Inhaled CO₂ Concentration While Wearing Face Masks: A Pilot Study Using Capnography

Cecilia Acuti Martellucci¹, Maria Elena Flacco¹, Mosè Martellucci², Francesco Saverio Violante³ and Lamberto Manzoli⁴

¹Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy. ²Department of Medicine and Surgery, University of Perugia, Perugia, Italy. ³Occupational Health Unit, Sant’Orsola Malpighi University Hospital, University of Bologna, Bologna, Italy. ⁴Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy.

ABSTRACT

BACKGROUND: Face masks are recommended based on the assumption that they protect against SARS-CoV-2 transmission, however studies on their potential side effects are still lacking. We aimed to evaluate the inhaled air carbon dioxide (CO₂) concentration, when wearing masks.

METHODS: We measured end-tidal CO₂ using professional side-stream capnography, with water-removing tubing, (1) without masks, (2) wearing a surgical mask, and (3) wearing a FFP2 respirator (for 5 minutes each while seated after 10 minutes of rest), in 146 healthy volunteers aged 10 to 90 years, from the general population of Ferrara, Italy. The inhaled air CO₂ concentration was computed as: ([mask volume − tidal volume] + tidal volume − mask volume) × ambient air CO₂/tidal volume.

RESULTS: With surgical masks, the mean CO₂ concentration was 7091 ± 2491 ppm in children, 4835 ± 869 in adults, and 4379 ± 978 in the elderly. With FFP2 respirators, this concentration was 13665 ± 3655 in children, 8502 ± 1859 in adults, and 9027 ± 1882 in the elderly. The proportion showing a CO₂ concentration higher than the 5000 ppm (8-hour average) acceptable threshold for workers was 41.1% with surgical masks, and 99.3% with FFP2 respirators. Adjusting for age, gender, BMI, and smoking, the inhaled air CO₂ concentration significantly increased with increasing respiratory rate (mean 10387 ± 3712 ppm among participants >18 breaths/minute, with FFP2 respirators), and among the minors.

CONCLUSION: If these results are confirmed, the current guidelines on mask-wearing should be reevaluated.

KEYWORDS: Carbon dioxide, face masks, capnography

Introduction

Most nations introduced the use of face masks in the community to contrast SARS-CoV-2 pandemic. ¹-³ Italy, one of the first countries to experience an epidemic wave, ³ required masks to be worn by all people older than 5 years, indoors and outdoors. ⁴

Surgical masks and respirators are assumed to reduce the spread of SARS-CoV-2, ² and are believed to decrease the incidence of other airborne infections. ³ On the other hand, a prolonged mask use has been associated with higher viral loads and more severe symptoms in infected people (possibly due to the re-inhalation of viral particles trapped in the mask), ⁶ with skin disorders due to pathogens contamination, a higher likelihood of frequent cough, sputum production, dyspnea, and panic attacks, ⁷,⁸ with delayed cognitive development in infants, ⁹,¹⁰ and with a substantial rise of inhaled carbon dioxide (CO₂), which in turn may cause other symptoms. ¹¹,¹²

Few studies, however, directly assessed CO₂ in air inhaled while wearing masks in the general population. ¹³,¹⁴ Three of these studies had an overall sample of 12 adults and 45 children, and used measurement tools which could not avoid the interference of water vapor, ¹⁴ which is typically high in exhaled air, ¹⁶ and is known to substantially decrease accuracy. ¹⁷ One study used a cardiopulmonary stress testing device, finding a negative impact of masks on cardiac and pulmonary parameters during exercise. ¹⁸ Finally, 4 further studies used capnography, the most appropriate instrument to verify changes in respiratory gases. ¹⁹ Early markers of hypventilation were observed after the 12-minute walking test among 22 children wearing FFP2 respirators, ²⁰ after the 6-minute walking test among 100 adults wearing surgical masks, ²² and even at rest in 30 adults and elderly with both types of masks. ²³

With the present study, we aimed at expanding the evidence about the potential inhalation of excess CO₂ as a consequence of wearing surgical masks or FFP2 respirators, among adults, children, and the elderly. Therefore, we used a professional
real-time capnograph, with water-removal tubing, in order to assess the inhaled air CO₂ concentration in a sample of healthy individuals wearing different types of masks.

**Materials and Methods**

**Study population**

The participants were healthy volunteers sequentially recruited by 3 general practitioners and 1 family pediatrician in the Province of Ferrara, Italy during April and May 2021, and June 2022. Inclusion criteria were: age between 10 and 90 years, forehead temperature <37.5°C, being able to wear a mask without assistance, and providing written informed consent (for the minors, the consent was requested to the legally responsible individual). Exclusion criteria were: pregnancy, and cardiac or respiratory comorbidities.

**Study design**

In this observational, descriptive study, we measured the end-tidal CO₂ (ETCO₂) in all participants (a) without masks; (b) wearing a surgical mask; (c) wearing a Filtering Face-Piece grade 2 (FFP2) respirator. Given that the masks constitute an added dead space of the airways, with different volumes depending on mask size and face shape, the concentration of CO₂ within this added dead space can be assessed measuring the ETCO₂, which indicates how much CO₂ is exhaled in the final phase of the expiration. The evaluations of ETCO₂ were performed after 10 minutes of rest, with participants seated, silent, and breathing only through the nose. To account for potential oscillations, which were however infrequent (n = 6), a trained physician (CAM) took measurements at minutes 3, 4, and 5, and the final value used in the analyses was the average of the 3 measurements.

All masks were identical and were provided by the investigators, who monitored and eventually adjusted the fit. The surgical mask was a 3-layer plane-shaped disposable face mask with ear loops (17.5 × 9.5 cm, conforming to UNI EN ISO 14683:2019 and AC:2019 regulations). The FFP2 was a 5-layer disposable respirator (15.0 × 10.0 cm, conforming to EN 149:2001 and A1:2009), equivalent to United States N95.

The measurement tool was a Rad-97™ capnograph with real-time side-stream gas measurement and water-removal tubing (Masimo Corp., Irvine, CA, USA). The sampling point (nasal cannulas) was positioned outside the exhaled air stream—below the lips of each subject—to ensure that the detected ETCO₂ was that of the volume of air within the masks (Figure 1). Consistently, participants were also required to keep their mouth closed, thus ensuring reproducibility by preventing any exhaled air stream from reaching the cannulas. The capnography device measured CO₂ in mmHg, which was converted to ppm using a standardized conversion formula.

The environmental CO₂ concentration (in ppm) was measured using an automatic Temtop mod. M2000C® sensor (Elitech Technology Inc., Milpitas, CA, USA). All measurements were taken into a room that was constantly and amply ventilated with external air.

For each participant, information was also collected on blood oxygen saturation and respiratory rate (measured at the same time points as the ETCO₂), age, gender, weight and height, and smoking (current—at least one cigarette per
Blood oxygen saturation was measured through a LTDS800® digital finger pulse oximeter (Dimed Co. Ltd., Cavriglia, AR, Italy).

Data analysis
The primary outcome was the mean inhaled air CO\textsubscript{2} concentration when wearing masks. The secondary outcome was the proportion of individuals with inhaled air CO\textsubscript{2} concentration exceeding 5000 ppm, which is the long-term (8-hours average) threshold indicated as Permissible Exposure Limit by the United States Department of Labor Occupational Safety and Health Administration (OSHA), and as Indicative Occupational Exposure Limit by the European Agency for Safety and Health at Work (EU-OSHA).\textsuperscript{30,31} Based on this standard, several states of the European Union produced their own binding laws, such as Italy’s Law 81 of 2008, which indicates the 8-hour exposure to CO\textsubscript{2} as the occupational limit.\textsuperscript{32}

CO\textsubscript{2} inhaled air concentration was computed as follows: \((\text{mask volume} \times \text{end tidal CO}_2) + \text{[tidal volume - mask volume]} \times \text{ambient air CO}_2) / \text{tidal volume}.\textsuperscript{33} The standard value of 7 ml/kg of weight was used for the tidal volume (the volume of air inhaled and exhaled with every respiration cycle).\textsuperscript{34,35} Similarly, a water displacement procedure performed on 5 participants indicated that the volume of the chosen masks approached the minimum average values reported by the literature, which were thus adopted: 50 ml for the surgical mask,\textsuperscript{36} and 98 ml for the FFP2 respirator.\textsuperscript{37}

The differences in the mean CO\textsubscript{2} concentration with and without masks were evaluated using Wilcoxon matched-pairs signed ranks test.\textsuperscript{38} The analyses were repeated separately for children (aged 10-18 years), adults (19-64 years), and elderly (65-90 years), assessing potential differences between the groups through Kruskal-Wallis tests. Multiple linear regression was then performed to investigate potential independent predictors of higher CO\textsubscript{2} content wearing surgical (model 1) or FFP2 (model 2) masks. All covariates were included a priori in the models, and a 2-tailed \(P\)-value \(< 0.05\) was considered significant for all analyses, which were carried out using Stata 15.1 (Stata Corp., College Station, TX, 2017).

We decided to enroll a minimum sample size of 100 subjects as it would allow 95% confidence interval to remain within \(\pm 10\%\) of the sample mean value, assuming an average inhaled air CO\textsubscript{2} concentration of 2000 ± 1000 ppm wearing surgical masks, and 3000 ± 1000 ppm wearing FFP2 respirators.\textsuperscript{14}

The study protocol was approved by the Ethics Committee of the Emilia-Romagna Region “Area Vasta Emilia Centrale” on February 12th, 2021 (code 78/2021/Oss/UniFe).

Results
Sample characteristics
Participation was requested to 151 eligible subjects; 146 provided the consent and were thus included in the study (47.3% males; mean age 46.2 ± 21.6 years). Twenty-four participants were aged 10 to 18 years, 38 were aged 65 to 90 years. The mean Body Mass Index (BMI) was 24.4 ± 4.8, and current or former smokers were 19.2%. The average respiratory rate was 16.8 ± 3.5 breaths per minute, with 37.0% breathing at or above 18 breaths per minute; the average blood oxygen saturation was 97.4 ± 1.0%, with 98.0% of the sample at or above 96% saturation.

Outcomes
The mean inhaled air CO\textsubscript{2} without masks was 460 ± 20 ppm. While wearing the surgical mask, the mean CO\textsubscript{2} was 5087 ± 1579 ppm (95% confidence interval 4828-5346 ppm), and exceeded 5000 ppm in 41.1% (33.0%-49.5%) of the measurements. While wearing the FFP2 respirator, the average CO\textsubscript{2} was 9653 ± 2874 ppm (9183-10123 ppm), and 98.6% (95.2%-99.8%) of the participants showed values higher than 5000 ppm (Table 1). Among the minors, the mean CO\textsubscript{2} concentration when wearing surgical masks was 7091 ± 2491 ppm (6039-8144 ppm), and was considerably higher than among the adults (4835 ± 869 ppm; \(P < .01\)), or the elderly (4379 ± 978 ppm; \(P < .001\)). A similar difference by age class was observed also for the FFP2 respirators (Table 2).

The CO\textsubscript{2} concentration varied also by respiratory rate: wearing surgical masks, inhaled air CO\textsubscript{2} was 4670 ± 750 ppm among the individuals with respiratory rate \(\leq 14\) breaths per minute, progressively rising to 5656 ± 2193 ppm when 18 or more breaths per minute were taken. A similar trend was observed for FFP2 respirators (Table 1).

Multivariate analyses substantially confirmed univariate results: a higher respiratory rate was significantly associated with higher inhaled air CO\textsubscript{2} wearing both masks. Regression coefficients for \(\geq 25\) compared to \(\leq 14\) breaths per minute were +570 (\(P < .05\)) and +1347 (\(P < .01\)), respectively, with surgical masks and FFP2 respirators (Table 1).

Male gender, age, overweight (BMI \(> 25\) vs \(< 25\)), and smoking were all associated with a significantly lower CO\textsubscript{2} concentration (model fit \(R^2 .45\) for both mask types). Wearing surgical masks, regression coefficients were: -658 (-1069 to -247) for male gender, -197 (-304 to -89) for 10-year increases in age, -1069 (-1544 to -594) for overweight, and -818 (-1320 to -317) for smoking. Wearing FFP2 respirators, coefficients were: -1071 (-758 to -383) for male gender, -308 (-487 to -128) for 10-year increases in age, -2440 (-3235 to -1645) for overweight, and -1754 (-2593 to -914) for smoking.

Finally, both respiratory rate and blood oxygen saturation did not differ substantially without or with the masks. Also, when wearing masks, the mean ETCO\textsubscript{2} remained within 33 mmHg.

Discussion
In our sample of healthy individuals, at rest, after 5 minutes of surgical masks use, the mean inhaled air CO\textsubscript{2} approached the occupational exposure limit of 5000 ppm in adults and the
## Table 1. Outcomes for the overall sample and results of the multiple linear regression predicting overall inhaled air CO₂ in ppm (N=146).

|                          | WITHOUT MASK | SURGICAL MASK | FFP2 RESPIRATOR |
|--------------------------|--------------|---------------|-----------------|
| **Mean CO₂ detected inside the mask in ppm** |               |               |                 |
| Mean ± SD (95% CI)       | 0 ± 0 (—)    | 42277 ± 4846 (41 483-43 069)* | 42889 ± 4653 (42 128-43 650)* |
| Estimated inhaled CO₂ in ppm |               |               |                 |
| Mean ± SD (95% CI)       | 460 ± 20 (457-464)b | 5087 ± 1579 (4828-5346)* | 9653 ± 2874 (9183-10 123)* |
| >5000 ppm, %             | 0.0          | 41.1          | 99.3            |
| Inhaled air CO₂ in ppm by respiratory rate, mean ± SD (95% CI) |               |               |                 |
| Slow (≤14 breaths per minute, n=25) | 462 ± 20 (455-469) | 4670 ± 750 (4408-4932) | 8738 ± 1618 (8174-9303) |
| Moderate (15-17 breaths per minute, n=43) | 458 ± 19 (453-463) | 4802 ± 1028 (4532-5072) | 9087 ± 2129 (8527-9647) |
| High (>18 breaths per minute, n=34) | 461 ± 22 (456-467) | 5656 ± 2193 (5057-6254) | 10837 ± 3712 (9824-11 850) |
| Coefficients for the linear regression |               |               |                 |
| Respiratory rate, 1 breath per minute increase — | 72 (14, 130)** | 162 (64, 259)** |
| Low (≤14 breaths per minute, n=25) — | 0.00 (Ref. cat.) | 0.00 (Ref. cat.) |
| Moderate (15-17 breaths per minute, n=43) — | 126 (−392, 643) | 353 (−514, 1219) |
| High (>18 breaths per minute, n=34) — | 570 (39, 1101)** | 1347 (457, 2236)** |

Abbreviation: FFP2, filtering face-piece grade 2 respirator.
*End-tidal CO₂ detected inside the face masks.
bOnly ambient air CO₂.
*P < .001 (Wilcoxon matched pairs signed-rank test) for the comparison of CO₂ parameters between without and with surgical or FFP2 masks. **P < .05 and ***P < .01 from the Wald test for the linear regression adjusted by gender, age, Body Mass Index, and smoking status.

## Table 2. Sample characteristics and outcomes by age-class.

|                          | CHILDREN (N=24) | ADULTS (N=84) | ELDERLY (N=38) |
|--------------------------|-----------------|---------------|----------------|
| **Mean CO₂ detected inside the mask in ppm** |               |               |                 |
| Without masks            | 0 ± 0 (—)       | 0 ± 0 (—)     | 0 ± 0 (—)      |
| With surgical mask       | 39967 ± 5260 (37 746-42 188) | 43577 ± 4056 (42 697-44 457) | 40 858 ± 5342 (39 103-42 615) |
| With FFP2 respirator     | 41118 ± 3540 (39 624-42 613) | 43468 ± 4988 (42 386-44 551) | 42 729 ± 4291 (41 318-44 139) |
| Inhaled air CO₂ in ppm, mean ± SD (95% CI) |               |               |                 |
| Without masks³           | 469 ± 21 (460-478)* | 461 ± 19 (457-465)* | 452 ± 21 (445-459)* |
| >5000 ppm, %             | 0.0             | 0.0           | 0.0            |
| Surgical mask            | 7091 ± 2491 (6039-8144)*** | 4835 ± 869 (4647-5023)*** | 4379 ± 978 (4057-4700)*** |
| >5000 ppm, %             | 91.7            | 35.7          | 21.1           |
| FFP2 respirator          | 13 665 ± 3655 (12 122-15 208)*** | 8502 ± 1851 (7894-9111)* | 9027 ± 1882 (8619-9436)*** |
| >5000 ppm, %             | 100             | 98.8          | 100            |

Abbreviation: FFP2, filtering face-piece grade 2 respirator.
*End-tidal CO₂ detected inside the face masks.
³Only ambient air CO₂.
*P < .001 (Wilcoxon matched pairs signed-rank test) for the comparisons between without and with surgical or FFP2 masks, and between the 2 types of masks. **P < .01 for the comparison between children and adults and between children and the elderly. ***P < .01 for the comparison between adults and the elderly only with surgical masks (Kruskal-Wallis test).
and one recent study found that the subjects wearing cotton ance, may contribute to the onset of dyspnea in mask-wearers,47 which however had very small sample sizes and used instruments that could not avoid the interference of water vapor.16,39 The available capnography studies report an increase in ETCO₂, suggesting that masks may impair ventilation to a certain degree, especially during physical activity.21,22,23 The present study was the first to quantify the CO₂ concentration within face masks by using capnography. Indeed, the explanation of the observed high CO₂ values lies in the combination of tidal and mask volumes: even though the 500 ml tidal volume of the average adult man is predominantly filled with low environmental concentrations of CO₂,25 the portion represented by the mask dead space had a CO₂ content so high that the overall inhaled air CO₂ increased substantially.40

Concerning the risk of hypoxia, several evaluations found that blood oxygen saturation decreases when donning face masks, and even more noticeably when wearing them for long periods or during physical activity.11,22,23,40 In contrast, the present study was performed at rest and for a short time, during which the recorded levels of CO₂ did not substantially alter blood oxygen saturation, as in similar studies.13,14,41 Nevertheless, the exposure to inhaled air CO₂ values higher than 5000 ppm, for long periods, is considered unacceptable for the workers, and is forbidden in several countries,30 because it frequently causes signs and symptoms such as headache, nausea, drowsiness, rhinitis, and reduced cognitive performance.42,43

Also, reports have been published about the negative impact of respirators on healthcare professionals, such as headache or respiratory distress.20 In fact, minors can be expected to be at a substantially higher risk of experiencing respiratory distress.20 In fact, minors can be expected to be at a disadvantage also in this evaluation, because their small build corresponds to a small tidal volume, which therefore provides less dilution of the excess CO₂ compared to the greater tidal volume of adults.34,35 Nonetheless, given the limited number of the included minors, this finding inevitably requires validation.

As regards the difference between mask types, of the above mentioned studies, one did not find differences in the CO₂ concentration between surgical and FFP2 (with valve) masks, but only one subject was analyzed.14 The other study did not include surgical masks in the evaluation.33 In fact, given the similar ETCO₂ between the 2 mask types, the larger dead space inside FFP2 respirators is expected to determine a sharp difference in CO₂ content between surgical and FFP2 masks.36,37 This is consistent with 3 previous studies: 1 on patients whose ETCO₂ increased with increasing mask dead space,48 and 2 on healthcare professionals (one of which used capnography) which observed CO₂ retention within FFP2 masks, whether with or without valve.49,50

In relation to the respiratory rate, no previous study specifically evaluated its association with CO₂ concentration in healthy individuals at rest. However, an increase of inhaled air CO₂ was found during physical activity with masks,38 and with higher respiratory rates in post-operative ventilated patients.51 In addition, it is well known that, besides mask use, slow breathing is associated with significantly lower inhaled air CO₂ concentration.52

Separate considerations are needed for smokers, whose lower CO₂ concentration in face masks appears counterintuitive. However, smoking a higher number of cigarettes has been associated with higher arterial and lower alveolar CO₂, consistent with the decreased pulmonary gas diffusion which characterizes smokers.53 Thus, the diminished capacity to eliminate CO₂ by the smokers may likely explain the lower concentrations in face masks.

Finally, concerning the minors, no study so far directly compared them to adults, and, apart from one relatively old research which showed increased ETCO₂ concentrations in young children wearing gas masks,34 the aforementioned capnography assessments of children identified no changes in physiologic parameters with surgical masks,21 but found increased CO₂ when walking with FFP2 masks, which is an earlier sign of alveolar hypoventilation than falling oxygen saturation and indicates respiratory distress.20 In fact, minors can be expected to be at a disadvantage also in this evaluation, because their small build corresponds to a small tidal volume, which therefore provides less dilution of the excess CO₂ compared to the greater tidal volume of adults.34,35 Nonetheless, given the limited number of the included minors, this finding inevitably requires validation.

As mentioned, OSHA allows CO₂ concentrations up to 5000 ppm for an 8-hour working day.30,31 However, throughout the pandemic, most people living in cities not only had to wear masks at work, but also on public transportation, in supermarkets and other public places, and sometimes even outdoor. This exceeds the 8-hour limit, and warrants careful evaluation of the potential benefits and harms when considering face mask requirements.

Analogous considerations apply to children, who may add to 5 hours of morning classes further time of indoor extra-curricular activities, and also a variable time on public transportation. The US Environmental Protection Agency reports an upper
limit for CO2 concentration in schools of 700 ppm above outdoor concentrations (conventionally around 400 ppm), as recommended in the Standard 62-2001 of the American Society of Heating, Refrigerating, and Air-Conditioning Engineers.55 In Europe, regulation EN 13779 also refers to the outdoor concentration and classifies indoor air quality as low with >1000 ppm, essentially in agreement with the US standard.56 Based on this regulation, further norms were developed on the maximum occupancy of non-residential buildings such as schools, and on the ventilation requirements, such as norm UNI EN ISO 16000-26, which recommends specific timings for air changes depending on room occupancy and use.57

Indeed, given the recent evidence suggesting that, in crowded rooms, 2 air-changes per hour may lower aerosol build-up more efficiently than the best performing masks,58 the choice of increased ventilation over mask-wearing could be taken into consideration when allowed by the environmental and epidemiological conditions, especially for the minors.

Moreover, the observed difference in inhaled air CO2 according to mask types suggests that, in the presence of mask mandates, and when the usage is protracted and/or physical activity is required, surgical masks should be used instead of FFP2 respirators, as they reduce the possible negative effects of high CO2 concentrations.59,60 Further suggestions, both for the general public and for people whose job requires the protracted use of masks, may include: reducing physical exertion, taking breaks to remove the masks and reduce CO2 build-up, staying hydrated (as dehydration worsens the symptoms due to high CO2 concentration),61 and possibly use powered air-purifying respirators, which were described as more comfortable than FFP2 respirators.44

Finally, if the relationship between CO2 levels and respiratory rate is verified, the current guidelines to control SARS-CoV-2 pandemic should be reevaluated.62 This would be particularly important for blue-collar workers and the elderly, who are known to breathe faster,62,63 possibly more so when wearing masks,11 and showed higher baseline CO2 concentrations.64 Indeed, the average respiratory rate at rest has been estimated around 15 breaths per minute in healthy adults,65 and, in the present assessment, 3 additional breaths per minute were enough to increase the mean CO2 content over 5000 ppm when wearing surgical masks.

The chosen capnography device had water-removal tubing, and real-time monitoring, ensuring reliable and reproducible CO2 measurements,28 as confirmed by the similarity of our findings with those of other capnography assessments of individuals at rest.20,22,23 Indeed, relative humidity ranges 42% to 91% in exhaled air,16 potentially altering CO2 assessments,17,19 which might explain the differences with the measurements of previous studies that used sensors for environmental CO2.12,15 Additionally, we examined the largest sample, so far, of healthy individuals of various ages, comparing both surgical masks and FFP2 respirators.13,14

This study has also limitations that must be considered. First, although the sample size is the largest yet, it is still relatively scarce, especially for the minors. Second, the volume of the dead space within the mask could not be assessed for each participant, and therefore we could not closely inspect the possible influence of face shape and individual added dead space on the inhaled air CO2. Third, the instrument’s precision of 1.5 mmHg (1974 ppm) widens the uncertainty around the measurements. Importantly, however, when 1974 ppm are subtracted to the mean inhaled air CO2, the CO2 in surgical masks decreases to about 3000 ppm, while it still exceeds the 5000 ppm threshold with FFP2 respirators.30 Fourth, the experimental conditions, with participants at complete rest, in a constantly ventilated room, exclusively nose breathing, were far from those experienced by workers and students during a typical day, normally spent in rooms shared with other people or doing some degree of physical activity. Since it was observed that speech and even low level physical activity are associated with increases in CO2 concentration, CO2 values in real life are likely to be higher than those recorded in this study.49,66 Fifth, the short measurement times (5 minutes for each type of mask) reflect our aim to calculate the CO2 concentration within face masks, for which we needed the ETCO2, a parameter which stabilizes after about 1 minute from the start of capnography. Therefore the present findings should be viewed only as preliminary evidence of the CO2 inhaled when wearing masks, and they do not allow to evaluate potential changes in physiological parameters, nor the ensuing compensatory mechanisms adopted by the respiratory system to contrast hypercapnia.67,68 Likewise, the exclusion of individuals with cardiac or respiratory diseases does not allow to generalize our findings to people with chronic obstructive pulmonary disease. These people may have impaired physiologic compensatory mechanisms, such as increased respiratory rate to compensate the increased ETCO220 and therefore it is plausible that their build-up of CO2 within face masks may be higher.69

Mask wearing is required in many countries throughout the working day, and during lectures in the case of students.2 Therefore, capnography and pulse oximeter monitoring of a general population sample should be extended to hours of observation, and not only in conditions of rest, in order to verify whether the ETCO2 detected within masks remains constant or has a tendency to increase with longer mask wearing and while performing habitual tasks. In addition, subjective symptoms such as headache and drowsiness should also be investigated.

As mentioned, the progressive rise in CO2 with increasing breaths per minute, and the higher CO2 in minors also requires validation from further studies with larger samples.

**Conclusions**

Shortly after wearing surgical masks, the inhaled air CO2 approached the highest acceptable exposure threshold recommended for workers, while concerningly high concentrations
were recorded in minors, and in virtually all individuals when wearing FFP2 masks. The CO₂ concentration was significantly higher among minors and the subjects with high respiratory rate. If these findings are confirmed, the current guidelines on masks use should be reevaluated.

Acknowledgements
The authors thank Luisa Rogari, Francesca and Marta Rosini, Lucia Ricciotti, and Giusi Giacomini for their help in data collection.

Author Contributions
CAM and LM: conceptualization and methodology. CAM, MEF, and MM: investigation. LM: funding acquisition and project administration. CAM and MEF: formal analysis. CAM, MEF, MM, FSV, and LM: data curation. LM and FSV: supervision and validation. CAM, MEF, and LM: writing—original draft. CAM, LM, and FSV: writing—review and editing. All authors critically revised the article for important intellectual content and gave final approval for the article. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Data Availability
The study data is available as supplementary material.

Supplemental Material
Supplemental material for this article is available online.

REFERENCES
1. Acuti Martellucci C, Flacco ME, Cappadona R, Bravi F, Mantovani I, Manzoli L. SARS-CoV-2 pandemic: an overview. Adv Biol Regul. 2020;77:100736.
2. Chu DK, Akl EA, Duda S, Solo K, Yacoub S, Schünemann HJ. Physical distancing, face masks, and eye protection to prevent person-to-person transmission of SARS-CoV-2 and COVID-19: a systematic review and meta-analysis. Lancet. 2020;395:1973-1987.
3. Flacco ME, Acuti Martellucci C, Bravi F, et al. Severe acute respiratory syndrome coronavirus 2 lethality did not change over time in two Italian provinces. In: Open Forum Infectious Diseases, Vol. 7, No. 12. Oxford University Press; 2020.
4. Italian Government. Ulteriori disposizioni attuative del decreto-legge 25 marzo 2020, n. 74, recante «Misure urgenti per fronteggiare l’emergenza epidemiologica da COVID-19», Gazzetta Ufficiale Serie Generale n.275. 2020. Accessed May 7 2022. https://www.gazzettaufficiale.it/eli/id/2020/03/30/20G05727/hg
5. Leung NHL, Chu DKW, Shiu EYC, et al. Respiratory virus shedding in exhaled breath and efficacy of face masks. Nat Med. 2020;26:676-680.
6. Fögen Z. The foegen effect: a mechanism by which facemasks contribute to the COVID-19 case fatality rate. Medicine. 2021;101:e28924.
7. Mouloiu DS, Pantzaoupolou I, Gourgoulanis KI. Medical/surgical, cloth and FFP/(K)N95 masks: unmasking preference, SARS-CoV-2 transmissibility and respiratory side effects. J Prev Med. 2022;12:325.
8. Perna G, Cuniberti F, Daccò S, Nobile M, Caldirola D. Impact of respiratory protective devices on respiration: implications for panic vulnerability during the COVID-19 pandemic. Jaffr J Disord. 2020;277:772-778.
9. Deoni SC, Beaucemín J, Volpe A, D’Alessio V, the RESONANCE Consortium. Impact of the COVID-19 pandemic on early child cognitive development: initial findings in a longitudinal observational study of child health. medRxiv. 2021;2021.08.10.21261846.
10. Stajdhaur A, Ganel T, Avidan G, Rosenbaum RS, Freud E. Face masks disrupt holistic processing and face perception in school-age children. Cog Res Princ Implic. 2022;7:9.
11. Kisielinski K, Giboni P, Prescher A, et al. Is a mask that covers the mouth and nose free from undesirable side effects in everyday use and free of potential hazards? Int J Environ Res Public Health. 2021;18:434.
12. Johnson AT. Respirator masks protect health but impact performance: a review. J Biol Eng. 2016;10:1-12. doi:10.1186/s13285-0016-0025-4
13. Rhee MSM, Lindquist CD, Silvestrini MT, Chan AC, Ong JFY, Sharma VK. Carbon dioxide increases with face masks but remains below short-term NIOSH limits. BMC Infect Dis. 2021;21:354.
14. Geiss O. Effect of wearing face masks on the carbon dioxide concentration in the breathing zone. Aerosol Air Qual Res. 2021;21:200403.
15. Walach H, Traindl H, Prentice J, et al. Carbon dioxide rises beyond acceptable safety levels in children under nose and mouth covering; results of an experiment study in healthy children. Environ Res. 2022;212:113564.
16. Mansour E, Vishinkin R, Rihet S, et al. Measurement of temperature and relative humidity in exhaled breath. Sens Actuators B Chem. 2020;304:127371.
17. Chan KL, Ning Z, Westerdahl D, et al. Dispersive infrared spectroscopy measurements of atmospheric CO₂ using a Fabry-Pérot interferometer sensor. Sci Total Environ. 2014;472:27-35.
18. Zhang G, Li M, Zheng M, et al. Effect of surgical masks on cardiopulmonary function in healthy young subjects: a crossover study. Front Physiol. 2021;12:710573.
19. Cherian SN. Physics of capnography - factors affecting IR spectrography. 2021. Accessed May 7 2022. https://www.capnography.com/chemical-method-of-co2-measurement?id=61
20. Lubrano R, Bloise S, Marcellino A, et al. Effects of N95 mask use on pulmonary function in children. J Pediatr. 2021;173:143-147.
21. Lubrano R, Bloise S, Torra A, et al. Assessment of respiratory function in infants and young children wearing face masks during the COVID-19 pandemic. JAMA Network Open. 2021;4:e210414.
22. Dirol H, Alkan E, Sindel M, Ozdemir T, Erbas D. The physiological and disturbing effects of surgical face masks in the COVID-19 era. Bratisl Lek Listy. 2021;122:821-835.
23. Sukul P, Bartels J, Fuchs P, et al. Effects of COVID-19 protective face-masks and wearing durations onto respiratory-haemodynamic physiology and exhaled breath constituents. Eur Respir J. Published online February 15, 2022. doi:10.1183/13993003.00443221.
24. Birgerson E, Tang EH, Lee WL, Sak JK. Reduction of carbon dioxide in filtering facepiece respirators with an active-venting system: a computational study. PLoS One. 2015;10:e013036.
25. Hall J. Gayton and Hall Textbook of Medical Physiology. 13th ed.; W B Saunders; 2015.
26. Kim KW, Choi HR, Bang SR, Lee JW. Comparison of end-tidal CO₂ measured by transportable capnometer (EMMA™ capnograph) and arterial CO₂ in general anesthesia. J Clin Monit Comput. 2016;30:737-741.
27. Zhuang Z, Bergman M, Brochu E, et al. Temporal changes in filtering-facepiece respirator fit. J Occup Environ Hyg. 2016;13:265-274.
28. Masimo. Rad-97 with Nomoline capnography - product information. 2021. Accessed May 7 2022. https://www.masimo.com/siteassets/us/documents/pdf/plm-10050b_product_information_rad-97_nomoline_capnography_us.pdf.
29. Breyse P, Lees P. Gases and vapors. Johns Hopkins Bloomberg School of Public Health; 2006. Accessed May 7, 2022. http://docplayer.net/23648808-Gases-and-vapors-patrick-n-breyse-phd-cih-peter-s-j-lees-phd-cih-johns-hopkins-university.html.
30. United States Department of Labor. Occupational safety and health administration occupational chemical database - carbon dioxide. September 9, 2021. Accessed May 7 2022. https://www.osha.gov/chemicaldata/183.
31. European Agency for Safety and Health at Work (EU-OSHA). Directive 2019/1831 - indicative occupational exposure limit values. Updated April 8, 2021; September 27, 2021. Accessed May 7 2022. https://osha.europa.eu/en/law- legislation/directive/directive20191831-indicative-occupational-exposure-limit-values.
32. Italian Government. Decreto 9 April 2008, n. 81. Actuation of art. 1 of Law 3 August 2007, n. 123, Concerning Health and Safety in Workplaces. Italian Government; 2008.
33. Tsuchi AS, Kayserilloglu A, Unal M, Ozer S, Akbay S. Ventilatory and metabolic response to rebreathing the expired air in the Sneekel. Int J Sports Med. 2003;24:162-165.
34. Hutchinson J. On the capacity of the lungs, and on the respiratory functions, with a view of establishing a precise and easy method of detecting disease by the spirometer. Med Chir Trans. 1846;29:337-252.
35. Needham CD, Rogan MC, McDonald I. Normal standards for lung volumes, intrapulmonary gas-mixing, and maximum breathing capacity. Thorax. 1954;9:313-325.
