinal microenvironment, enabling the investigation of dynamic relationships between host and microbial communities.

Lipidomics, the comprehensive analysis of cellular lipids, is a critical approach to unravel the molecular effects of microgravity on cellular function (Holčapek, M. et al., 2018). Lipids play pivotal roles in maintaining cells membrane integrity, signaling pathways, and energy metabolism. Furthermore, alterations in lipid composition can affect cellular homeostasis, membrane fluidity, and interactions between cells and microbes (Zhang, Y.M. et al., 2008; Šajbidor, J., 1997). Despite the recognized importance of lipids, research on how microgravity affects lipid metabolism in host-microbe co-culture systems remains limited.

This study seeks to fill this gap by exploring how simulated microgravity affects the lipidomic profiles of Caco-2 cells co-cultured with the pathogenic enterohemorrhagic *E. coli* O157:H7. By integrating advanced lipidomics techniques with a co-culture model, we examined how lipid changes induced by microgravity influence both host cell function and microbial activity. The results will provide valuable insights into the molecular mechanisms of host-microbe interactions in space and could help develop strategies to address health risks associated with extended space missions.

2. Materials and methods

2.1. Chemicals

Analytical LC grade methanol, chloroform, acetonitrile, 2-propanol, ammonium acetate, ammonium formate and formic acid were obtained from Sigma Aldrich (Milan, Italy). Cell culture materials were obtained from Euroclone (Milan, Italy). The *E. coli* O157:H7 bacterial strain was obtained from ATCC (Manassas, VA, USA). Ultrapure bi-distilled water was produced using a MilliQ purification system (Millipore, Milan, Italy).

2.2. Cell cultures

Intestinal Caco-2 cells (ECACC, Salisbury, Wiltshire, UK) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated bovine serum, 2 mM L-glutamine, 1% non-essential amino acids, 100 U/mL penicillin, and 100 mg/mL streptomycin. The cells were maintained as monolayers at 37 °C in a humidified atmosphere containing 5% CO2, with the culture medium being replaced twice weekly (Incani et al., 2016). For experimental studies, Caco-2 cells at passages 25–40 were plated and utilized 18–21 days after seeding, once fully differentiated. The cells were cultured in 25 mL flasks, and upon full differentiation, each flask was completely filled with culture medium before being placed in the clinostat. Following this procedure, the culture medium was aspirated from each flask, and the cellular material was collected by adding 2 mL of trypsin to detach the cells. Trypsin activity was then neutralized by adding 4 mL of complete culture medium. The cell suspension was transferred to centrifuge tubes and centrifuged at 300 × g for 5 minutes at 4°C. Immediately after centrifugation, the resulting cell pellets were rapidly resuspended in cold methanol and incubated for 10 minutes at -20°C to ensure enzymatic inactivation and metabolic quenching. This step was critical to preserve the native lipid profile by halting lipid remodeling, oxidation, and signaling processes. The quenched cell suspensions were then stored at -80°C until further lipidomic analysis.

For co-culture experiments, Caco-2 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% non-essential amino acids, and antibiotics (penicillin/streptomycin) at 37°C in a humidified incubator with 5% CO_2. Upon reaching confluence, cells were seeded at a density of 1×10^5 cells/cm² in 12-well plates and allowed to differentiate for 21 days. At least 24 hours prior to bacterial infection, the culture medium was replaced with antibiotic-free DMEM to ensure bacterial viability and allow effective host-pathogen interaction. From a strain of *E. coli* (ATCC 43895) seeded on Nutrient agar and incubated for 18 hours at 37°C, a suspension was prepared with an optical density of 0.5 (OD600), corresponding to 1.5 × 108 CFU/mL. Through serial dilutions, the concentration of 10^6 CFU/mL was arrived at, from which 1 mL was taken and added directly to the differentiated Caco-2 monolayers. The cultures were incubated for 4 h under standard conditions, after which the supernatants and cell layers were collected for further analysis.

2.3 Simulated microgravity condition

The RPM is a well-established instrument designed to simulate microgravity conditions on Earth by continuously altering the orientation of the samples in a randomized manner, thereby minimizing the influence of gravitational vectors (Calvaruso, M. et al., 2021). It achieves this by continuously and randomly altering the orientation of a sample along multiple axes, thereby averaging the gravitational vector to zero over time. This process effectively minimizes the influence of gravity on the sample, replicating the microgravity environment found in space. The RPM comprises an outer frame capable of independent rotation along multiple axes and an inner frame that holds the sample. Both frames rotate unpredictably at varying speeds and directions, creating a randomized motion pattern. This continuous and irregular rotation ensures an even distribution of gravitational forces across all directions, effectively minimizing gravitational influence on the sample. The device features a control system that enables precise adjustment of

rotation speeds and movement patterns. In this study, the RPM was configured to operate in Random Walk mode at a rotation speed of 80 degrees per second (rpm), regulated through dedicated software. Caco-2 cells served as the biological model in this study. Approximately 50×10^4 cells were seeded in 75 cm^2 plug-seal screw cap flasks containing DMEM low-glucose medium supplemented with L-glutamine and sodium pyruvate. These flasks were carefully placed on the RPM's inner frame and maintained in a dedicated room at a controlled temperature of 37 °C. Control samples were prepared under identical conditions but were kept stationary on a static bar at 1 g to experience the same level of vibration as the microgravity samples, ensuring consistent environmental conditions for both groups. After 24 h of exposure, both the microgravity-treated (0 g) and control (1 g) samples were collected. The cells were detached from the culture flasks using 2 mL of trypsin, followed by centrifugation to obtain a cell pellet. The harvested cell pellets were immediately stored at -80 °C until further use in lipidomic analysis.

2.4. Sample extraction

Cellular lipid extraction was carried out using a modified Folch method, which involves extraction with a chloroform-methanol mixture (Ulmer C.Z. et al., 2015). Specifically, 3 mg of cells were extracted using 375 μL of a methanol and chloroform mixture (2:1, v/v). To facilitate lipid homogenization and extraction, samples were vortexed intermittently every 15 minutes for 1 hour. Following the initial extraction, 350 μL of chloroform and 90 μL of a 0.2 M potassium chloride (KCl) solution were added to induce phase separation, enabling the isolation of the organic phase containing lipids. The mixture was then centrifuged at 17,700 × g for 10 minutes to ensure a clear separation of the aqueous and organic phases.

Subsequently, 400 μL of the organic layer was carefully transferred into a glass vial, and the solvent was evaporated under a gentle nitrogen stream to prevent lipid oxidation and degradation. The dried lipid residue was reconstituted in 20 μL of a methanol-chloroform mixture (1:1, v/v) to

ensure complete solubilization. Finally, 80 µL of a solvent mixture consisting of 2-propanol, acetonitrile, and water (2:1:1, v/v) was added to enhance compatibility with LC-MS analysis.

2.6. Untargeted UHPLC- QTOF/MS analysis

Cell samples were analyzed in quadruplicate using an Agilent UHPLC-QTOF/MS 6560 system coupled with a 1290 Infinity II LC system (Agilent Technologies, Palo Alto, US). To optimize sensitivity and ionization efficiency, 1 µL of sample was injected for electrospray ionization (ESI) in positive mode, while 4 µL was injected for ESI in negative mode. Chromatographic separation was performed on a C18 Kinetex column (1.5 µm, 100 mm × 2.1 mm, Phenomenex, Bologna, Italy), maintained at 50 °C with a flow rate of 0.4 mL/min. For positive ionization mode, the mobile phase consisted of (A) 10 mM ammonium formate in 60% Milli-Q water and 40% acetonitrile, and (B) 10 mM ammonium formate in 90% 2-propanol and 10% acetonitrile. The solvent gradient began with 80% A, decreasing linearly to 50% A over 2.1 min, followed by a reduction to 30% A over the next 10 min, and further decreased to 1% A over 1.9 min before re-equilibrating to the initial conditions within 1 min. For negative ionization mode, 10 mM ammonium acetate was used instead of ammonium formate in both mobile phases to enhance ionization efficiency.

An Agilent JetStream ion source was employed with optimized parameters, including a gas temperature of 200 °C, nitrogen gas flow of 10 L/min, nebulizer pressure at 50 psig, sheath gas temperature of 300 °C, and sheath gas flow of 12 L/min. The capillary voltage was set to 3500 V for positive ionization and 3000 V for negative ionization, with a nozzle voltage of 0 V. Additional settings included a fragmentor voltage of 150 V, a scan mass range of 50–1700 m/z, and a collision energy of 20 eV for positive mode and 25 eV for negative mode.

Data acquisition was performed using the Agilent MassHunter LC/MS Acquisition console. The dual ionization modes enabled comprehensive detection of both polar and non-polar lipid species.

This methodology ensured high-resolution chromatographic separation and sensitive mass spectrometric detection, facilitating robust and reproducible lipidomic profiling.

2.7 Data Analysis

The data were pre-processed using Mass Profinder 10.0 software (Agilent Technologies, Santa Clara, CA, USA), which facilitates mass recalibration and deconvolution, thereby generating a data matrix that includes all features detected across all samples. This matrix was subsequently analyzed using SIMCA 15.0 software (Sartorius, Umeå, Sweden). The data were initially autoscaled using UV scaling, followed by Principal Component Analysis (PCA), an unsupervised technique that enables visualization of sample and variable distributions within a multivariate space, and aids in the identification of outliers and distinctive features. Subsequently, Partial Least Squares Discriminant Analysis (PLS-DA) and its orthogonal extension, Orthogonal PLS-DA (OPLS-DA), were applied (**Figures S1-S4**). These supervised classification models were employed to identify and evaluate variables that discriminate between samples assigned to different classes. The variable importance in projection (VIP) scores were calculated, with values exceeding 1.0 being considered indicative of discriminatory features.

3. Results and discussion

3.1 Effect of microgravity on the co-culture

Understanding the lipidomic changes in intestinal epithelial cells during interactions with bacterial species is critical for elucidating host-pathogen dynamics and their impact on cellular functions. Lipids play essential roles in membrane integrity, cellular signaling, and metabolic regulation, making lipidomic profiling a valuable tool for investigating host responses to microbial interactions (Han, X. et al.,2005).

By an UHPLC-QTOF/MS platform we investigated the lipidomic alterations in Caco-2 cells co-cultured with *E. coli* under terrestrial gravity (1g) compared with those maintained under simulated microgravity (SMC) to provide insights into bacterial modulation of host lipid metabolism.

In comparison to controls maintained under terrestrial gravity, Caco-2 co-cultures with *E. coli* under simulated microgravity conditions showed a significant increase in complex lipids, including products of phosphatidylcholine hydrolysis, such as lysophosphatidylcholine (LysoPC), specifically LysoPC 14:0, LysoPC 16:0, LysoPC 16:1, LysoPC 17:0, LysoPC 18:0, LysoPC 18:1, LysoPC 20:1 (**Table 1**). Interestingly, Uruno et al. conducted a metabolomics study on plasma from wild-type and *Nrf2* knockout mice collected during and after spaceflights and observed an elevation of the different glycerophosholipids and sphingolipids. In particular the following plasma lysophosphocholines were found upregulated such as LysoPC 14:0, LysoPC 16:0, LysoPC 16:1, LysoPC 17:0, and LysoPC 18:0 (Uruno, A., et al., 2021). These results are also in accordance with our previous data showing an up regulation of the levels of lysophosphatidylcholines LysoPC 16:0, LysoPC 18:0, LysoPC 18:1, in Caco-2 cells under simulated microgravity conditions (Tolle, G. et al., 2024). These results also agree with the reduction of the levels of the following phosphatidylcholines PC 14:0/14:0, PC 14:0/16:1, PC 16:0/16:1, PC 18:0/18:1 which are hydrolyzed to the corresponding LysoPC (see **Table 1**). These shifts suggest bacterial-induced modulation of membrane dynamics and potential activation of autophagy pathways (Siqueira, M.

D.S. et al., 2018). Furthermore, cone shaped LysoPCs may influence membrane curvature and fluidity, and their levels might change as cells adapt to the microgravity environment. Additionally, LPCs are also bioactive lipids that participate in several signaling pathways, including those involved in inflammation, cell proliferation, and apoptosis acting through various receptors, such as G-protein-coupled receptors (GPCRs), to trigger intracellular signaling cascades (van Meer et al., 2008; Li, Y. et al., 2016; Schmitz & Ruebsaamen, 2010).

We previously reported that, following a simulated microgravity experiment with Caco-2 cells, ceramide levels were upregulated, while sphingomyelin levels were reduced. (Tolle, G. et al., 2024). In the experiment with the co-culture of Caco-2 cells with *E. coli* we observed the same trend. In particular levels of Cer 18:1;O_2/16:0, Cer 18:1;O_2/24:0, Cer 18:2;O_2/24:0 were found strongly upregulated and accordingly the levels of sphingomyelins SM 18:1;O_2/16:0, SM 18:1;O_2/24:0, SM 18:2;O_2/24:0 were found downregulated. The change of these complex lipids are attributable to the microgravity effects rather than the effect of *E coli.* Again, these results are in accordance with Uruno et al. (Uruno, A. et al., 2021).

The upregulation of CE 22:6 (Cholesteryl Ester 22:6, tentatively annotated as docosahexaenoic acid-esterified cholesterol) in Caco-2 cells co-cultured with *E. coli* under simulated microgravity suggest key metabolic adaptations influenced by both bacterial presence and microgravity-induced stress (**Table 1**). CE 22:6 is a storage form of docosahexaenoic acid (DHA), an omega-3 fatty acid with anti-inflammatory and membrane-stabilizing properties (Stephens, M. et al., 2020). DHA is typically involved in cholesterol homeostasis, lipid transport, and membrane remodeling, particularly in response to oxidative stress or lipid metabolism shifts (Chen, Z. et al., 2022). Again, Uruno et al. in their metabolomics study experiment conducted on the International Space Station (ISS) on mice plasma found different cholesteryl esters upregulated including CE(22:6) (Uruno, A. et al., 2021).

The increased levels of the bacterial diacylglyceryl trimethylhomoserines (DGTS), i.e. DGTS 16:0/19:1 and DGTS 19:1/19:1 when Caco-2 cells co-cultured with *E. coli* under simulated microgravity (see **Table 1**) indicate a distinctive adaptation of bacteria membrane lipids under microgravity-induced stress. Additionalym DGTS is typically upregulated in response to environmental stress, especially oxidative stress (Sato, N., 1992).

Attribution	**Theor.** ***(m/z)***	**Exp,** ***(m/z)***	**Adduct**	**Δ ppm**	**Rt (min)**	**VIP**	**UP regulation**
LPC 14:0	468.3076	468.3074	[M+H]	-0.42	1.65	1.15	g0
LPC 16:0	496.3396	496.3390	[M+H]	-1.20	2.16	1.39	g0
LPC 16:1	494.3235	494.3232	[M+H]	-0.60	1.82	1.33	g0
LPC 17:0	510.3547	510.3545	[M+H]	-0.39	2.58	1.17	g0
LPC 18:0	524.3702	524.3700	[M+H]	0.57	3.13	1.28	g0
LPC 18:1	522.3547	522.3544	[M+H]	-0.57	2.48	1.50	g0
LPC 20:1	550.3680	550.3683	[M+H]	0.54	3.35	1.22	g0
PC 14:0/14:0	678.5060	678.5063	[M+H]	0.44	8.00	1.44	g1
PC 14:0/16:1	704.5225	704.5220	[M+H]	-0.70	8.29	1.21	g1
PC 16:0/16:1	732.5537	732.5536	[M+H]	-0.13	9.85	1.12	g1
PC 18:0/18:1	788.6168	788.6167	[M+H]	-0.12	12.9	1.24	g1
Cer 18:1;O_2/16:0	538.5194	538.5190	[M+H]	-0.74	4.24	1.24	g0
Cer 18:1;O_2/24:0	650.6446	650.6442	[M+H]	-0.61	8.15	1.44	g0
Cer 18:2;O_2/24:0	648.6289	648.6288	[M+H]	-0.15	7.84	1.09	g0
SM 18:1;O_2/16:0	703.5733	703.5730	[M+H]	-0.42	9.15	1.46	g1
SM 18:1;O_2/24:0	815.7000	815.7001	[M+H]	-0.12	14.48	1.42	g1
SM 18:2;O_2/24:0	813.6843	813.6840	[M+H]	-0.36	13.93	1.38	g1
CE 22:6	697.5918	697.5916	[M+H]	-0.28	17.32	1.05	g0
DGTS 16:0/19:1	752.6385	752.6380	[M+H]	-0.66	12.70	1.43	g0
DGTS 19:1/19:1	792.6689	792.6687	[M+H]	-0.25	13.76	1.12	g0

Table 1. Comparison of lipid species upregulated in Caco-2 cells co-cultured with E. coli under simulated microgravity (g_0) and terrestrial gravity (g_1). The data reflects bacterial influence on lipid metabolism, including alterations in cholesteryl ester (CE), diacylglyceryl-trimethylhomoserine (DGTS), lysophosphatidylcholine (LPC), ceramide (Cer) and downregulation in phosphatidylcholines (PC) and sphingomyelins (SM). Data includes theoretical and experimental *m/z* values, adduct types, retention times (Rt), and variable importance in projection (VIP) scores.

3.2 Effect of *E.coli* on Caco-2 cells under simulated microgravity conditions

Under simulated microgravity we revealed significant lipid change when we compared the monoculture of Caco-2 cells with the co culture of pathogenic *E. coli*. The most notable changes were observed in the positive ionization mode with the up regulation of the levels of phosphatidylethanolamines (PE) and phosphatidylserines (PS) which are important components of cell membranes and are involved in various cellular functions. In particular, the following phosphatidylethanolamines: PE 14:0/16:1, PE 14:0/17:1, PE 16:0/16:1 and PE 18:1/18:1 showed the highest VIP values of variable importance in the projection (VIP) ranging from 1.35 to 1.52 (**Table 2**). These phosphatidylethanolamines were of bacterial origin because they were found strongly upregulated in the controls when we compared the lipid profiles of *E. coli* in microgravity conditions (*E. coli* G0 vs *E.coli* G1) (**Table S1**). The membrane lipid phosphatidylethanolamines are used by bacterial cells as a source of the metabolically compound ethanolamine (Randle, C.L. et al., 1969). In response to environmental stress such as microgravity, *E. coli* can modulate the lipid composition, to maintain membrane integrity. Under conditions like high temperature or osmotic stress, cells adapt PE levels to maintain membrane fluidity and functionality (Rowlett, V.W. et al., 2017; Los, D.A., et al., 2013). PE are synthesized in the Golgi from CDP-ethanolamine by ethanolamine phosphotransferase 1 (EPT1) in the presence of diacylglycerols (DG). Consistently, we observed a reduction of the levels of annotated DG in Caco-2 cells alone with VIP values ranging from 1.23 to 1.53 (**Table 2**). Additionally, we observed a downregulation of the levels of LysoPE from which PE are synthesized by the lysophospholipid acyltransferase (LPEAT1) (**Figure 1**) or through the acylation of lyso-PE by acyl-CoA transferase enzymes, LPCAT3, and LPCAT4 (St Germain, M., et al., 2023).

Levels of the phosphatidylserines PS 16:0/18:1 and PS 18.0/18:1 were also found to be upregulated in the co-culture with *E. coli* while their metabolic precursors phospatidylcholines PC 16:0/18:1 and PC 18.0/18:1 were strongly downregulated (**Table 2**). These PS are also of bacterial origin

because found strongly upregulated in the controls when we compared the lipid profiles of *E. coli* in microgravity conditions (**supplementary materials**). In bacteria, phosphatidylserines are crucial in maintaining membrane integrity and asymmetry, regulating membrane dynamics and curvature, and participating in bacterial stress response. Phosphatidylserines can be found in the intestinal mucus and are the principal source of carbon and nitrogen for Salmonellae and *Escherichia coli*. (Krivan, H.C et al., 1992).

In the negative ionization mode, in the coculture we found that the levels of fatty acids such as FA 18:1 and FA 19:1 and interestingly fatty acids hydroxy fatty acids, FAHFA 16:0/18:2, FAHFA 18:0/20:2, FAHFA 18:1/20:3 were upregulated.

These findings highlight the impact of bacterial co-culture under simulated microgravity on lipid metabolism in intestinal epithelial cells, providing a deeper understanding of host-microbe interactions in altered gravitational environments.

Attribution	**Theor.** ***(m/z)***	**Exp,** ***(m/z)***	**Adduct**	**Δ ppm**	**Rt (min)**	**VIP**	**UP regulation**
PE 14:0/16:1	662.4746	662.4741	[M+H]	-0.75	8.53	1.44	caco+e.coli
PE 14:0/17:1	676.4905	676.4902	[M+H]	-0.44	9.50	1.52	caco+e.coli
PE 16:0/16:0	692.5225	692.5220	[M+H]	-0.72	11.38	1.2	caco+e.coli
PE 16:0/16:1	690.5069	690.5068	[M+H]	-0.14	10.13	1.25	caco+e.coli
PE 16:0/17:1	704.5219	704.5218	[M+H]	-0.14	10.88	1.21	caco+e.coli
PE 16:1/17:1	702.5063	702.5062	[M+H]	-0.14	9.78	1.02	caco+e.coli
PE 16:0/18:1	718.5384	718.5382	[M+H]	-0.27	11.57	1.05	caco+e.coli
PE 16:1/18:1	716.5225	716.5220	[M+H]	-0.69	10.32	1.23	caco+e.coli
PE 16:0/19:1	732.5539	732.5538	[M+H]	-0.13	12.52	1.13	caco+e.coli
PE 17:1/18:1	730.5382	730.5381	[M+H]	-0.13	11.23	1.18	caco+e.coli
PE 18:0/18:1	746.5704	746.5701	[M+H]	0.53	13.08	1.29	caco+e.coli
PE 18:1/18:1	744.5541	744.5540	[M+H]	-2.24	12.22	1.35	caco+e.coli
PE 18:1/18:2	742.5401	742.5402	[M+H]	0.13	10.76	1.21	caco+e.coli
PE 16:0/20:4	740.5219	740.5215	[M+H]	-0.54	10.41	1.15	caco+e.coli
PE 18:1/19:1	758.5699	758.5698	[M+H]	-0.13	12.67	1.1	caco+e.coli
PE 19:1/19:1	772.5889	772.5880	[M+H]	-1.16	13.56	1.24	caco+e.coli
PE 18:0/20:4	768.5540	768.5538	[M+H]	1.04	11.94	1.02	caco+e.coli
PE 18:0/20:5	766.5381	766.5380	[M+H]	-0.13	10.62	1.09	caco+e.coli
PE 18:1/20:5	764.5249	764.5248	[M+H]	-0.13	10.11	1.16	caco+e.coli
PE 18:1/22:5	794.5702	794.5701	[M+H]	-0.12	12.05	1.17	caco+e.coli
PE 18:0/22:6	792.5536	792.5530	[M+H]	-0.75	11.64	1.19	caco+e.coli
PE 18:1/22:6	790.5376	790.5374	[M+H]	-0.25	10.32	1.24	caco+e.coli

PS 16:0/16:1	734.4942	734.4941	[M+H]	-0.13	8.13	1.26	caco+e.coli
PS 16:0/18:1	762.5275	762.5274	[M+H]	-0.13	9.62	1.12	caco+e.coli
PS 18:0/18:1	790.5603	790.5601	[M+H]	-0.25	11.09	1.19	caco+e.coli
PC 14:0/14:1	676.4900	676.4902	[M+H]	0.29	7.64	1.05	caco
PC 14:0/15:0	692.5215	692.5211	[M+H]	-0.57	8.79	1.43	caco
PC 14:0/16:0	706.5382	706.5381	[M+H]	-0.14	9.59	1.28	caco
PC 15:0/16:0	720.5536	720.5534	[M+H]	-0.27	10.37	1.13	caco
PC 15:0/16:1	718.5382	718.5380	[M+H]	-0.27	9.09	1.5	caco
PC 16:0/16:0	734.5694	734.5692	[M+H]	-0.27	11.15	1.46	caco
PC 16:1/16:1	730.5382	730.5380	[M+H]	-0.27	8.57	1.05	caco
PC 16:0/17:0	748.5844	748.5841	[M+H]	-0.40	11.62	1.11	caco
PC 15:0/18:1	746.5693	746.5690	[M+H]	-0.40	10.64	1.02	caco
PC 16:1/17:1	744.5537	744.5532	[M+H]	-0.67	9.36	1.4	caco
PC 16:0/18:0	762.6001	762.6004	[M+H]	0.39	12.70	1.2	caco
PC 16:0/18:1	760.5850	760.5852	[M+H]	0.26	11.36	1.16	caco
PC 16:1/18:1	758.5694	758.5692	[M+H]	-0.26	10.26	1.5	caco
PC 16:1/18:2	756.5530	756.5532	[M+H]	0.26	9.24	1.02	caco
PC 14:0/20:4	754.5354	754.5355	[M+H]	0.13	8.56	1.12	caco
PC 14:0/20:5	752.5211	752.5210	[M+H]	-0.13	7.59	1.42	caco
PC 18:0/17:1	774.6008	774.6007	[M+H]	-0.12	11.86	1.51	caco
PC 17:1/18:1	772.5851	772.5850	[M+H]	-0.12	10.86	1.19	caco
PC 18:1/18:1	786.5996	786.5990	[M+H]	-0.76	12.29	1.06	caco
PC 18:1/18:2	784.5850	784.5854	[M+H]	0.50	10.78	1.13	caco
PC 18:2/18:2	782.5676	782.5674	[M+H]	-0.25	9.45	1.21	caco
PC 16:1/20:4	780.5539	780.5532	[M+H]	-0.89	9.16	1.06	caco
PC 16:1/20:5	778.5366	778.5365	[M+H]	-0.12	8.26	1.01	caco
PC 18:1/19:0	802.6308	802.6304	[M+H]	-0.49	13.62	1.16	caco
PC 18:1/19:1	800.6308	800.6304	[M+H]	-0.87	12.3	1.47	caco
PC 17:1/20:3	796.5847	796.5845	[M+H]	-0.25	12.17	1.15	caco
PC 18:1/20:0	816.6471	816.6470	[M+H]	-0.12	14.14	1.01	caco
PC 18:1/20:1	814.6324	814.6322	[M+H]	-0.24	13.56	1.06	caco
PC 16:1/22:2	812.6149	812.6148	[M+H]	-0.12	11.96	1.24	caco
PC 18:0/20:4	810.5994	810.5991	[M+H]	-0.37	10.97	1.37	caco
PC 18:1/20:4	808.5850	808.5852	[M+H]	0.24	10.33	1.12	caco
PC 16:0/22:6	806.5698	806.5692	[M+H]	-0.74	9.80	1.31	caco
PC 18:0/22:5	836.6156	836.6155	[M+H]	-0.11	11.84	1.22	caco
PC 18:1/22:5	834.5997	834.5596	[M+H]	-0.11	11.35	1.55	caco
PC 18:1/22:6	832.5845	832.5840	[M+H]	-0.60	10.00	1.13	caco

Table 2. Comparison of lipid species between Caco-2 cells alone and Caco-2 cells co-cultured with E. coli under simulated microgravity conditions (g_0). Highlighted are significant changes in lipid composition induced by bacterial interaction, including ceramides (Cer), diacylglycerols (DG), phosphatidylcholines (PC), and lysophosphoetanolamine (PE), Phosphatidylserine (PS) and sphingomyelins (SM). Data includes theoretical and experimental *m/z* values, adduct types, retention times (Rt), and variable importance in projection (VIP) scores.

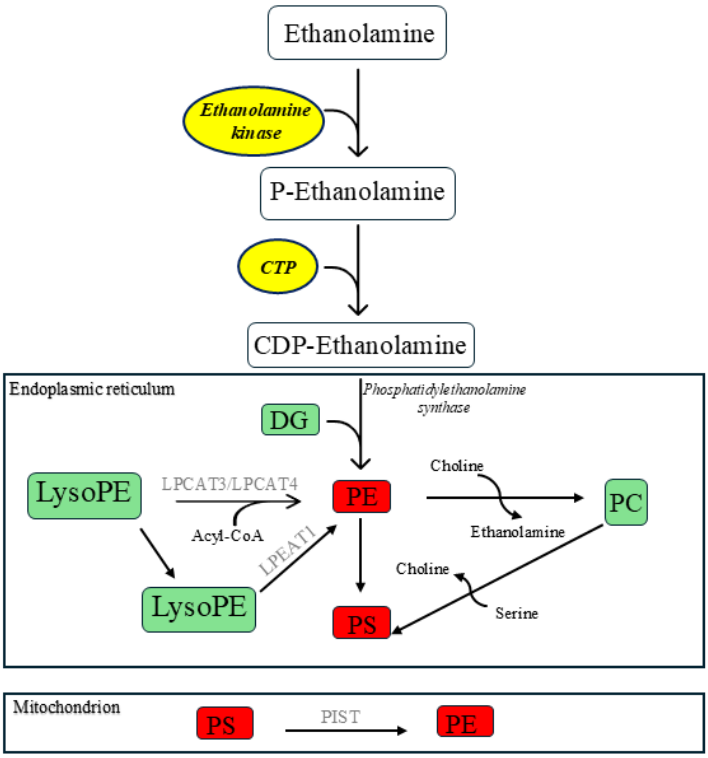


Figure 1. Metabolic pathway for the synthesis of phosphatidylethanolamine (PE) and phosphatidylserine (PS) from ethanolamine. The process begins with the phosphorylation of ethanolamine by ethanolamine kinase, followed by activation with CTP to form CDP-ethanolamine. In the endoplasmic reticulum, PE is synthesized via phosphatidylethanolamine synthase and can undergo further conversions into lysophosphatidylethanolamine (LysoPE), phosphatidylserine (PS), and phosphatidylcholine (PC). Additionally, PS is transported to mitochondria, where it is converted into PE through the PIST pathway. Lipids highlighted in red are upregulated in co culture with *E.coli* , while those in green are downregulated.

3.3. Effect of *E.coli* on Caco-2 cells at terrestrial gravity

When comparing the monoculture of Caco-2 cells and the co colture of Caco-2 cells with *E. coli* at terrestrial gravity, we observe an upregulation of diacylglyceryl-trimethylhomoserine (DGTS), lysophosphatidylcholine (LPC), and phosphatidylethanolamine (PE) in the co-culture, while diacylglycerols (DG), lysophosphatidylethanolamine (LPE), phosphatidylcholine (PC), and sphingomyelin (SM) in Caco-2 cells monoculture (**Table 3**). These alterations suggest a distinct remodeling of membrane lipids in response to bacterial co-culture, which may reflect changes in membrane dynamics, signaling pathways, and metabolic adaptations (Zhou et al., 2021). The presence of *E. coli* has been shown to modulate tight junction and protein expression, leading to increased permeability and activation of inflammatory pathways (Berkes, J. et al., 2003).

Attribution	Theor. (*m/z*)	Exp, (*m/z*)	Adduct	Δ ppm	Rt (min)	VIP	UP regulation
DGTS 16:0/19:1	752.6385	752.6382	[M+H]	-0.39	12.7	1.36	caco+e.coli
DGTS 19:1/19:1	792.6689	792.6688	[M+H]	-0.12	13.76	1.25	caco+e.coli
DG 16:0/16:0	586.5403	586.5401	[M+H4N]	-0.34	14.28	1.23	caco
DG 16:0/19:1	626.5718	626.5715	[M+H4N]	-0.47	14.07	1.4	caco
DG 19:1/19:1	666.6031	666.6030	[M+H4N]	-0.15	15.77	1.47	caco
LPC 14:0	468.3076	468.3075	[M+H]	-0.21	1.65	1.42	caco+e.coli
LPC 15:0	482.3235	482.3232	[M+H]	-0.62	3.09	1.28	caco+e.coli
LPC 16:0	496.3396	496.3394	[M+H]	-0.80	2.16	1.15	caco+e.coli
LPC 18:0	524.3702	524.3701	[M+H]	-0.19	3.13	1.24	caco+e.coli
LPC 18:1	522.3547	522.3540	[M+H]	-1.34	2.48	1.12	caco+e.coli
LPE 16:0	454.2927	454.2924	[M+H]	-0.66	2.37	1.14	caco
LPE 18:0	482.3238	482.3235	[M+H]	-0.62	3.28	1.14	caco
LPE 18:1	480.3076	480.3074	[M+H]	-0.41	2.37	1.19	caco
PC 16:0/16:0	734.5694	480.5691	[M+H]	-0.40	11.15	1.25	caco
PC 16:0/16:1	732.5537	732.5534	[M+H]	-0.40	9.85	1.28	caco
PC 16:1/16:1	730.5382	730.5380	[M+H]	-0.27	8.57	1.18	caco
PC 18:0/18:1	788.6168	788.6164	[M+H]	-0.50	12.9	1.4	caco
PC 18:1/18:1	786.5996	786.5994	[M+H]	-0.25	12.29	1.23	caco
PC 18:1/18:2	784.5850	784.5851	[M+H]	0.12	10.78	1.18	caco
PE 16:0/16:0	692.5225	692.5220	[M+H]	-0.72	1.13	1.15	caco+e.coli

PE 16:0/16:1	690.5069	690.5064	[M+H]	-0.72	1.01	1.09	caco+e.coli
PE 18:0/18:1	746.5704	746.5701	[M+H]	-0.40	1.3	1.28	caco+e.coli
PE 18:1/18:1	744.5541	744.5540	[M+H]	-0.13	1.17	1.14	caco+e.coli
PE 18:1/18:2	742.5401	742.5404	[M+H]	0.40	1.07	1.17	caco+e.coli
PE 16:0/20:4	740.5219	740.5216	[M+H]	-0.40	1.04	1.07	caco+e.coli
SM 18:0;O_2/16:0	705.5903	705.5900	[M+H]	-0.42	9.98	1.04	caco
SM 18:1;O_2/16:0	703.5733	703.5731	[M+H]	-0.28	9.15	1.32	caco
SM 18:1;O_2/18:0	731.6052	731.6050	[M+H]	-0.27	10.98	1.21	caco
SM 18:1;O_2/20:0	759.6346	759.6344	[M+H]	-0.26	12.59	1.18	caco

Table 3. Identification of lipid species that exhibit significant changes between Caco-2 monoculture and co-culture with *E. coli* under terrestrial gravity (g_1). The data reflects bacterial influence on lipid metabolism, including alterations in diacylglyceryl-trimethylhomoserine (DGTS), diacylglycerols (DG), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), phosphatidylcholines (PC), hosphatidylethanolamine (PE), sphingomyelins (SM). Data includes theoretical and experimental *m/z* values, adduct types, retention times (Rt), and variable importance in projection (VIP) scores.

4. Conclusions

This study provides a detailed lipidomic characterization of Caco-2 cells co-cultured with *E. coli* under SMC compared to samples maintained at terrestrial gravity, revealing significant modifications in lipid metabolism. The observed changes suggest an adaptive cellular response to microgravity conditions, which may influence membrane composition, signaling pathways, and metabolic homeostasis. These findings contribute to a deeper understanding of host-microbe interactions and underscore the potential of lipidomics as a tool for investigating cellular responses to bacterial challenge. Future studies should explore the functional implications of these lipidomic alterations in the context of intestinal health and disease.

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Funding

This work has been developed within the framework of the project eINS- Ecosystem of Innovation for Next Generation Sardinia (cod. ECS 00000038) funded by the Italian Ministry for Research and Education (MUR) under the National Recovery and Resilience Plan (PNRR) - MISSION 4 COMPONENT 2, “From research to business” INVESTMENT 1.5, “Creation and strengthening of Ecosystems of innovation” and construction of “Territorial R&D Leaders”. This work was funded under the National Operational Programme (PON) Research and Innovation DM 1061 of 10.8.2021. The research leading to these results has received funding from the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to Pierluigi Caboni CUP F53C22000750006 University of Cagliari. The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

G.T performed her activity in the framework of the PhD in Innovation Sciences and Technologies at the University of Cagliari, Italy. We acknowledge the CeSAR (Centro Servizi d’Ateneo per la Ricerca) of the University of Cagliari (Italy) for the QTOFMSMS experiments performed with an Agilent 6560. We also acknowledge Partenariato Esteso ‘’Heal Italia – health extended alliance for innovative therapies, advanced lab research, and integrated approaches of precision medicine’’– PE_00000019.