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Correlation of Respiratory Aerosols and Metabolic Carbon Dioxide

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Abstract: Respiratory aerosols from breathing and talking are an important transmission route for viruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Previous studies have found that particles with diameters ranging from 10 nm to 145 µm are produced from different regions in the respiratory system and especially smaller particles can remain airborne for long periods while carrying viral RNA. We present the first study in which respiratory aerosols have been simultaneously measured with carbon dioxide (CO_2) to establish the correlation between the two concentrations. CO_2 concentrations are easily available through low-cost sensors and could be used to estimate viral exposure through this correlation, whereas source-specific aerosol measurements are complicated and not possible with low-cost sensors. The increase in both respiratory aerosols and CO_2 was linear over ten minutes in a 2 m^3 chamber for all participants, suggesting a strong correlation. On average, talking released more particles than breathing, with 14,600 ± 16,800 min^{-1} (one-σ standard deviation) and 6210 ± 5630 min^{-1} on average, respectively, while CO_2 increased with 139 ± 33 ppm min^{-1} during talking and 143 ± 29 ppm min^{-1} during breathing. Assuming a typical viral load of 7×10^6 RNA copies per mL of oral fluid, ten minutes of talking and breathing are estimated to produce 1 and 16 suspended RNA copies, respectively, correlating to a CO_2 concentration of around 1800 ppm in a 2 m^3 chamber. However, viral loads can vary by several orders of magnitude depending on the stage of the disease and the individual. It was therefore concluded that, by measuring CO_2 concentrations, only the number and volume concentrations of released particles can be estimated with reasonable certainty, while the number of suspended RNA copies cannot.

Keywords: respiratory aerosols; carbon dioxide; airborne transmission; indoor air quality; COVID-19

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1. Introduction

A key reason for the difficulty of controlling the COVID-19 pandemic is the airborne spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Particles are emitted from the human respiratory system not only by coughing and sneezing but also by talking and breathing [2–4]. These particles can remain airborne for long periods and travel far outside the personal space of the emitter [5]. SARS-CoV-2 has been found in these particles on several occasions [6,7] and the virus remains viable in aerosol droplets for hours, with a half-life of 1.1 h [8]. Before the COVID-19 pandemic, similar airborne transmission properties were also found for SARS [9], MERS [10] and influenza [11]. Particle concentrations are typically expressed as either number of particles per air volume in (m^{-3}) or mass per air volume in (µg m^{-3}). With a known average concentration of

7×10^6 RNA copies mL^{-1} of oral fluid [12], the volume concentration in ($mL\,m^{-3}$) is particularly of interest.

As CO_2 is a relatively abundant emission of the human body and is easy and cheap to measure, its concentration can be a good indicator of other anthropogenic bioeffluents in indoor spaces, as the only other sources would be open flames, pets or combustion devices. Because CO_2 concentrations increase with the number of occupants in a space, and one or more of the occupants could potentially be infected, it is logical that respiratory disease risk scales with indoor CO_2 levels. Du et al. [13], for example, demonstrated that when CO_2 was reduced to less than 1000 ppm, it was associated with a 97 % decrease in tuberculosis incidence among contacts.

In 2003, Rudnick and Milton [14] introduced a model that uses CO_2 levels to estimate the risk of airborne transmission of influenza and other respiratory diseases. Peng and Jimenez [15] used this model to estimate the COVID-19 infection risks for different scenarios and found that, even though the CO_2 level corresponding to a given infection risk may vary by over two orders of magnitude depending on the environment and activity, it is still a useful metric to assess ventilation. During the COVID-19 pandemic, several researchers estimated the transmission risk of SARS-CoV-2 in such spaces as nail salons [16], office buildings [17] and schools [18] based on occupant respiration rates and activities. For instance, Harrichandra et al. [16] concluded that increased outdoor airflow rates and the use of face masks by both employees and customers could substantially reduce SARS-CoV-2 transmission in New York City nail salons. These studies demonstrated the value of CO_2 sensing for assessing airflow as a factor in transmission risk.

There are numerous studies that have directly investigated respiratory aerosols from breathing, talking and coughing and found particles in the range of 10 nm to 145 µm [3,4,19]. As mentioned above, there are also a large number that recommend the use of CO_2 as a proxy for infection risk or use CO_2 as a tracer for simulation of the spread of droplet nuclei [15,20–23].

The link between CO_2 and respiratory aerosols has not been studied in detail however, and the uncertainty around virus exhalation rates are large [14,15]. We present the first study investigating respiratory aerosols while simultaneously measuring metabolically produced carbon dioxide. The aim of our study was to find the correlation between the two concentrations and estimate the suspended fluid volume as well as the suspended SARS-CoV-2 RNA number of a hypothetical infected person at a given CO_2 concentration. This relationship could be used to improve the modelling of airborne infection transmission and the determination of threshold limits for CO_2 concentrations in different scenarios. Furthermore, the increase in respiratory particles over time was measured for the first time in a a closed space, offering a realistic setting opposed to the direct particle measurements in exhaled air that have been performed previously.

2. Methods

2.1. Setup

An airtight chamber with 1 m length, 2 m width and 1 m height was used as a sampling environment to simultaneously measure expelled particles from either breathing or talking and the CO_2 exhaled in the process. Inside the chamber, three fans were installed to mix the air along three Cartesian axes, facing each other to assure mixing. Next to this array, a probe for relative humidity and temperature was installed as well as an infrared sensor (Sensirion SCD30) for the measurement of CO_2 concentrations. In the middle of the chamber, an AirBubbl (AirLabs, Copenhagen and London, air cleaning device with F8 grade filters and $30\,m^3\,h^{-1}$ clean air delivery rate) was placed for the removal of particles before experiments. The general array of used instrumentation is schematically shown in Figure 1. The inlet of an optical particle sizer (OPS 3330, TSI Incorporated, Shoreview, Minnesota) was connected with 3/16 inch inner diameter, 5/16 inch outer diameter and conductive tubing to the chamber, and the sampling air was recirculated back inside after passing a HEPA in-line filter to keep the pressure in the chamber constant. Even though

particles below the range of the OPS are known to be produced [4], they are not expected to significantly contribute to volume and suspended RNA concentrations and were therefore not measured. With a flow rate of 1 L min^{-1}, the cleaning effect through the instrument was negligible. A second connection port in the walls of the chamber was used to either increase the humidity or add filtered, dry air to create a slight overpressure. One of the chamber walls featured a quadratic opening with 80 cm width and height. This opening was covered with a sheet of Teflon, which had been prepared with a circular cutout for a short tube, that was fixed to the Teflon film in an airtight manner to create a connection between the inside and the outside of the chamber. From the outside, a rubber face mask (Moldex 7002M) was attached to the tube so that an airtight connection could be established between a participant's mouth and nose with the chamber. When no subject was wearing the mask, a tapered rubber plug was placed into the tube opening to seal the chamber. The setup was at a height that allowed participants to comfortably sit on a chair with adjustable height while wearing the mask. Inside the chamber, opposite the Teflon sheet, two printed DIN A3 pages with a text about particulate matter were attached to the wall as material to be read out loud for the experiments including talking.

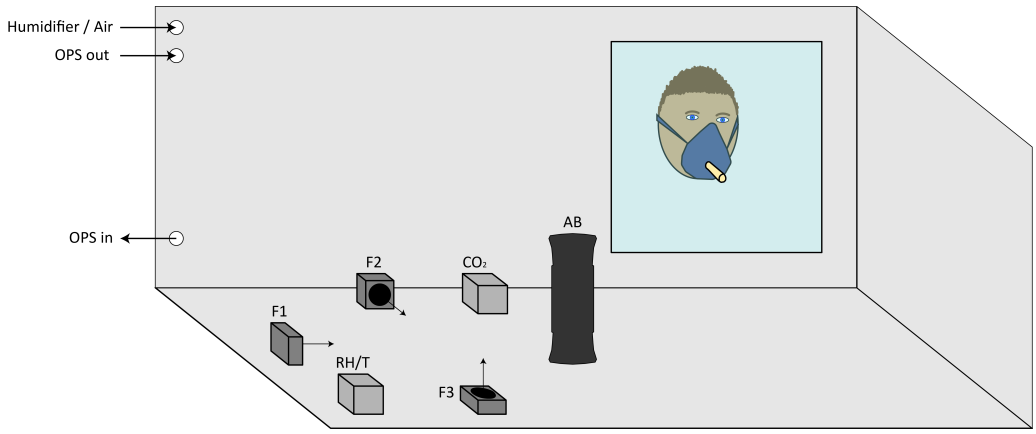


Figure 1. 2 m^3 chamber setup for measurement of respiratory particles and CO_2. OPS: Optical Particle Sizer, F1–F3: mixing fans, T: temperature probe, RH: relative humidity probe, CO_2: infrared CO_2 sensor, AB: AirBubbl.

2.2. Protocol

On each day of the study, 1 of the 16 volunteers (8 male, 8 female) participated in a breathing and a talking experiment. The subjects were of different nationalities but all fluent in English on a professional level. Before the subject arrived, a background measurement of particle leakage into the chamber was taken. The OPS was measuring with a sample length and frequency of 1 min, while CO_2 concentrations were registered every 30 s. With all fans running and the tube plug removed, air from a humidifier was released into the chamber until a relative humidity (RH) of 50 % was reached. With reinserted tube plug, the AirBubbl was then turned on to clean the air until the particle concentration was below 0.02 cm^{-3}. The AirBubbl was then turned off and the background increase in particles was measured for at least 30 min. The AirBubbl was then turned on again to reduce particle concentration below 0.02 cm^{-3} after making sure that the relative humidity remained at 50 %. Then, the AirBubbl was turned off and three to five samples of background concentrations were taken. During this, clean air was introduced into the chamber through a HEPA filter to create a slight overpressure. This assured that clean air from the chamber was exiting the chamber instead of outside air entering when the participant removed the tube plug and put on the mask. For the first experiment, the subject was instructed to breath normally through the nose for ten minutes while their mouth stayed naturally closed. Afterwards, the mask was taken off and the tube plug reinserted. After taking at least three more samples, the chamber was flushed with room air to remove the additional CO_2, rehumidified to 50 % RH and cleaned with the AirBubbl to reduce the particle concentration. The procedure of

the first experiment was then repeated but with the participant continuously reading out loud the printed text for the entire 10 min inside the chamber.

Between subjects, the chamber was sterilised using an ozone generator, which has been shown to effectively remove both viruses [24] and bacteria [25]. The mask, tube and plug were thoroughly washed with water and soap and then disinfected.

2.3. Data Treatment

All data were analysed in Python. For all linear regression models, the scikit-learn package [26], which uses ordinary least squares linear regression, was implemented. First, a linear regression model for background PM leakage into the chamber over at least 30 min was determined for each measurement day. Before each breathing and talking experiment, the average background concentration of particles was measured with the AirBubbl turned off immediately before establishing the connection between subject and chamber. This background concentration was then subtracted from the data of the respective experiment. Then, linear regression models for respiratory aerosols and CO_2 over time were created. The background leakage slope for PM was subtracted from the aerosol slope to yield the actual increase from the respective activity. Since two measurement instruments were used and their sampling intervals did not exactly align, the particle increase over CO_2 ρ_{PM/CO_2} in $cm^{-3}\,ppm^{-1}$ was calculated as

$$\rho_{PM/CO_2} = \frac{r_{PM}}{r_{CO_2}}, \tag{1}$$

where r_{PM} is the background leakage corrected increase in particles over time in $cm^{-3}\,min^{-1}$ and r_{CO_2} is the increase in CO_2 over time in $ppm\,min^{-1}$. The particle number density n_{PM} in cm^{-3} could then be calculated with

$$n_{PM}(x_{CO_2}) = \rho_{PM/CO_2}(x_{CO_2} - b_{CO_2}) + b_{PM}, \tag{2}$$

where x_{CO_2} is the CO_2 concentration in ppm, ρ_{PM/CO_2} is the particle increase over CO_2 in $cm^{-3}\,ppm^{-1}$, calculated from Equation (1), b_{CO_2} is the intercept of the CO_2 linear regression model in ppm and b_{PM} is the intercept of the particle linear regression model in cm^{-3}.

2.4. Estimation of Volume Concentrations

The setup was not designed to measure volume concentrations as particles were too diluted in the 2 m^3 chamber to obtain a size distribution. Instead, the size distribution described by Johnson et al. [19] was used to estimate the volume of airborne particles. Of their BLO-model, only modes B (bronchiolar fluid film burst) and L (larynx mode) were used for particles from speech, as the O (oral) mode was measured using droplet deposition analysis and these particles are not expected to be airborne. The volume distributions were estimated with

$$\frac{dCv}{d\ln D} = \ln 10 \times \sum_{i=1}^{2} \frac{\pi}{6} D^3 \left(\frac{Cn_i}{\sqrt{2\pi}\ln GSD_i} \right) \exp\left(-\frac{(\ln D - \ln CMD_i)^2}{2(\ln GSD_i)^2} \right), \tag{3}$$

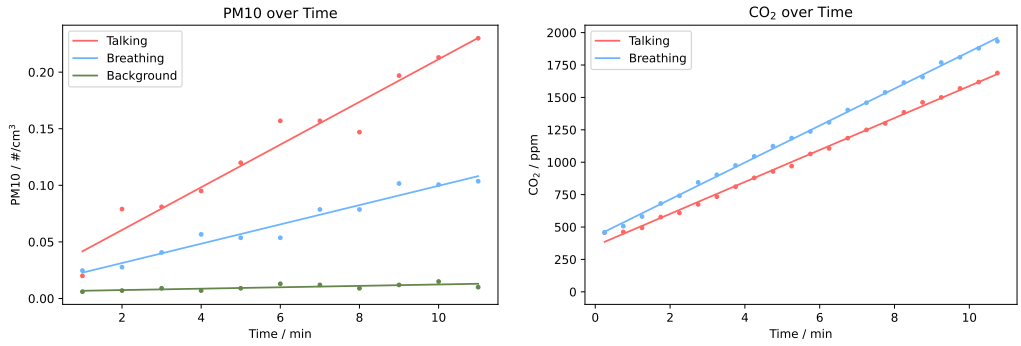
where D is the particle diameter and Cn_i is the number concentration. GSD_i and CMD_i are the geometric standard deviations and count median diameters of modes B and L and have been taken from Johnson et al. [19]. Volume concentrations were then calculated by simply integrating over the distributions.

For an estimate of the volume concentrations from breathing, only the B mode was considered, which is expected to overestimate the actual volume, as people were instructed to breathe through their mouth in Johnson et al., while they were breathing through their nose in this study. Mouth breathing is known to produce slightly larger particles [2,27] and the volume concentration from breathing should be seen as an upper limit and means of comparison.

3. Results

3.1. Respiratory Aerosol and CO_2 Increase over Time

In a 2 m^3 chamber, particle and CO_2 concentrations were simultaneously measured over ten minutes while a subject was either breathing or talking. A total of 16 volunteers (eight male, eight female) participated; one data set was corrupt and excluded from analysis. A comparison of the typical increase in PM10 (particulate matter with an aerodynamic diameter smaller than 10 µm) from background leakage and the breathing and talking experiments of one of the participants are shown in Figure 2A. The corresponding CO_2 concentrations over time are shown in Figure 2B. During breathing experiments, the relative humidity remained constant at 50 %, while it increased by a few percentage points during talking experiments. However, no increase in the production rate was registered within the 10 min time span of any individual experiment and the effect of the slight change in humidity, if any, was found to be negligible.



(**A**) (**B**)

Figure 2. Typical experimental data for one of the fifteen participants. (**A**): Increase in respiratory aerosols over time in a 2 m^3 chamber from background leakage, a breathing and a talking experiment. (**B**): Typical increase in CO_2 over time during the same breathing and talking experiments.

The distribution of respiratory aerosol production rates, corrected by background leakage for breathing and talking, as well as the corresponding distributions of CO_2 production rates for all subjects are shown in Figures 3A and B. The displayed data are summarised in Table 1, which includes the average production rates as concentrations over time, the standard deviations and the average R^2 values of the linear regression models used to obtain the reported slopes. Note that these deviations do not represent an error but the variation of particle and CO_2 production rates between participants. The average volume production rates have been calculated with the BLO-model of Johnson et al. [19], which assumes a bimodal distribution of particles with mode diameters of 1.6 and 2.5 µm, respectively, for particles from speech. Breathing only produces particles in the smaller mode, which is linked to the bronchiolar fluid burst mechanism and amplifies the difference between the two activities, although the actual volume concentration may be even lower as the model is based on mouth breathing. The exact formula for calculating volume concentrations can be found in the methods section.

For 13 out of 15 people tested, talking produced more particles than breathing, whereas one person emitted particles at the same rate for both activities and one person emitted more from breathing, resulting in an average increase of 2.98 $\pm$ 2.74 (one-σ standard deviation) in particle production for talking. There was not a clear pattern in the rate of CO_2 production as five people produced more CO_2 while talking, whereas one person produced equal amounts from both activities and nine people produced more CO_2 from breathing, resulting in an average ratio of 0.979 $\pm$ 0.185. The ratios are summarised in Figure 3C. Note that CO_2 production can be higher for either activity, but the standard deviation of 0.185 is low in comparison to the standard deviation of 2.74 for particle production rate ratios. Taking the definition of Asadi et al.[3], three subjects in this study can be described

as talking superemitters as their production rates exceed the mean by more than one standard deviation. With this definition, the same three people can be considered breathing superemitters. When taking the proposed definition of Holmgren et al. [4] instead, only one participant qualifies as a talking superemitter as their production rate exceeds the mean by more than two standard deviations and no participant would be a breathing superemitter.

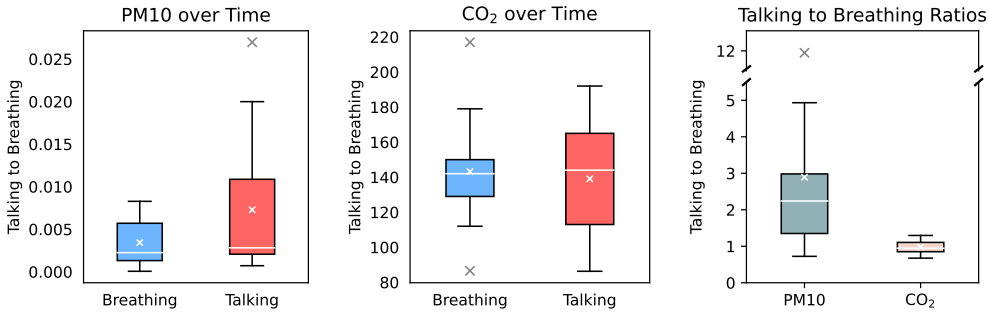


Figure 3. Boxplots of production rates and ratios from 15 participants. The median and mean are represented by a white line and a white cross, respectively, while the lower and upper box limits correspond to the lower and upper quartiles. Outliers are shown for data points exceeding the upper quartile plus 1.5 times the interquartile range (IQR) and data points lower than the lower quartile minus 1.5 times the IQR. (**A**): Increase in respiratory aerosols over time from respective breathing and talking experiments of 15 participants in a 2 m^3 chamber. (**B**): CO_2 production rates of 15 participants during the respective experiments. (**C**): Ratios of production rates from talking compared to breathing for each subject. Notice the break on the *y* scale, which was used to include an outlier.

Table 1. Average production rates r of respiratory aerosols and CO_2 over time from 15 volunteers in a 2 m^3 chamber and their standard deviations σ.

Parameter	Breathing	Talking	Unit
r_{PM}	3.11×10^{-3}	7.29×10^{-3}	$cm^{-3}\,min^{-1}$
$\sigma\,(r_{PM})$	2.82×10^{-3}	8.41×10^{-3}	$cm^{-3}\,min^{-1}$
$R^2\,(r_{PM})$	0.627	0.806	
$r_{PM,V}$	9.26×10^{-3}	1.12×10^{-1}	$\mu m^3\,cm^{-3}\,min^{-1}$
$\sigma\,(r_{PM,V})$	8.40×10^{-3}	1.29×10^{-1}	$\mu m^3\,cm^{-3}\,min^{-1}$
r_{CO_2}	143	139	$ppm\,min^{-1}$
$\sigma\,(r_{CO_2})$	29.4	33.2	$ppm\,min^{-1}$
$R^2\,(r_{CO_2})$	0.998	0.998	

With eight male and eight female participants, the influence of biological gender can be investigated. The average production rates for men are 2.61×10^{-3} and 5.83×10^{-3} $cm^{-3}\,min^{-1}$ for breathing and talking, respectively, while those for women are 3.67×10^{-30} and 8.95×10^{-3} $cm^{-3}\,min^{-1}$ for breathing and talking, respectively. The higher production rates for female participants, however, is largely created by the strongest superemitter, which was a woman. Without her contribution, the female production rates are only 3.91×10^{-4} and 1.13×10^{-4} $cm^{-3}\,min^{-1}$ higher for breathing and talking, respectively, than those of the male participants. This suggests that there may not be a significant difference between men and women, but with eight subjects from each gender, the test group may not be large enough to be representative in this aspect.

3.2. Correlation of Respiratory Aerosols and CO_2 and Estimation of Suspended RNA

Figure 4A shows the increase in respiratory aerosols in relation to metabolically produced CO_2 for the breathing and talking experiments of the same representative subject of Figure 2. The distribution of production rates for all participants, mathematically determined with Equation (1), is shown in Figure 4B. The corresponding average production rates and their standard deviations are shown in Table 2. These deviations, again, do not represent errors but the different production rates among volunteers. The table also includes average volume production rates with CO_2 dependency, which have been determined by correlating the volume production rates of Table 1 with the CO_2 production rates. They can be used to estimate the amount of suspended SARS-CoV-2 virions by multiplying the exhaled volume with the viral RNA concentration in oral fluid. With an average viral load of 7×10^6 mL^{-1} and a maximum viral load of 2.35×10^9 mL^{-1}, the concentrations strongly vary between individuals and depend on the progression of the infection [12]. The resulting, respective, average and maximum amounts of 16 and 5300 theoretically released RNA copies after 10 min of talking correlate with an approximate CO_2 concentration of 1800 ppm (1390 ppm above the background). Less copies can be expected from breathing, which, after 10 min, is estimated to result in 1 RNA copy on average or a maximum of 440 RNA copies, correlating to 1840 ppm (1430 ppm above the background). Keep in mind, the RNA production rate from breathing is also likely to be overestimated as the volume was estimated by the BLO model. The above calculated values have been determined for our 2 m^3 chamber with homogenised air but can be theoretically scaled to any room size, assuming the air is well mixed. The theoretically produced number of RNA copies per cubic metre per ppm of CO_2 r_{RNA} are summarised in Table 2.

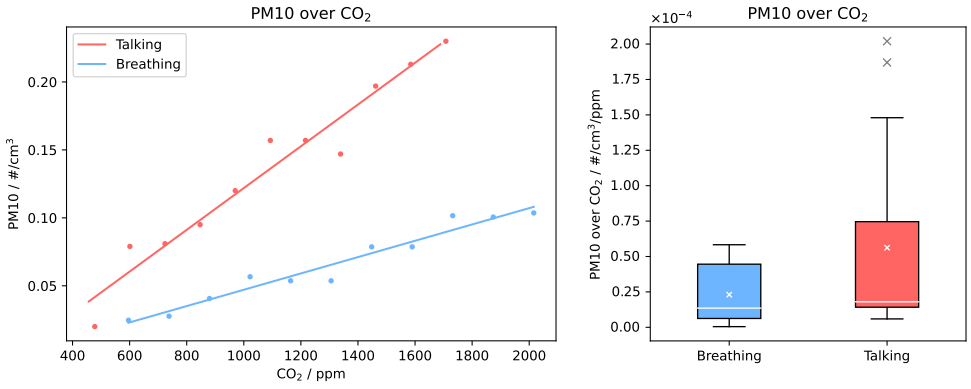


Figure 4. Correlation of respiratory aerosols and CO_2. (**A**) Increase in respiratory aerosols over CO_2 from talking and breathing in a 2 m^3 chamber for the same representative subject from Figure 2. (**B**) Distribution of respiratory aerosol production rates in relation to metabolic CO_2 in a 2 m^3 chamber for 15 participants. The median and mean are represented by a white line and a white cross, respectively, while the lower and upper box limits correspond to the lower and upper quartiles. Outliers are shown for data points exceeding the upper quartile plus 1.5 times the interquartile range (IQR) and data points lower than the lower quartile minus 1.5 times the IQR.

Table 2. Average number and volume production rates r of respiratory aerosols over CO_2 from 15 volunteers in a 2 m^2 chamber and their standard deviations σ.

Parameter	Breathing	Talking	Unit
r_{PMCO_2}	2.30×10^{-5}	5.62×10^{-5}	$cm^{-3}\,ppm^{-1}$
$\sigma\,(r_{PMCO_2})$	2.12×10^{-5}	6.84×10^{-5}	$cm^{-3}\,ppm^{-1}$
$r_{PMCO_2,V}$	6.85×10^{-5}	8.65×10^{-4}	$\mu m^3\,cm^{-3}\,ppm^{-1}$
$\sigma\,(r_{PMCO_2,V})$	6.31×10^{-5}	10.5×10^{-4}	$\mu m^3\,cm^{-3}\,ppm^{-1}$
r_{RNA}	4.8×10^{-4}	6.1×10^{-3}	$m^{-3}\,ppm^{-1}$
$r_{RNA,max}$	0.16	2.0	$m^{-3}\,ppm^{-1}$

4. Discussion

The results presented here show that, in a closed space, both respiratory aerosols and metabolic CO_2 increase linearly over time and that, on average, talking creates more particles than breathing. As both concentrations correlate linearly with time, they correlate linearly with each other and CO_2 can be used as a proxy for airborne exhaled particles.

The results of this study can be compared to the results from other recent studies looking at respiratory aerosols. The higher particle concentrations from talking, compared to breathing, observed in this study were expected, as previous studies reported the same hierarchy [2,3]. It was also expected to see large variations between subjects, as standard deviations twice as high as the average production rates and outliers further than three standard deviations from the mean have been reported in other studies [3,4,28]. These outliers have been termed superemitters or superspreaders and they might be the cause of infection in superspreading events, which have been reported in several studies [29,30].

Two of the most thorough studies investigating the particle concentrations from exhaled air have been carried out using the Expired Droplet Investigation System (EDIS), developed by Morawska et al. [2]. During their first study, average concentrations of 0.307 cm^{-3} during talking and 0.05 cm^{-3} during breathing were reported. Multiplying these values with the approximate exhalation rates of 13.5 and 12.0 L min^{-1} for talking and breathing, respectively [31], leads to particle production rates of 4.14×10^3 and 6.00×10^2 min^{-1}, respectively. The production rates of this study can be expressed independently of the specific chamber by multiplying them with its volume, leading to absolute rates of 6.22×10^3 and 1.46×10^4 particles min^{-1} for breathing and talking, respectively. Our production rate during speech is slightly higher than that of Morawska et al. but lies well within the same order of magnitude. Our production rate from breathing differs slightly more, lying just outside one order of magnitude with 6.21×10^3 min^{-1}. Looking at speech in the EDIS, volunteers were instructed to perform 10 s of voiced counting alternating with 10 s of breathing, meaning subjects were only speaking half the time and bringing the production rates of their experiment and this study closer together. In a later study that focused on the development of the BLO model in the EDIS, a particle concentration of 0.16 cm^{-3} was reported for speech [19], resulting in the slightly lower production rate of 2.16×10^3 min^{-1}, which still lies within the same order of magnitude as our findings. Similar concentrations for breathing were observed by Holmgren et al. [4], who measured 0.06 cm^{-3} for normal breathing and 5.3 cm^{-3} for airway closure breathing, correlating to production rates of 7.2×10^2 and 6.4×10^4 min^{-1}, respectively, whereas the former is more relevant to this study than the latter. Asadi et al. [3] investigated the particle emission from speech in relation to voice amplitude and found an average production rate of 240 min^{-1} at 85 dB. As described by the authors, however, 80 % of the particles were removed due to the sampling mechanism of the Aerodynamic Particle Sizer used for detection and further particles may have escaped through the only semi-confined sampling environment.

Volume production rates and RNA production rates emphasise the difference between breathing and talking experiments, which might seem small when considering only number

concentrations. These differ by only slightly more than a factor of two, while volume production rates differ more than one order of magnitude. This is due to the different modes during these activities as described by Johnson et al. [19]. While breathing only creates small particles from the bronchiolar fluid film burst mechanism (B mode) with a median diameter of 1.6 µm, talking also releases particles from the larynx (L mode) with a median diameter of 2.5 µm. Therefore, it makes sense that talking has been estimated to produce more than ten times the amount of airborne RNA copies. The median diameter of the B mode relates to mouth breathing, and nose breathing is likely to produce even smaller particles, amplifying the described difference between activities. Notice that our estimation for maximum production assumes the maximum SARS-CoV-2 RNA copy concentration found by Wölfel et al. [12] but still uses the average particle production rate. If a superemitter of particles also happens to carry a high concentration of RNA copies in their oral fluid, their airborne RNA production rate can be even higher. At the same time, suspended RNA concentrations can be much lower than the average as their concentration in oral fluid increases from zero to a maximum and back to zero throughout the progression of COVID-19 [12].

Since the CO_2 production rate barely differs between breathing and talking, it is necessary to know the average time that people spend talking in a given setting to estimate the particles they produce. Applying the partitioning to the production rates for breathing and talking would result in a useful estimation of suspended particles, which increases in reliability with the number of present people as individual differences would average out. The suspended SARA-CoV-2 RNA concentration, however, depends on the number of infected people, their individual particle production rates and their stage of infection, creating large uncertainties in the estimation of an infection risk.

This study shows that there is a strong argument for keeping indoor spaces well ventilated or using supplementary air cleaning technology. Typically, the anthropogenic metabolism is the only indoor source of CO_2 and, therefore, is a great proxy for suspended particles as well as indoor air quality in general. Ambient indoor particulate matter, on the other hand, has many sources and comes in concentrations several orders of magnitude higher than respiratory aerosols [32], making it impossible to monitor human expired aerosols directly. Even though specific concentrations of suspended RNA copies from an infected person may vary strongly between individuals due to different particle production rates and viral concentrations in oral fluid, CO_2 was shown to strongly correlate with the airborne particle concentration. The estimation of suspended RNA based on CO_2 measurements alone is unreliable as masks and air cleaners, but especially varying and individual RNA concentrations, complicate the correlation. However, it has been shown previously that CO_2 can be used as a proxy for infection risk indoors, and the results from this study should improve future models based on this link [15]. Overall, high CO_2 concentrations measured with low-cost sensors should always be a reason for concern and action should be taken to ventilate the room.

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Institutional Review Board Statement: The University of Copenhagen Research Ethics Committee for Science and Health approved this study (Case 504-0250/21-5000) and all of the experiments were performed in accordance with relevant guidelines and regulations. All volunteers gave informed consent to participate in the study and their data have been collected anonymously.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data are included in this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Morawska, L.; Milton, D.K. It Is Time to Address Airborne Transmission of Coronavirus Disease 2019 (COVID-19). *Clin. Infect. Dis.* **2020**, *71*, 2311–2313. [CrossRef] [PubMed]
2. Morawska, L.; Johnson, G.R.; Ristovski, Z.D.; Hargreaves, M.; Mengersen, K.; Corbett, S.; Chao, C.Y.; Li, Y.; Katoshevski, D. Size distribution and sites of origin of droplets expelled from the human respiratory tract during expiratory activities. *J. Aerosol Sci.* **2009**, *40*, 256–269. [CrossRef]
3. Asadi, S.; Wexler, A.S.; Cappa, C.D.; Barreda, S.; Bouvier, N.M.; Ristenpart, W.D. Aerosol emission and superemission during human speech increase with voice loudness. *Sci. Rep.* **2019**, *9*, 2348. [CrossRef] [PubMed]
4. Holmgren, H.; Ljungström, E.; Almstrand, A.C.; Bake, B.; Olin, A.C. Size distribution of exhaled particles in the range from 0.01 to 2.0 µm. *J. Aerosol Sci.* **2010**, *41*, 439–446. [CrossRef]
5. Xie, X.; Li, Y.; Chwang, A.T.; Ho, P.L.; Seto, W.H. How far droplets can move in indoor environments—Revisiting the Wells evaporation-falling curve. *Indoor Air* **2007**, *17*, 211–225. [CrossRef] [PubMed]
6. Santarpia, J.L.; Rivera, D.N.; Herrera, V.L.; Morwitzer, M.J.; Creager, H.M.; Santarpia, G.W.; Crown, K.K.; Brett-Major, D.M.; Schnaubelt, E.R.; Broadhurst, M.J.; et al. Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. *Sci. Rep.* **2020**, *10*, 12732. [CrossRef]
7. Liu, Y.; Ning, Z.; Chen, Y.; Guo, M.; Liu, Y.; Gali, N.K.; Sun, L.; Duan, Y.; Cai, J.; Westerdahl, D.; et al. Aerodynamic analysis of SARS-CoV-2 in two Wuhan hospitals. *Nature* **2020**, *582*, 557–560. [CrossRef]
8. van Doremalen, N.; Bushmaker, T.; Morris, D.H.; Holbrook, M.G.; Gamble, A.; Williamson, B.N.; Tamin, A.; Harcourt, J.L.; Thornburg, N.J.; Gerber, S.I.; et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. *N. Engl. J. Med.* **2020**, *382*, 1564–1567. [CrossRef]
9. Yu, I.T.; Li, Y.; Wong, T.W.; Tam, W.; Chan, A.T.; Lee, J.H.; Leung, D.Y.; Ho, T. Evidence of Airborne Transmission of the Severe Acute Respiratory Syndrome Virus. *N. Engl. J. Med.* **2004**, *350*, 1731–1739. [CrossRef]
10. Kim, S.H.; Chang, S.Y.; Sung, M.; Park, J.H.; Kim, H.B.; Lee, H.; Choi, J.P.; Choi, W.S.; Min, J.Y. Extensive Viable Middle East Respiratory Syndrome (MERS) Coronavirus Contamination in Air and Surrounding Environment in MERS Isolation Wards. *Clin. Infect. Dis.* **2016**, *63*, 363–369. [CrossRef]
11. Yan, J.; Grantham, M.; Pantelic, J.; De Mesquita, P.J.B.; Albert, B.; Liu, F.; Ehrman, S.; Milton, D.K. Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 1081–1086. [CrossRef]
12. Wölfel, R.; Corman, V.M.; Guggemos, W.; Seilmaier, M.; Zange, S.; Müller, M.A.; Niemeyer, D.; Jones, T.C.; Vollmar, P.; Rothe, C.; et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* **2020**, *581*, 465–469. [CrossRef]
13. Du, C.R.; Wang, S.C.; Yu, M.C.; Chiu, T.F.; Wang, J.Y.; Chuang, P.C.; Jou, R.; Chan, P.C.; Fang, C.T. Effect of ventilation improvement during a tuberculosis outbreak in underventilated university buildings. *Indoor Air* **2020**, *30*, 422–432. [CrossRef] [PubMed]
14. Rudnick, S.N.; Milton, D.K. Risk of indoor airborne infection transmission estimated from carbon dioxide concentration. *Indoor Air* **2003**, *13*, 237–245. [CrossRef] [PubMed]
15. Peng, Z.; Jimenez, J.L. Exhaled CO_2 as a COVID-19 Infection Risk Proxy for Different Indoor Environments and Activities. *Environ. Sci. Technol. Lett.* **2021**, *8*, 392–397. [CrossRef]
16. Harrichandra, A.; Ierardi, A.M.; Pavilonis, B. An estimation of airborne SARS-CoV-2 infection transmission risk in New York City nail salons. *Toxicol. Ind. Health* **2020**, *36*, 634–643. [CrossRef]
17. Burrdige, H.C.; Fan, S.; Jones, R.L.; Noakes, C.J.; Linden, P. Predictive and retrospective modelling of airborne infection risk using monitored carbon dioxide. *arXiv* **2021**, arXiv:2009.02999.
18. Vouriot, C.V.M.; Burridge, H.C.; Noakes, C.J.; Linden, P.F. Seasonal variation in airborne infection risk in schools due to changes in ventilation inferred from monitored carbon dioxide. *Indoor Air* **2021**, *31*, 1154–1163. [CrossRef]
19. Johnson, G.R.; Morawska, L.; Ristovski, Z.D.; Hargreaves, M.; Mengersen, K.; Chao, C.Y.; Wan, M.P.; Li, Y.; Xie, X.; Katoshevski, D.; et al. Modality of human expired aerosol size distributions. *J. Aerosol Sci.* **2011**, *42*, 839–851. [CrossRef]
20. Deng, X.; Gong, G.; He, X.; Shi, X.; Mo, L. Control of Exhaled SARS-CoV-2-Laden Aerosols in the Interpersonal Breathing Microenvironment in a Ventilated Room with Limited Space Air Stability. *J. Environ. Sci. (China)* **2021**, *108*, 175–187. [CrossRef]
21. Bhagat, R.K.; Wykes, M.S.D.; Dalziel, S.B.; Linden, P.F. Effects of ventilation on the indoor spread of COVID-19. *J. Fluid Mech.* **2020**, *903*. [CrossRef]
22. Li, X.; Niu, J.; Gao, N. Co-Occupant's Exposure to Exhaled Pollutants with Two Types of Personalized Ventilation Strategies under Mixing and Displacement Ventilation Systems. *Indoor Air* **2013**, *23*, 162–171. [CrossRef] [PubMed]
23. Ai, Z.; Mak, C.M.; Gao, N.; Niu, J. Tracer Gas Is a Suitable Surrogate of Exhaled Droplet Nuclei for Studying Airborne Transmission in the Built Environment. *Build. Simul.* **2020**, *13*, 489–496. [CrossRef] [PubMed]
24. Hudson, J.B.; Sharma, M.; Petric, M. Inactivation of Norovirus by ozone gas in conditions relevant to healthcare. *J. Hosp. Infect.* **2007**, *66*, 40–45. [CrossRef] [PubMed]
25. Sharma, M.; Hudson, J.B. Ozone gas is an effective and practical antibacterial agent. *Am. J. Infect. Control* **2008**, *36*, 559–563. [CrossRef] [PubMed]

26. Pedregosa, F.; Varoquaux, G.; Gramfort, A.; Michel, V.; Thirion, B.; Grisel, O.; Blondel, M.; Prettenhofer, P.; Weiss, R.; Dubourg, V.; et al. Scikit-learn: Machine Learning in Python. *J. Mach. Learn. Res.* **2011**, *12*, 2825–2830.
27. Papieni, R.S.; Rosenthal, F.S. The Size Distribution of Droplets in the Exhaled Breath of Healthy Human Subjects. *J. Aerosol Med.* **1997**, *10*, 105–116. [CrossRef]
28. Edwards, D.A.; Man, J.C.; Brand, P.; Katstra, J.P.; Sommerer, K.; Stone, H.A.; Warded, E.; Scheuch, G. Inhaling to mitigate exhaled bioaerosols. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 17383–17388. [CrossRef]
29. Lloyd-Smith, J.O.; Schreiber, S.J.; Kopp, P.E.; Getz, W.M. Superspreading and the effect of individual variation on disease emergence. *Nature* **2005**, *438*, 355–359. [CrossRef]
30. Miller, S.L.; Nazaroff, W.W.; Jimenez, J.L.; Boerstra, A.; Buonanno, G.; Dancer, S.J.; Kurnitski, J.; Marr, L.C.; Morawska, L.; Noakes, C. Transmission of SARS-CoV-2 by inhalation of respiratory aerosol in the Skagit Valley Chorale superspreading event. *Indoor Air* **2020**, *31*, 314–323. [CrossRef]
31. Gupta, J.K.; Lin, C.H.; Chen, Q. Characterizing exhaled airflow from breathing and talking. *Indoor Air* **2010**, *20*, 31–39. [CrossRef] [PubMed]
32. Patel, S.; Sankhyan, S.; Boedicker, E.K.; Decarlo, P.F.; Farmer, D.K.; Goldstein, A.H.; Katz, E.F.; Nazaroff, W.W.; Tian, Y.; Vanhanen, J.; et al. Indoor Particulate Matter during HOMEChem: Concentrations, Size Distributions, and Exposures. *Environ. Sci. Technol.* **2020**, *54*, 7107–7116. [CrossRef] [PubMed]