Articles

Translocation of black carbon particles to human intestinal tissue

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Summary

Background Evidence is accumulating that elevated levels of particulate air pollution, including black carbon, have been linked to gastrointestinal disorders and a lower intestinal bacterial richness and diversity. One of the hypothesized underlying mechanisms is the absorption of air pollution-related particles from the gastrointestinal tract.

Methods We visualized and quantified black carbon particles via white light generation under femtosecond-pulsed laser illumination in ileum and colon biopsies of five human patients. The biodistribution was assessed in three different layers (*i.e.*, mucosa, submucosa, and muscularis propria).

Findings Black carbon particles could be identified in all three tissue layers of the ileum and colon biopsies of five participants (two men and three women; mean \pm standard deviation age, 76.40 \pm 7.37 years), and their carbonaceous nature was confirmed via emission fingerprinting. The median (\pm SD) black carbon load was borderline statistically significantly higher in the ileum compared to the colon (1.21 \times 10⁵ \pm 1.68 \times 10⁴ particles/mm³ versus 9.34 \times 10⁴ \pm 1.33 \times 10⁴ particles/mm³; p = 0.07) and was driven by a difference in black carbon load in the submucosa layer (p = 0.01). Regarding the three tissue layers, loads were higher in the submucosa, compared with the mucosa (ileum: +76%, p < 0.0001; colon: +70%, p = 0.0001) and muscularis propria (ileum: +88%, p < 0.0001; colon: +88%, p < 0.0001). In ileum, loads were borderline higher in the mucosa versus muscularis propria (p = 0.09).

Interpretation This explorative study provides real-life evidence that black carbon particles can reach the intestinal tissue and accumulate in different intestinal tissue layers. These findings support further research into how particulate air pollution directly affects gastrointestinal health.

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Introduction

Ambient and household air pollution are the biggest environmental threats to human health¹ as they contribute to 6.7 million annual premature deaths worldwide.² High levels of fine particulate matter ($PM_{2.5}$), one of the most noxious compounds due to its small size,³ have been associated with gastrointestinal disorders such as ulcerative colitis⁴ and colorectal cancer.⁵ Additionally, air pollution exposure has been linked to a lower intestinal bacterial richness and diversity both in children⁶ and adults,⁷ which might impact human health as a balanced bacteriome is paramount for efficient energy production (*e.g.*, short-chain fatty acids) and pathogen defense (*e.g.*, antimicrobial peptides).^{8,9} One of the hypothesized mechanisms via which air pollution can affect gastrointestinal health is the direct translocation of air pollution-related particulates.





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Research in context

Evidence before this study

We searched Pubmed and Web of Science to evaluate the presence of studies published in English that examined the translocation of exogenous particles into gastrointestinal tissue until April 23, 2024. Herefore, we used the search terms ("particle" or "particulate") plus ("translocation" or "accumulation") plus ("gastrointestinal" or "intestine") for the title and abstract. Previous research in laboratory animals and humans has shown that exogenous particles, including silica, titanium dioxide, and polystyrene particles, can accumulate in gastrointestinal tissue. For instance, a study in female Sprague-Dawley rats reported the presence of fluorescent polystyrene microspheres in intestinal tissue after daily oral administration for 10 days. Furthermore, the accumulation of black pigment (i.e., aluminium and magnesium-rich silicates) in macrophages in the lamina propria and submucosa was seen in human ileum biopsies collected during colonoscopy. To date, the real-life translocation of air pollution-related particles, such as black carbon, into human intestinal tissue has never been investigated.

Added value of this study

We present the first study in which black carbon particles were quantified in three different tissue layers (mucosa, submucosa, and muscularis propria) in ileum and colon biopsies of five human patients. Hereby, we provide real-life evidence on the accumulation of air pollution-related particles in intestinal tissue. This research shows that although the average exposure to particulate matter in Flanders (Belgium) is below the annual average threshold imposed by the European Union (10.8 μ g/m³ in 2022 compared to 20 μ g/m³), black carbon particles can still translocate to the intestinal tissue.

Implications of all the available evidence

Even at low ambient particulate matter exposure, this study shows in real life the accumulation in different intestinal tissue layers. This finding supports further research into how air pollution directly affects gastrointestinal health, as air pollution has already been linked to intestinal disorders, such as ulcerative colitis and colorectal cancer, and lower intestinal bacterial richness and diversity.

It is postulated that particulates originating from combustion, such as black carbon within the PM2.5 fraction, can reach the intestines via multiple pathways. First, inhaled black carbon particles3 can be ingested via mucociliary clearance after deposition in the lungs.10 Second, black carbon particles can also reach the intestinal lumen via the ingestion of particle-contaminated food and water.^{3,11} In the lumen, these particles may be taken up by a variety of transport mechanisms, including para- and transparacellular transport,12 or they can be engulfed and transported by immune cells (e.g., dendritic cells and macrophages).13 Besides ingestion, small black carbon particles (<1 µm) can enter the lung alveoli,³ bypass the lung-blood barrier,^{3,14} and translocate to distal body sites via the systemic circulation as substantiated by their presence in the kidney,15 brain,16 and fetal tissues.17

The translocation of particulate air pollution into human intestinal tissue has never been investigated. We present a study that used tissue from the small (*i.e.*, ileum) and large intestine (*i.e.*, colon) from five patients and demonstrated the presence of black carbon particles in three different layers (mucosa, submucosa, and muscularis propria).

Methods

Study population and samples

In this explorative study, five anonymized patients who underwent a right hemicolectomy for colorectal cancer in Ziekenhuis Oost-Limburg (ZOL) Genk in Belgium were included. All participants signed a chart of no objection to the use of residual tissue samples for scientific research purposes. The sample collection and use was approved by the ethical committee of ZOL and conducted in accordiance to the principles outlined in the Helsinki Declaration.18 Transmural biopsies from the small (*i.e.*, ileum) and large intestine (*i.e.*, colon) were taken from the removed intestinal tissue at a considerable distance from the tumor. For black carbon quantification, frozen biopsies were fixed in 4% formaldehyde on ice for at least 24 h before being dehydrated and paraffin-embedded. 5 µm sections were cut using a microtome (Leica Microsystems, UK; 2 per biopsy), mounted on histological glass slides, dried overnight at 37 °C, and stored at room temperature until analysis. This study followed the Strengthening the Reporting of Observational Studies in Epidemiolgy (STROBE) reporting guidelines.

Black carbon quantification and validation

Black carbon particles were quantified in ileum and colon biopsies using label-free white light generation under femtosecond pulsed illumination (Figure S1), as previously described by our group.¹⁹ All images were captured at room temperature using a Zeiss LSM880 NLO scan head mounted to the rear port of an inverted laser-scanning microscope (Zeiss Axio Observer. Z1 motorized stand; Carl Zeiss) equipped with a twophoton femtosecond pulsed laser (810 nm, 120 fs, 80 MHz, MaiTai DeepSee, SpectraPhysics, USA) and a Plan-ApoChromat 20×/0.8 M27 air objective (Carl Zeiss). Three different tissue layers, i.e., mucosa, submucosa, and muscularis propria, were imaged for

each ileum and colon biopsy (Fig. 1). A total of eight 850.19 × 850.19 μ m² tile scans in two slides (*i.e.*, four per slide) were acquired for each layer with a pixel dwell time of 2.05 μ s. Two-photon-induced white light emission of black carbon particles was acquired in the non-descanned mode after spectral separation and emission filtering using 400–410 nm (second harmonic generation (SHG) channel) and 450–650 nm (two-photon excited autofluorescent (TPAF) channel) bandpass filters. The number of black carbon particles was calculated using a peak-finding algorithm in Matlab (Matlab R2017b, MathWorks), which counts pixels above a certain threshold value: 0.5% lower than the

highest intensity value for TPAF, and 45% (mucosa and muscularis propria) or 0.5% (submucosa) lower than the highest intensity value for SHG. A higher threshold was used for the submucosa layer due to the higher level of vascularization leading to a higher background signal.²⁰ The detected pixels in both channels are compared, and only the overlapping pixels are identified as black carbon particles. The effectively imaged intestinal area was assessed using TPAF images in Fiji (ImageJ, Madison, WI) and the focal volume based on the point spread function of the optical system (*i.e.*, 2.37 µm). Finally, the number of black carbon particles per mm³ tissue was defined for the three tissue layers. Black carbon loads of



Fig. 1: Histology of the intestine. Representative images of (A) all intestinal layers and a detail of the (B) mucosa, (C) submucosa, and (D) muscularis propria. Paraffin-embedded 5 μ m sections were stained with hematoxylin and eosin and imaged at × 2.5 and × 10 magnifications.

the different tissue layers were measured separately and also averaged to obtain the number of particles per mm³ ileum or colon tissue. As previously described by our research group,¹⁷ the carbonaceous nature of the detected black carbon particles was confirmed via emission fingerprinting, *i.e.*, we assessed that the emitted white light ranges across the entire visible spectrum. Additionally, the signal originating from the black carbon particles was compared with that of commercially engineered conductive carbon black nanopowder (<2.5 µm; US Research Nanomaterials) as the signals should be consistent.¹⁹

Statistics

Black carbon loads are expressed as median values (±SD). The black carbon loads between the two intestinal regions (*i.e.*, ileum and colon) were compared via a Paired Samples t-test, while the black carbon loads between the different tissue layers (*i.e.*, mucosa, submucosa, and muscularis propria) were compared via a two-way repeated measures ANOVA using RStudio (version 4.2.3; R Core Team). Figures were generated using the commercially available GraphPad Prism version 8 (GraphPad Software Inc.).

Role of funding source

The funders had no role in study design, sample collection, data analysis, or manuscript writing.

Results

Black carbon detection and validation

To study the translocation of black carbon particles to the intestinal tissue in humans, ileum and colon biopsies were employed from five participants: three women and two men with a mean \pm standard deviation age of 76.40 \pm 7.37. All participants live in Limburg (Belgium). We were able to detect black carbon in all three tissue layers of the ileum and colon biopsies: mucosa (Fig. 2A), submucosa (Fig. 2B), and muscularis propria (Fig. 2C). We confirmed the carbonaceous nature of the detected black carbon particles via emission fingerprinting. As seen in Figure S2, carbon-containing particles, such as black carbon and commercially engineered carbon black, generated white light that spans the entire visible spectrum when illuminated with a near-infrared femtosecond pulsed laser. In contrast, the emission fingerprint of the background signal of the intestinal tissue consists of a distinct peak that does not continuously range over all wavelengths.

Black carbon distribution in intestinal tissue

The black carbon load was borderline statistically significantly higher in ileum tissue compared to colon tissue $(1.21 \times 10^5 \pm 1.68 \times 10^4 \text{ particles/mm}^3 \text{ versus})$ $9.34 \times 10^4 \pm 1.33 \times 10^4$ particles/mm³; p = 0.07) (Fig. 3A). With regard to the three tissue layers, black carbon loads were highest in the submucosa (ileum: $8.87 \times 10^4 \pm 1.38 \times 10^4$ particles/mm³, and colon: $6.58 \times 10^4 \pm 1.68 \times 10^4$ particles/mm³), followed by the mucosa (ileum: $2.12 \times 10^4 \pm 1.52 \times 10^4$ particles/mm³, and colon: $2.00 \times 10^4 \pm 1.26 \times 10^4$ particles/mm³), and lowest in the muscularis propria (ileum: $1.09 \times 10^4 \pm 2.98 \times 10^4$ particles/mm³, and colon: $7.58 \times 10^3 \pm 8.02 \times 10^3$ particles/mm³) (Fig. 3B). The differences with the submucosa were statistically significant: submucosa versus mucosa (ileum: +76%, p < 0.0001; colon: +70%, p = 0.0001) and submucosa versus muscularis propria (ileum: +88%, p < 0.0001; colon: +88%, p < 0.0001). The difference in black carbon load between mucosa and muscularis propria was borderline statistically significant in the ileum (p = 0.09)but not in the colon (p = 0.30) biopsies. The difference in black carbon load between ileum and colon biopsies



Fig. 2: Evidence of black carbon particles in different intestinal regions: (A) mucosa, (B) submucosa, and (C) muscularis propria. White light generation originating from black carbon particles (white, indicated with a white arrow) under femtosecond pulsed laser illumination (excitation 810 nm) was observed. Images represent the overlap of the two-photon autofluorescence of the tissue (green, emission 450–650 nm) and second harmonic generation of collagen (red, emission 400–410 nm). Scale bar: 10 μm.



Fig. 3: Boxplot representing the (A) average number of black carbon particles per mm³ ileum or colon tissue, or (B) the average number of black carbon particles per mm³ stratified per tissue layer: mucosa, submucosa, and muscularis propria. Violin boxes span from the lowest to the highest value, with the horizontal line inside the box representing the median value. n = 5. *indicates p-value ≤ 0.05 and indicates p-value ≤ 0.10 .

was driven by a statistically significant higher black carbon load in the submucosa of the ileum compared to the colon (p = 0.01). The average loads did not differ statistically significantly for mucosa (p = 0.64) and muscularis propria (p = 0.83).

Discussion

Despite the postulation of different mechanisms via which air pollution-related particulates can reach the intestine, their translocation in a real-life situation in humans has not been shown. We present the first study in which black carbon particles were visualized and quantified in three different tissue layers (mucosa, submucosa, and muscularis propria) in ileum and colon biopsies of five human patients. We were able to identify black carbon particles in all biopsies and their carbonaceous nature was confirmed via emission fingerprinting. Black carbon loads were significantly higher in ileum tissue compared with colon tissue. With regard to the three tissue layers, loads were highest in the submucosa, followed by mucosa and muscularis propria. As the investigated transmural biopsies were taken at a considerablde distance from the tumor, the cancer process itself probably did not result in an elevated black carbon absorption.

In this study, black carbon particles were quantified in three gastrointestinal wall tissue layers. The innermost layer, *i.e.*, the mucosa, acts as a barrier for nutrition absorption and maintaining contaminants in the lumen. The mucosa rests on the submucosa, which is loose connective tissue containing large blood vessels, lymphatics, nerves, and secretory glands.²¹ The third and last layer, the muscularis propria, consists of two smooth muscle layers, an inner circular layer and a outer longitidual layer, ensuring peristalsis.²¹

Different mechanisms via which black carbon particles can reach the intestines are postulated. First, inhaled 5-10 µm black carbon particles can reach the intestinal lumen after mucociliary clearance10 and subsequent swallowing. Particles can also reach the intestinal lumen via the ingestion of particle-contaminated food and water,3,11 resulting in an estimated daily ingestion of 10¹²–10¹⁴ particles.¹¹ In the lumen, particles can be taken up via nutrient-related epithelial transport mechanisms, such as paracellular transport, transcellular transport, and carrier-mediated transport²² as demonstrated in animal studies.23-25 For instance, Li et al.23 showed the presence of polystyrene nanoparticles in the jejunum of mice after 32 weeks of exposure to nanoparticles via their drinking water. As approximately 95% of all nutrients are absorbed in the small intestine and further transported to the blood and lymphatic vessels in the submucosa,26 this route of transportation is a plausible explanation given our finding that more black carbon particles are seen in ileum than colon biopsies and that the load was highest in the submucosa. Besides nutrient-related transport, black carbon particles can also reach the intestinal tissue after active transport through M-cells. M-cells are part of Pever's patches,²⁷ lymphoid follicles mainly located in the small intestine that are responsible for antigen surveillance in the intestinal lumen and immune response regulation. When antigens are transported through M-cells, they can be taken up by macrophages and dendritic cells, who present them to B-cells and T-cells in the germinal center of the Peyer's patch to initiate an immune response.27 Previous studies already showed that, besides antigens, exogenous particles (e.g., silica, titanium dioxide, and aluminium) can also be taken up by these immune cells as they have been visualized in Peyer's patches.^{13,28} At least 46% of all Peyer's

patches are located in the distal 25 cm of the human ileum and the patches extend from the mucosa all the way into the submucosa.²⁹ Hence, this uptake mechanism further supports our finding of higher black carbon loads in the ileum tissue and the submucosa layer. Lastly, as previously stated, black carbon particles belonging to the ultrafine particle range can reach the systemic circulation via bypassing the lung-blood barrier,^{3,14} after which they are transported to distal organs. Intestinal tissue, and specifically the submucosa, has a rich vasculature for the transportation of absorbed nutrients.³⁰ Since 95% of all nutrients are absorbed in the small intestine through the network of villi and microvilli,²⁶ the vascularization is more extensive there. Therefore, the results of our study support this transportation route.

This study's patients were elderly, and it is suggested that advanced age may be associated with prolonged intestinal transit time,^{31,32} which could potentially increase th intestinal uptake of black carbon particles. For instance, research by Nandhra et al.³¹ investigated intestinal transit time in healthy volunteers aged 21-88 year and found that increasing age was associated with longer regional and total colonic transit time, and whole gut transit time. Nevertheless, some studies33 did not find a significant association between age and intestinal transit time. Moreover, age does not appear to significantly alter intestinal uptake, as evidenced by an in vivo study that assessed the gastrointestinal site-specific permeability (i.e., small intestinal, colonic, and whole gut) between adults and the elderly, which reported no significant differences.³⁴ Additionally, a study by Massonet et al.35 investigated the distribution of fatty acid and cholesterol transporters in post-mortem intestinal samples of humans aged 37-83 years, finding no correlation between transporter levels and age.

Our findings offer valuable insights into the translocation of air pollution-related particles, supporting further research into how particulate air pollution directly affects gastrointestinal health. Nevertheless, we acknowledge some study limitations. First, ileum and colon biopsies from only five participants were included in this study and all of whom were recruited from the same hospital, potentially limiting the generalization of the findings. Second, as the samples were obtained from anonymized patients we did not have data on modeled residential air pollution. Furthermore, we cannot exclude that a fraction of the measured particles originates from other sources than air pollution, such as the consumption of carbon black particles used as food coloring agent.³⁶ Last, we were unable to discriminate between the different transport mechanisms via which air pollutants might reach the intestinal tissue. However, since black carbon particles are observed within the mucosa layer, there appears to be a potential for direct transportation from the gastrointestinal lumen into the intestinal tissue for at least a part of the air pollutionrelated particles. Moreover, upon comparing the black carbon load in the intestines with those found in other organs, such as the brain¹⁶ and kidney,¹⁵ we can conclude that the loads are of a higher magnitude. This further supports the notion of an additional uptake pathway besides the systemic circulation. Yet, for full proof of the intestinal biodistribution of black carbon particles, we should analyze the colocalization of black carbon particles with cell types, such as endothelial cells, immune cells, or epithelial cells, by staining the cells with fluorescentlabeled antibodies. It should also be noted that ambient air pollution exposure can alter the intestinal microbiome composition as seen in multiple publications.^{6,7,37} These alterations could affect the transport and retention of black carbon particles.38,39

Conclusion

In this explorative study, we provided real-life evidence of the accumulation of black carbon particles in intestinal tissue. Black carbon loads were higher in ileum tissue compared to colon tissue. With regard to tissue layers, most particles accumulated in the submucosa. Our findings support further research into how particulate air pollution directly affects gastrointestinal health.

Contributors

TVP: conceptualisation, data curation, formal analysis, funding acquisition, visualization, writing–original draft, writing–review and editing. KV: investigation, writing–review and editing. LR: investigation, writing–review and editing. PVE: data curation, resources, writing– review and editing. JH: conceptualisation, formal analysis, supervision, writing–review and editing. PC: data curation, writing–review and editing. MA: methodology, writing–review and editing. MP: supervision, writing–review and editing. TSN: conceptualisation, formal analysis, funding acquisition, supervision, writing–review, and editing. All authors read and approved the final version of the manuscript. Both TVP and TSN had access to the data.

Data sharing statement

As intestinal biopsies were collected from anonymized patients, no participant data will be shared.

Declaration of interests

MA and TSN declare that aspects of the work mentioned in the paper are the subject of an awarded patent (Method for detecting or quantifying carbon black and/or black carbon particles, reference codes: EP3403068B1 and US11002679B2) filed by Hasselt University (Hasselt, Belgium) and KU Leuven (Leuven, Belgium). All other authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi. org/10.1016/j.ebiom.2024.105464.

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