



Effects of COVID-19 protective face masks and wearing durations on respiratory haemodynamic physiology and exhaled breath constituents

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While assumed to protect against SARS-CoV-2 transmission, face masks cause various physiometabolic side-effects and changes in exhaled VOC profiles. Effects are more pronounced in FFP2 masks and are profound at age ≥ 60 years. <https://bit.ly/33fzOMA>

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Abstract

Background While assumed to protect against coronavirus transmission, face masks may have effects on respiratory–haemodynamic parameters. Within this pilot study, we investigated immediate and progressive effects of FFP2 and surgical masks on exhaled breath constituents and physiological attributes in 30 adults at rest.

Methods We continuously monitored exhaled breath profiles within mask space in older (age 60–80 years) and young to middle-aged (age 20–59 years) adults over the period of 15 and 30 min by high-resolution real-time mass-spectrometry. Peripheral oxygen saturation (S_{pO_2}) and respiratory and haemodynamic parameters were measured (noninvasively) simultaneously.

Results Profound, consistent and significant ($p \leq 0.001$) changes in S_{pO_2} (≥ 60 _FFP2-15 min: $5.8 \pm 1.3\%$ ↓, ≥ 60 _surgical-15 min: $3.6 \pm 0.9\%$ ↓, < 60 _FFP2-30 min: $1.9 \pm 1.0\%$ ↓, < 60 _surgical-30 min: $0.9 \pm 0.6\%$ ↓) and end-tidal carbon dioxide tension (P_{ETCO_2}) (≥ 60 _FFP2-15 min: $19.1 \pm 8.0\%$ ↑, ≥ 60 _surgical-15 min: $11.6 \pm 7.6\%$ ↑, < 60 _FFP2-30 min: $12.1 \pm 4.5\%$ ↑, < 60 _surgical-30 min: $9.3 \pm 4.1\%$ ↑) indicate ascending deoxygenation and hypercarbia. Secondary changes ($p \leq 0.005$) to haemodynamic parameters (*e.g.* mean arterial pressure (MAP) ≥ 60 _FFP2-15 min: $9.8 \pm 10.4\%$ ↑) were found. Exhalation of bloodborne volatile metabolites, *e.g.* aldehydes, hemiterpene, organosulfur, short-chain fatty acids, alcohols, ketone, aromatics, nitrile and monoterpene mirrored behaviour of cardiac output, MAP, S_{pO_2} , respiratory rate and P_{ETCO_2} . Exhaled humidity (*e.g.* ≥ 60 _FFP2-15 min: $7.1 \pm 5.8\%$ ↑) and exhaled oxygen (*e.g.* ≥ 60 _FFP2-15 min: $6.1 \pm 10.0\%$ ↓) changed significantly ($p \leq 0.005$) over time.

Conclusions Breathomics allows unique physiometabolic insights into immediate and transient effects of face mask wearing. Physiological parameters and breath profiles of endogenous and/or exogenous volatile metabolites indicated putative cross-talk between transient hypoxaemia, oxidative stress, hypercarbia, vasoconstriction, altered systemic microbial activity, energy homeostasis, compartmental storage and washout. FFP2 masks had a more pronounced effect than surgical masks. Older adults were more vulnerable to FFP2 mask-induced hypercarbia, arterial oxygen decline, blood pressure fluctuations and concomitant physiological and metabolic effects.

Introduction

Since early 2020, face masks have gradually become an integral part of our new-normal lifestyle as a component of the public health and social measures employed during the current coronavirus disease 2019 (COVID-19) pandemic [1, 2]. During the second wave of the pandemic, use of surgical and/or FFP2/N95/KN95 masks were strictly mandatory attributes while in public. National and/or global policymakers have



recommended even adapting FFP3 masks for further protection considering the emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants [3, 4]. In Germany, the government has recommended the use of FFP2 masks for up to 75 min at a stretch, and surgical masks at all times in public; the same guidelines are applied to school-attending children and individuals aged >60 years [5]. A recent meta-analysis demonstrated nearly 70% reduction of infection risk in healthcare workers and an overall reduction of infection risk [6]. Nevertheless, this systemic review was based on only case-control studies, which were not adjusted for bundled approach and aerosol-generating procedures. Conversely, a randomised controlled trial demonstrated that nonoccupational use ($\sim 3 \text{ h} \cdot \text{day}^{-1}$) of surgical masks in adults along with some degree of social distancing did not reduce transmission [7]. In the last week of January 2021, Austria and Bavaria (south-east Germany) mandated respirator masks (FFP2 or KN95) in stores and on public transport. Although FFP2 masks were recommended to all members of the general public, there was no association with any preventive effect during explosive third waves (and death toll) of COVID-19 in Austria and Bavaria during the spring of 2021. In line with that, a recent analysis of cases and fatalities in western European countries without mask mandates could not find increases in numbers of infections or deaths in countries adopting generalised mask mandates [8].

While being recommended as protective against COVID-19 transmission, masks are inducing variable side-effects on our cardiorespiratory physiology [9–11], bronchopulmonary gas-exchange [12] and *in vivo* metabolic processes [13, 14]. Studies have shown effects of surgical masks on cardiopulmonary parameters, oxygen (O_2)–carbon dioxide (CO_2) homeostasis, blood pH and thermoregulation [15]. Additionally, studies have shown that conditions such as resistive breathing and/or hypoxia-driven hyperventilation, respiratory alkalosis and increased oxidative stress could cause immediate immune suppression [16–19] and might lead to metabolic alkalosis [20].

Studies have indicated both complementary and/or conflicting results on the side-effects of different masks. CHAN *et al.* [21] reported near-zero impact of nonmedical cloth masks and surgical masks on peripheral oxygen saturation (S_{pO_2}) and CO_2 tension in 50 young adults during sitting and brisk walking for 10 min and minimal effects of nonmedical masks on S_{pO_2} (self-monitored by subjects) in 25 older adults (>65 years) for 1 h in community settings. RHEE *et al.* [22] have demonstrated significant increases in CO_2 (measured by nasal canula) concentrations in 11 healthy subjects within 15 min under FFP2 conditions, which remained within National Institute for Occupational Safety and Health limits for short-term use, but exceeded the long-term exposure threshold of 0.5%. BLAD *et al.* [23] could not find considerable differences in inhaled CO_2 within mask space (of medical masks) while experimenting *via* breathing simulator, particle generator and manikin head, but they found a rise in CO_2 rebreathing of $\sim 10\,000$ ppm (1% CO_2) overall. In contrast, a comprehensive review by KISIELINSKI *et al.* [15] has demonstrated a significantly measurable ($p < 0.05$) decrease in O_2 saturation under fabric, surgical and N95 masks in 17%, 22% and 44% of studies, respectively. They hypothesised a mask-induced exhaustion syndrome (MIES) which refers to consistent, recurrent and uniform presentation of psychological and physical deterioration and symptoms from multidisciplinary observations. In other studies, blood O_2 levels dropped significantly ($p < 0.05$ and $p < 0.01$) below lower limits, with S_{pO_2} values ranging from 92.1% to 93.2% in mask users compared to values from individuals without masks, ranging from 95.8% to 97.6% [24–26].

Other human studies have demonstrated clinically concerning side-effects of FFP2 masks on at-risk populations suffering from COPD and other lung conditions, patients undergoing dialysis as well as significant physiometabolic effects on pregnant women and/or healthcare workers and on healthy subjects doing exercise and/or intensive athletics. Those effects triggered compensatory responses, *e.g.* mild increase in heart rate and increase in the rate of perceived exertion in trained young athletes, reduction in exercise capacity [10] and dyspnoea during short walk [27] in healthy nonathlete adults, significant respiratory compromise in mild COPD, asthma, chronic rhinitis [28] and severe obstructive lung disease [29] patients. During the 2002–2004 SARS outbreak, effects of N95 masks were reported on 39 end-stage renal disease (ESRD) patients, undergoing 4 h of haemodialysis. While dyspnoea was common under N95 masks, during haemodialysis, 70% of patients had significant reduction in arterial oxygen partial pressure, 19% had reached various degrees of hypoxaemia, 11 patients experienced chest discomfort and 17 patients had respiratory distress [30]. Recent analysis on 4747 ESRD patients (1925 on haemodialysis) depicted that universal use of surgical masks in haemodialysis units along with other preventive measures were effective against SARS-CoV-2 transmission in the Republic of Ireland [31]. Pregnancy-driven physiological changes in normal breathing patterns (uplift of diaphragm), increase in O_2 , nutrient and energy demand (by the developing embryo) and efforts to eliminate additional CO_2 (from fetal respiration) in the mid–end phase of gestation often leads to a higher breathing rate (physiological hyperventilation) and increased cardiac output as principal respiratory compensation phenomena. Recent studies have shown significant compromise in such compensation under N95 masks. Prospective observations on 297 pregnant

women (37–41 weeks of gestation) have demonstrated moderate (S_{pO_2} up to 93%) and major ($S_{pO_2} < 92\%$) decreases in S_{pO_2} under surgical and N95 respirators, respectively [32]. Consequently, N95 respirators were removed to recalibrate the oxygen levels in those cases. Similar observations from a controlled trial on healthy pregnant healthcare workers (27–32 weeks' gestation) have shown significant reductions in tidal volume, minute ventilation (without significant change in respiratory rate (f_R)), O_2 consumption and CO_2 production under N95 masks [33]. Such effects were more pronounced under low-intensity work (3 metabolic equivalents), which also reduced exhaled O_2 exhalation by 3.2% and increased CO_2 exhalation by 8.9%. A recent pilot observation of mask-driven cardiopulmonary effects in 12 healthy subjects (age 40.8 ± 12.4 years) at rest and during exercise are interpreted as significant, but modest [11].

However, those studies could not offer an insight into metabolic changes at the downstream level. In order to understand the immediate physiometabolic effects of face masks, we need to monitor continuous changes in metabolic markers along with simultaneous changes in respiratory and haemodynamic parameters. In this context, high-resolution mass-spectrometry based real-time analysis of exhaled volatile organic compounds (VOCs) could offer a unique insight into the body's immediate physiological [34–38] and metabolic [39, 40] status. Several studies report on the potential of VOC analysis for SARS-CoV-2 detection, but data on the influence of masks on these profiles are missing. Continuous and breath-resolved measurements allow us to track changes in exhaled metabolic markers over the durations of mask use. As endogenous VOCs are known to originate from metabolic pathways and are influenced by physiological processes, changes in exhaled concentrations due to mask wearing could indicate effects of physiometabolic attributes. Combining VOC profiling with simultaneous pulse oximetry, capnography and haemodynamic monitoring could enable a broader unconventional understanding of clinically relevant effects of face masks.

We applied online high-resolution mass-spectrometry (*i.e.* proton transfer reaction (PTR) time-of-flight (ToF) mass spectrometry (MS))-based breathomics in parallel with noninvasive measurements of S_{pO_2} , f_R , end-tidal CO_2 partial pressure (P_{ETCO_2}), exhaled humidity and oxygen, cardiac output, stroke volume, pulse rate and blood pressure. The physiometabolic side-effects of FFP2 and surgical masks over 15–30 min in healthy human subjects aged 20–80 years are addressed in detail. Effects of wearing duration and age from both mask types are compared.

Methods

Human subjects

All experiments in this pilot study were conducted according to the amended Declaration of Helsinki guidelines and signed informed consent from 30 subjects (aged 20–80 years) were obtained (approval number A2021-0012, issued by the institutional ethics committee of University Medicine Rostock, Germany) prior to inclusion. Inclusion criteria specified adults (male and female) aged up to 80 years. Exclusion criteria specified that included subjects were not suffering from any acute diseases/health condition (during the 6 months before participation) and were not undertaking any special diet and/or medication during inclusion. Among the subjects aged >60 years, three had mild COPD (one male and two females) and two (females) had chronic bronchitis in the past. At the time of inclusion, these five participants had no symptoms, pathological findings or ongoing medications. They had described and confirmed their good health condition (over the past ≥ 1 year).

Determination of sample size

We applied ANOVA for calculation of sample size. For a minimum detectable difference in mean substance intensities of 450 counts per second, a standard deviation of 300 was estimated. To attain an α -value of 0.005 and a test power of 0.99, two experimental groups, considering a population of 100 000, required a sample size of 26 (with minimal group size of ≥ 10 each). In this study, we included 30 subjects for analysis in order to detect $<5\%$ differences in exhaled VOCs up to low parts per trillion by volume levels.

Assignment of groups

We divided the study population in two groups, namely young to middle-aged adults (aged <60 years) and older adults (aged ≥ 60 years). Anthropometric data were confirmed by participants during inclusion and are presented in table 1.

Experimental setup

Three devices were synchronised for real-time measurements of several parameters simultaneously (figure 1): continuous monitoring of breath VOCs, O_2 , CO_2 and humidity *via* PTR-ToF-MS; noninvasive

TABLE 1 Anthropometric information of subjects

	Subjects (n)	Age (years)	BMI ($\text{kg}\cdot\text{m}^{-2}$)	Lifestyle habits/life events					
				Smoking	Alcoholic	Special diet	Acute, chronic disease/ medication/past history	Contraception	Pregnancy
Young to middle-aged adults									
Male	8	20–59	18–25	Yes (n=1) No (n=7)	No	No	No	N/A	N/A
Female	9	20–59	19–29	Yes (n=3) No (n=6)	No	No	No	No	No
Older adults									
Male	7	60–80	21–27	Yes (n=2) No (n=5)	No	No	Mild COPD (n=1) Chronic bronchitis (n=2)	N/A	N/A
Female	6	60–80	23–29	Yes (n=2) No (n=4)	No	No	Mild COPD (n=2)	N/A	N/A

Data are presented as n or range. BMI: body mass index; N/A; not applicable.

measurements of haemodynamic parameters *via* volume clamp method; and S_{pO_2} monitoring *via* pulse oximetry. Mainstream capnography (for P_{ETCO_2}) was performed immediately before and after mask use. Data acquisition was initiated in parallel.

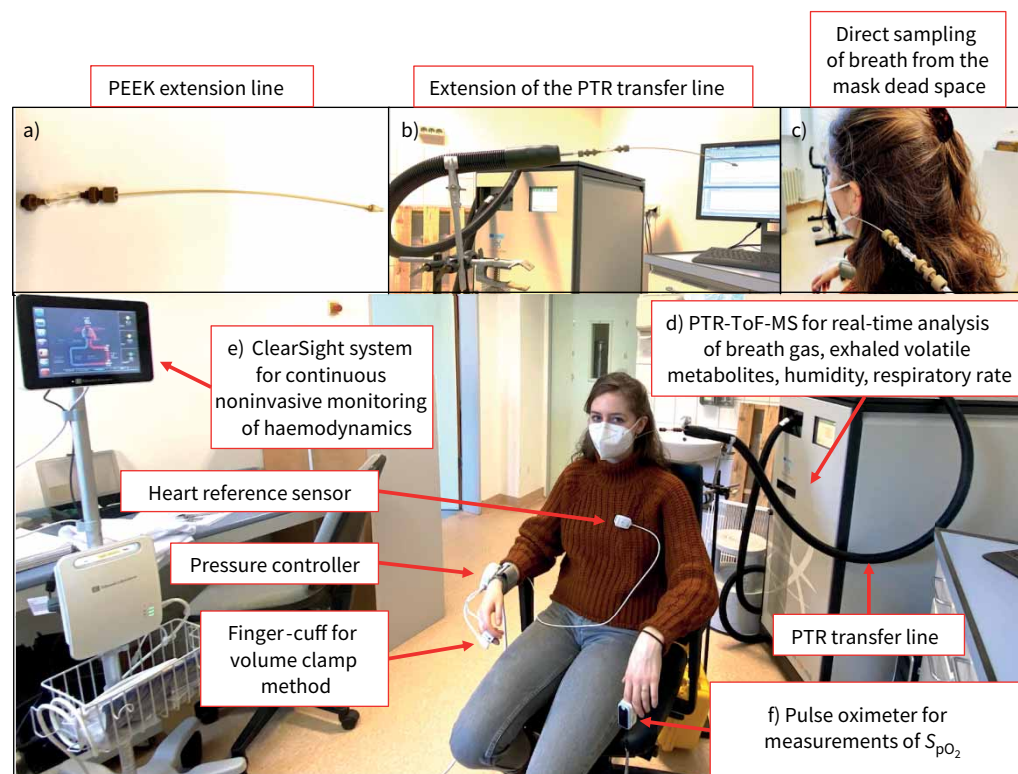


FIGURE 1 Instrumentation and experimental setup of the study. a,b) Customised polyetheretherketone (PEEK) extension of the proton transfer reaction (PTR) transfer line for c) direct sampling from the mask dead space. d) Continuous real-time breathomics *via* PTR-time-of-flight (ToF)-mass spectrometry (MS) in parallel with continuous noninvasive monitoring of e) haemodynamics *via* the ClearSight system and f) pulse oximetry. S_{pO_2} : peripheral oxygen saturation.

Breath sampling protocol

Volunteers rested by sitting on a chair for ≥ 10 min before actual sampling. Each participant was instructed to maintain the sitting posture [41] and then wore a face mask to breathe only by mouth. They spontaneously inhaled and exhaled only *via* the mouth [42].

The transfer-line of PTR-ToF-MS was connected (*via* polyetheretherketone (PEEK) finger-tight fittings) to a PEEK extension line (*i.e.* 30 cm long, with an outer diameter of 1 mm and inner diameter of 0.75 mm) in order to directly sample breath-resolved VOCs from the mask dead space (figure 1). The PTR transfer line was fixed (*via* metal clamps) at the back of subject's head (at a level below the left/right earlobe). The PEEK line was placed along the subject's right/left cheek (following the maxillary line) and was inserted within the mask dead space up to the front of the subject's lips. The tip of this sampling line was encased within a conical PEEK ferrule in order to avoid any unwanted contact with mask surface or with subject's lips. These extension lines were sterilised for reuse.

In each volunteer, measurements with two different masks (FFP2 and surgical) were conducted on two consecutive days and at the same time. The recruitment of subjects in FFP2 and surgical mask experiments were at random. Some subjects participated in the FFP2 mask experiment on the first day and others participated in the surgical mask experiment. Young to middle-aged adults were measured for 30 min and older adults were measured for 15 min. The measurements in older adults were stopped once they attained $S_{pO_2} < 94\%$.

PTR-ToF-MS measurements of breath VOCs

Breath VOCs were measured continuously *via* a PTR-ToF-MS 8000 (Ionicon Analytik, Innsbruck, Austria) and with pre-optimised experimental conditions [36, 43], *i.e.* continuous side-stream mode of sampling *via* a 6-m heated (at 75°C) silico-steel transfer line connected to a sterile mouthpiece. A continuous sampling flow of 20 mL·min⁻¹ was applied and the time resolution of the PTR-ToF-MS measurements was 200 ms. Thus, data points were generated after every 200 ms and at each data point hundreds of compounds were measured at their trace abundances (in both expiratory and room air). The ion source current was set to 4 mA and the water flow was set to 6 mL·min⁻¹. Drift tube temperature was set to 75°C, voltage was 610 V and the pressure was 2.3 mbar. The resulting electric field/particle density (E/N) ratio was 139 Td. After every minute a new data file was recorded automatically and the mass scale was recalibrated after each run (60 s). We used the following masses for mass calibration: 21.0226 (H₃O⁺-isotope), 29.9980 (NO⁺) and 59.049 (C₃H₆O).

VOC data processing

VOCs were measured in counts per seconds and corresponding intensities were normalised onto primary ion (H₃O⁺) counts. Raw data was processed *via* PTR-MS viewer software (version 3.4). As PTR-MS continuously records both exhaled breath and inhaled room-air, the "breath tracker" algorithm (based on Matlab version 7.12.0.635, R2011a) was applied to identify expiratory and inspiratory phases [36]. Here, acetone was used as the tracker mass, as it is an endogenous substance, which has significantly higher signal intensity at expiration than inhalation. As the mass resolution of PTR-ToF-MS (4000–5000 $\Delta m m^{-1}$) can assign volatiles upon their measured mass and corresponding sum formula with high precision [42], compound names are used while discussing results. VOCs were quantified *via* multicomponent mixture of standard reference substances. Quantification under adapted sample humidity (as in exhaled breath) using a liquid calibration unit (Ionicon Analytik) is our pre-established state-of-the-art process [44].

Selection of VOCs for analysis

We considered compounds with expiratory abundances significantly above the inspiratory/room-air abundance. Out of those markers, 32 substances were selected. These VOCs are well-known breath markers in clinical breathomics and reflect different origins, physicochemical characters and dependencies on physiology, metabolism, pathology, therapy and lifestyle/habits [39, 40, 42, 45, 46]. None of these VOCs were contributed from the applied masks, as we examined the mask emissions for direct comparisons.

Continuous haemodynamic monitoring

Noninvasive measurements of haemodynamic parameters, *e.g.* cardiac output, stroke volume, pulse rate and mean arterial pressure (MAP) were performed *via* our pre-optimised setup by using the volume clamp method (ClearSight system-EV1000; Edwards Lifesciences, California, USA) [35, 41].

Mainstream capnography

Mainstream capnography was performed just before and after each mask use *via* a small portable capnograph (EMMA PN 3639; Masimo Sweden, Danderyd, Sweden) attached to a sterile breathing mouthpiece. P_{ETCO_2} values were recorded in mmHg units. Absolute values are considered from the third breath onwards, as first two to three breaths are used to calibrate the CO_2 and f_{R} sensor.

Statistical analysis

Analytical mean values (of measured parameters) from each participant were calculated over each minute of breath-resolved measurement. Data from every fifth minute were included for statistical analysis. In cases of nonparametric distribution of data, median values were considered for statistical analysis.

In order to reduce the evident intra-individual variations in measured variables, each participant was used as his/her own control. Thus, variables from each subject were normalised onto the corresponding initial values (of the first minute). Normalisation was performed separately for each mask types (FFP2 and surgical) and in each age group (young to middle-aged and older adults).

As the distribution of measured data in each group is contributed by every individual (of that group), the relative standard deviations (RSDs) in VOC abundances from each group were also calculated for each substance. The RSDs were calculated (as a percentage) by rating sample standard deviations over corresponding sample means.

Statistically significant differences within groups were assessed *via* repeated measurement ANOVA on ranks (Friedman repeated-measures ANOVA on ranks, Shapiro–Wilk test for normal distribution and *post hoc* Student–Newman–Keuls method for pairwise multiple comparisons between all groups; $p \leq 0.005$) in SigmaPlot software (version 14).

For all measured variables, from all pairwise comparisons, the differences are presented by referring to the corresponding values at the first minute of each mask use and within each age group.

In order to compare between groups (*i.e.* the effects of both mask types on both age groups), relative changes (percentage) over time (with respect to initial values) were calculated for selected variables in each group. Here, we have selected the principal physiometabolic denominators and candidate VOCs that are potentially originating from several *in vivo* metabolic processes. Relative changes were calculated at 15th and 30th minutes in young to middle-aged adults and at the 15th minute in older adults. The changes in P_{ETCO_2} values were calculated immediately before and after mask use. In case of intergroup comparisons, one-way ANOVA was applied, due to unequal group size. All groups were compared to each other. From all pairwise comparisons between groups, the differences are presented by referring to the corresponding percentage of changes caused by FFP2 masks on older adults.

In order to understand the correlations between exhaled VOCs and physiological parameters within each mask type, dimension reduction factor analyses (factor extraction *via* the principal components method, factor scores *via* the regression method and one-tailed significance at $p \leq 0.005$) were performed in SPSS.

Results

Figure 2 shows heatmaps of relative changes (normalised mean values) of physiological parameters such as P_{ETCO_2} , S_{pO_2} , f_{R} , cardiac output, stroke volume, pulse rate, MAP and relative changes in exhaled alveolar abundances of 32 VOCs of interest during the use of FFP2 and surgical face masks by young to middle-aged and older adults. Measured variables from each volunteer were normalised onto corresponding median values from the first minute. The mean of those normalised values from every fifth minute is presented in the heatmaps. P_{ETCO_2} values are depicted from immediately before and after the mask use and are placed at the first and final minute of heatmaps for direct comparisons. The changes in RSDs of all measured parameters are presented as heatmaps in supplementary figure S1.

Figure 3 depicts absolute or normalised values of physiological parameters and of alveolar concentrations of exhaled VOCs in every fifth minute (starting from the first minute) in four groups. The first two groups consist of data from FFP2 masks on young to middle-aged and older adults and the later two groups contain data from surgical masks on young to middle-aged and older adults. P_{ETCO_2} values are presented from immediately before and after the use of masks. Figure 3a represents the physiological parameters absolute values (with units) of S_{pO_2} , P_{ETCO_2} and f_{R} and normalised haemodynamics values. Figure 3b represents aliphatic aldehydes and organosulfur; figure 3c represents hemiterpene, ketone and smoking-/environment-related VOCs, exhaled humidity and oxygen; and figure 3d represents aliphatic acids,

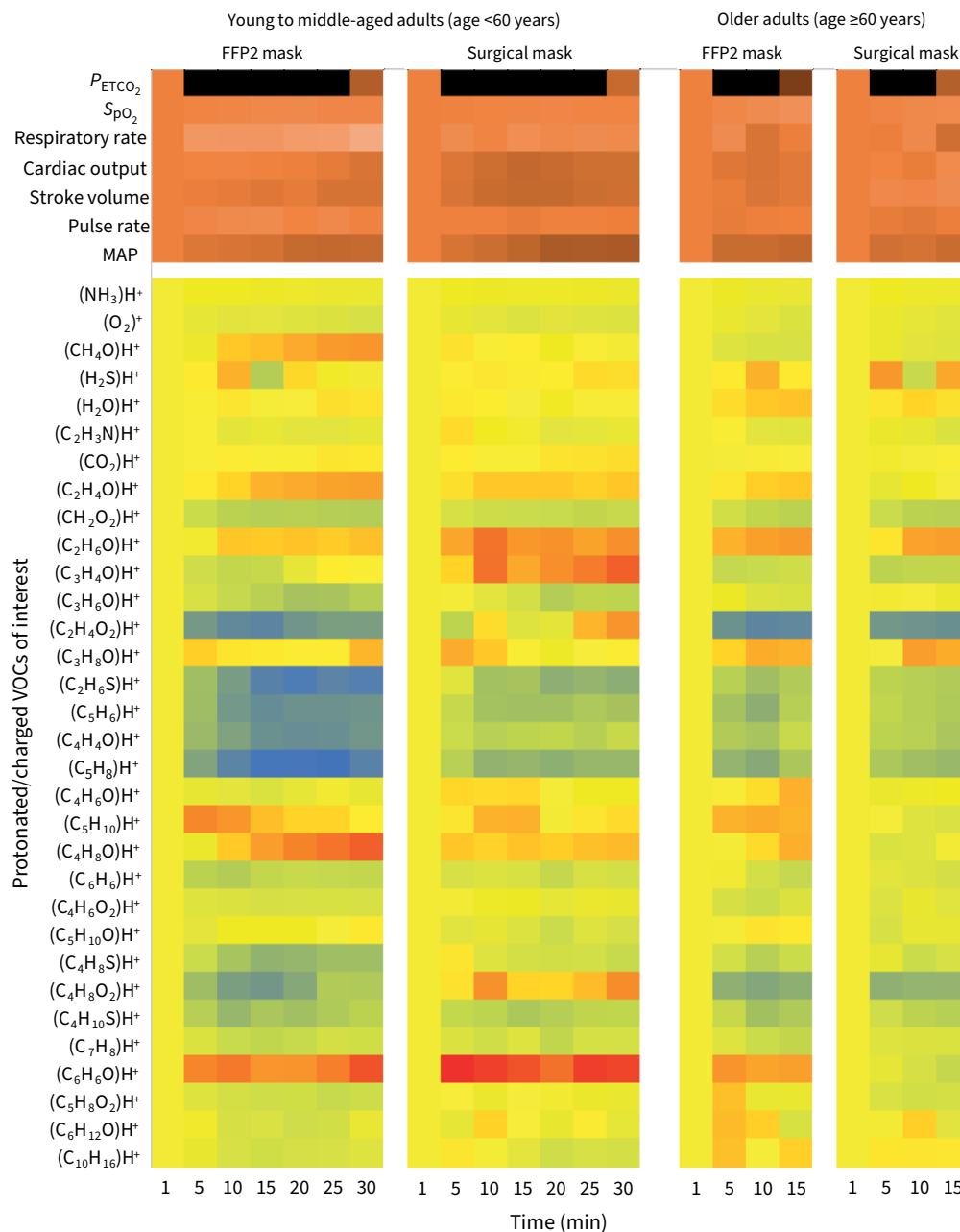


FIGURE 2 Relative changes in normalised mean values of physiological parameters and of exhaled alveolar volatile organic compound (VOC) abundances during the use of coronavirus disease 2019 protective face masks by young to middle-aged and older adults. The y-axis represents the physiological parameters end-tidal carbon dioxide tension (P_{ETCO_2}), peripheral oxygen saturation (S_{pO_2}), respiratory rate, cardiac output, stroke volume, pulse rate, mean arterial pressure (MAP) values and the protonated/charged VOCs of interest. VOCs were tentatively identified according to their mass/charge ratio. For each individual, VOC data were normalised onto corresponding median values from the first minute. Likewise, respiratory and haemodynamic parameters and S_{pO_2} were normalised; the means of those normalised values from every fifth minute are presented. Only P_{ETCO_2} values are presented from immediately before and after mask use and are placed at the first and final minutes of the heatmaps. Red and blue colours symbolise relatively higher and lower abundances of VOCs, respectively. Similarly, dark and light brown colours symbolise relatively higher and lower values of physiological parameters, respectively. In case of P_{ETCO_2} , the points without any measurements are coloured in black.

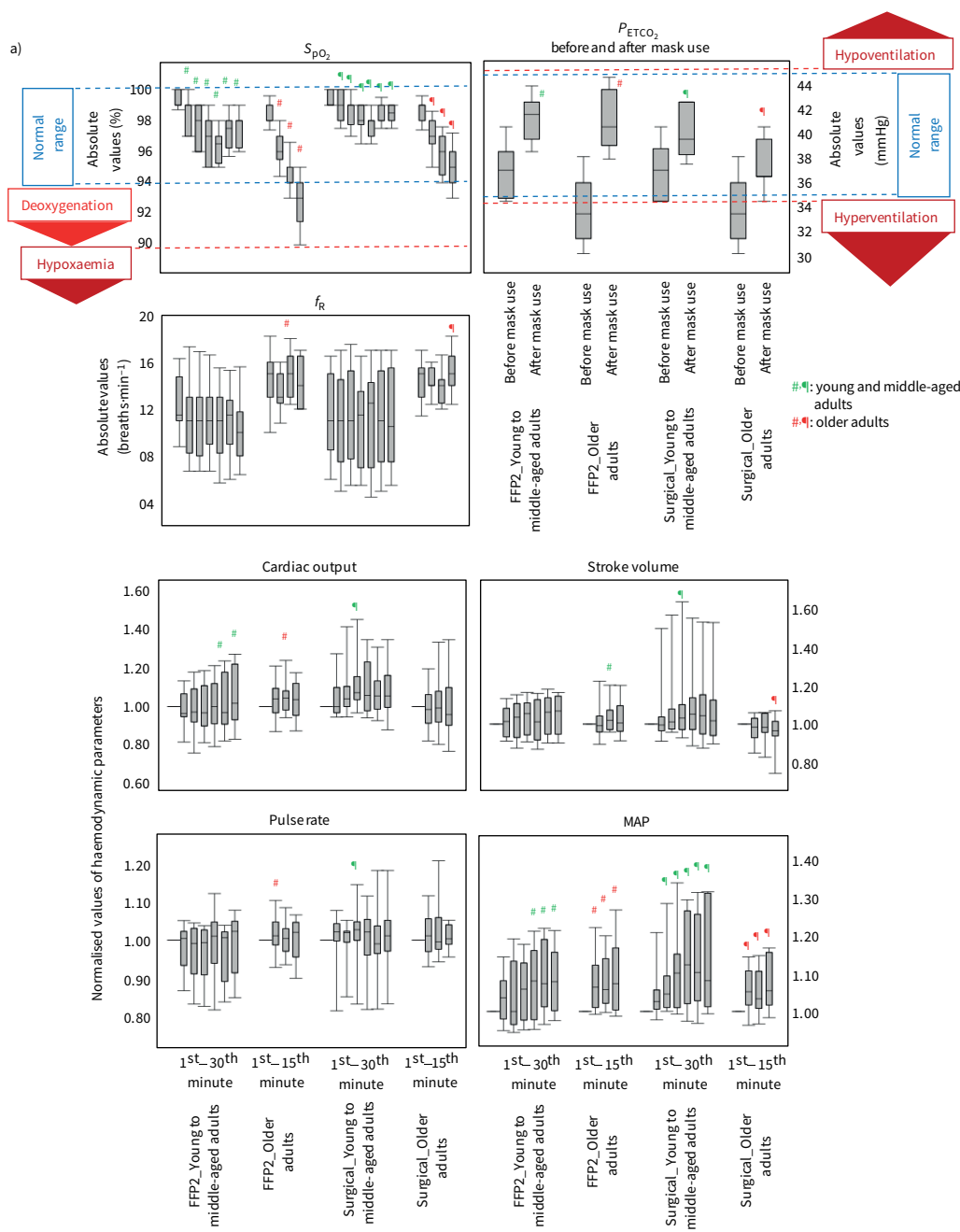


FIGURE 3 Comparison of differences in physiological parameters and in exhaled alveolar volatile organic compound (VOC) concentrations during the use of coronavirus disease 2019-protective face masks by young to middle-aged and older adults. **a)** physiological parameters; **b)** aliphatic aldehydes and organosulfur; **c)** hemiterpene, ketone, nitrile, aromatics, exhaled humidity and oxygen; and **d)** aliphatic acids, alcohols and monoterpene. The x-axes represent measurement time in four groups: two mask types (FFP2 and surgical) used by two age cohorts (young to middle-aged and older adults). The y-axes represent absolute values (with units) or normalised (onto corresponding initial values) values of measured parameters from every fifth minute, starting from the first minute. For both mask types, young to middle-aged and older adults were measured for 30 min and 15 min, respectively. End-tidal carbon dioxide tension (P_{ETCO_2}) values were measured immediately before and after the use of masks and absolute values (with units) are presented. Measured values within each group were compared. P_{ETCO_2} values before and after mask use are compared statistically. Statistical significance was tested by means of repeated measurement ANOVA on ranks ($p \leq 0.005$). From all pairwise-multiple comparisons, statistically significant differences with respect to the “first minute” are indicated as follows. #: FFP2 masks; ¶: surgical masks.

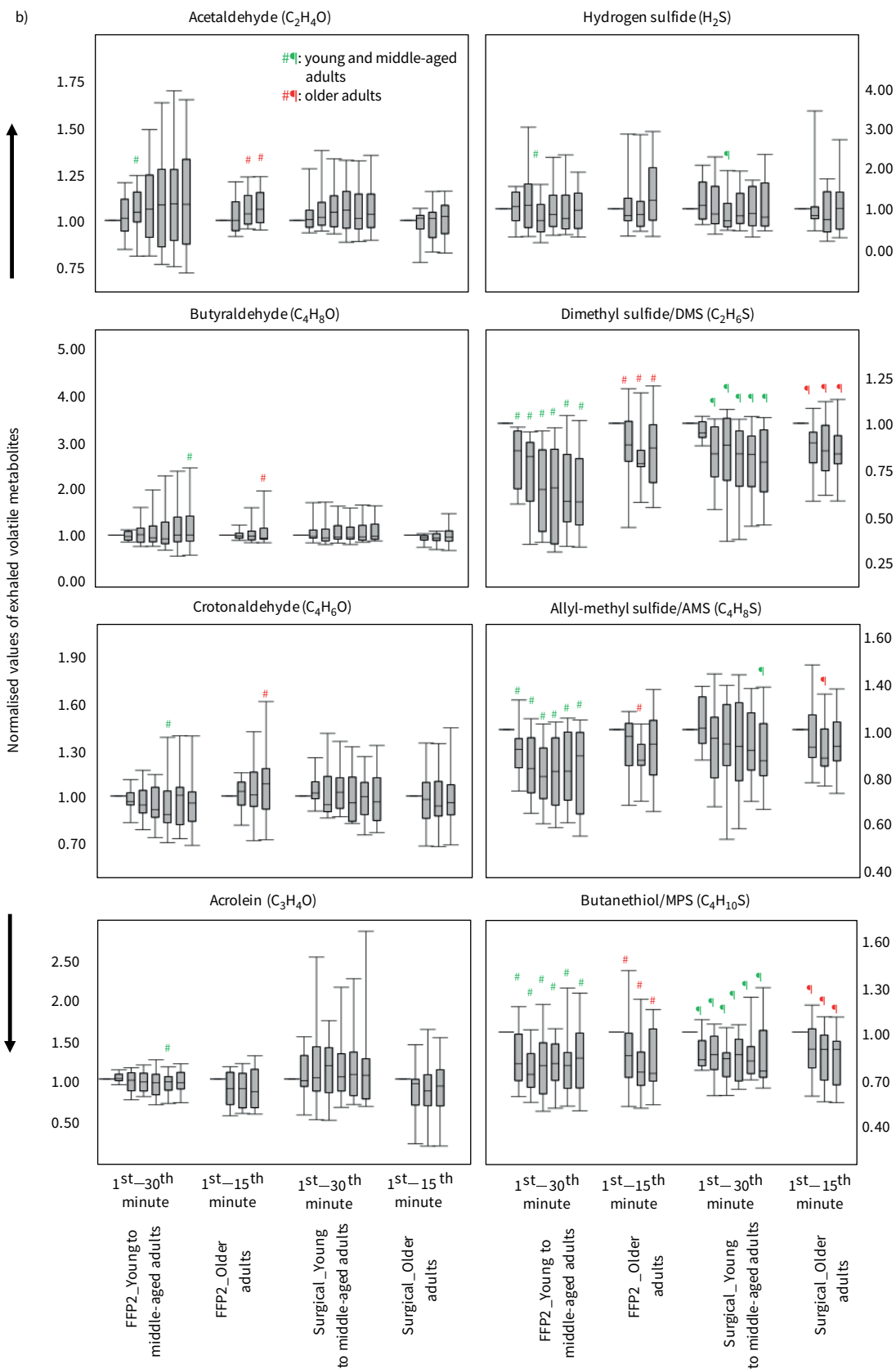


FIGURE 3 continued

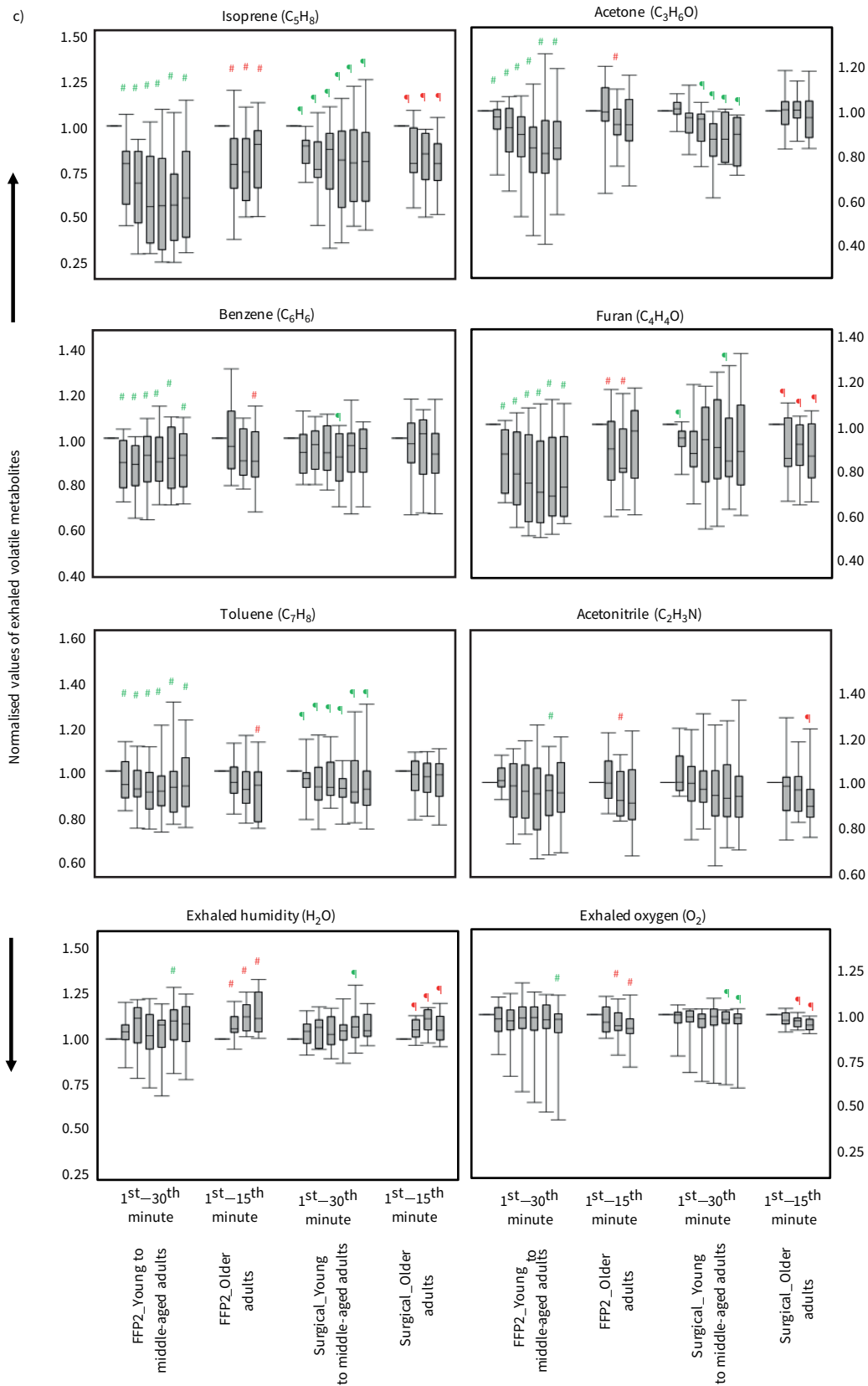


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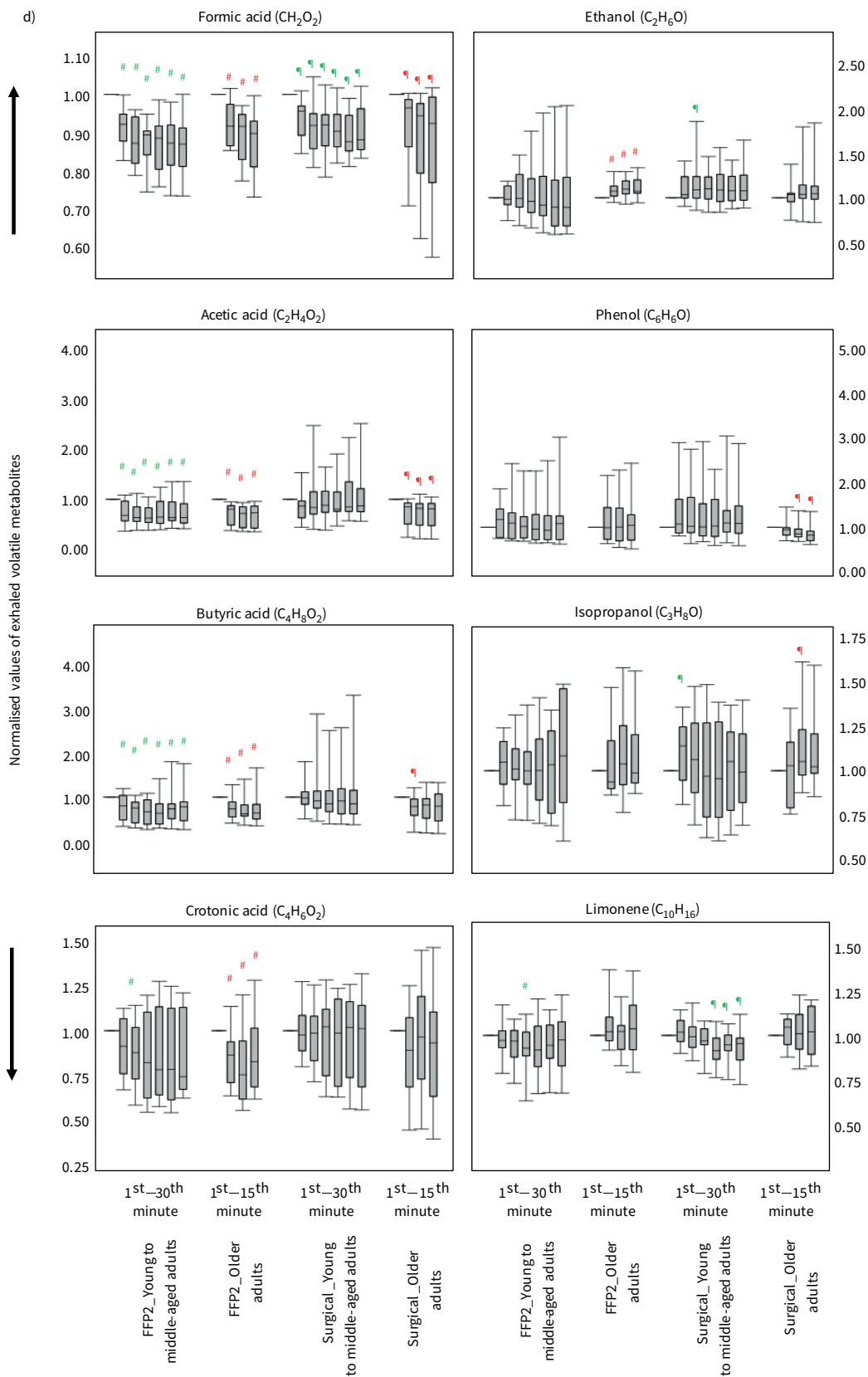


FIGURE 3 continued

alcohols and monoterpene. Absolute values are only considered for parameters, which are less likely to be affected by inter- or intra-day variations within each individual. From all pairwise comparisons, the differential expressions (statistically significant at $p \leq 0.005$) in each variable within each group is indicated with respect to the corresponding “first minute” of measurement.

The correlation coefficients and respective p-values between physiological parameters and VOCs of interest are presented in table 2 and between relevant physiological parameters are presented in table 3. Detailed inter-VOC correlations (with respect to physiological parameters) along with corresponding p-values are presented in supplementary tables S1 and S2. It is noteworthy that cardiac output showed strong positive correlation to stroke volume under both masks, whereas pulse rate remained only moderately related to cardiac output under FFP2 conditions. Cardiac output, stroke volume and pulse rate showed relatively higher variations under the surgical masks, whereas pulse rate remained completely unrelated to cardiac output. While f_R remained unrelated to other physiological parameters under both masks, MAP has shown significant and moderate negative correlations to S_{pO_2} only under FFP2 masks and good positive correlations to cardiac output and stroke volume under surgical masks.

Figure 4 depicts comparison of relative changes (percentages) in physiological parameters and in selected metabolic breath markers within four groups: FFP2 masks on young to middle-aged and older adults and surgical masks on young to middle-aged and older adults. Relative changes (with respect to initial values) in young to middle-aged adults at the 15th and 30th minutes and in older adults at the 15th minute are presented. For P_{ETCO_2} , relative changes between measured values immediately before and after use of masks are presented. Figure 4a presents physiometabolic parameters and figure 4b presents exhaled alveolar volatile organic metabolites. From all pairwise-multiple comparisons, statistically significant differences are indicated with respect to changes caused by FFP2 masks on older adults. Statistical significance (with corresponding p-values) of differences in all variables between groups are indicated with respect to the “15th minute in older adults with FFP2 mask” in supplementary table S3.

Discussion

FFP2 and surgical face masks immediately affected physiological and metabolic attributes. Effects progressed with the course of mask-wearing time. The most pronounced effects were observed with FFP2 masks, and especially in older adults. Profound and consistent decreases in S_{pO_2} and increases in P_{ETCO_2} indicated hypercapnia-induced right shift of the oxyhaemoglobin saturation curve in all subjects, which is caused mainly due to rebreathing of CO_2 [22, 47–49] from mask dead space and a change in normal breathing patterns. Older adults with past history of mild COPD and/or chronic bronchitis were not solely responsible for the most reduced S_{pO_2} and/or most increased P_{ETCO_2} . Despite significant increases in f_R (as compensatory responses to increased arterial CO_2 partial pressure) in older adults, P_{ETCO_2} increased most significantly after 15 min of FFP2 mask-wearing in our setup, indicating compromised respiratory compensation. Notably, at the end of participation, our volunteers described altered breathing patterns and respiratory discomfort as their breathing experiences under both masks, and more pronouncedly under FFP2 masks. Deeper and slower inhalation and exhalation patterns (slow breathing) were experienced by young to middle-aged adults, whereas the older adults described breathing resistance, dyspnoea and relatively faster breathing with deeper inhalations (*i.e.* extended inspiratory time) and inspiratory efforts (*i.e.* shallow/thoracic breathing) under FFP2 masks. Although we could not conduct breath-resolved spirometry and capnography within the mask space, the aforementioned facts along with observed significant decrease in O_2 exhalation and constant increase in exhaled humidity suggest hypercapnia-like effects (profound increase in P_{ETCO_2}) due to rebreathing from mask dead space. Haemodynamic parameters such as cardiac output and MAP changed in counter (homeostatic)-response/secondary to those respiratory effects. Due to the decline in arterial O_2 saturation, cardiac output increases as a physiological response to counterbalance the biological oxygen demand. PHILLIPS *et al* [50] demonstrated a dose-response increase in cardiac output under acute isocapnic hypoxaemia in healthy adults. Correlations (table 3) indicated that stroke volume was the principal determinant of cardiac output under both masks. During spontaneous breathing, changes in intrathoracic pressure (*i.e.* secondary to negative pressure ventilation) gives rise to stroke volume variations (SVVs) *via* rise and fall in arterial pulse pressure at expiration and inspiration, respectively. As face masks induce additional (and variable according to mask fittings and tightness) upper-airway resistance against breathing and change normal breathing patterns, high SVVs are reasonable within our setup. Previously, we observed high SVVs while applying upper-airway resistances against breathing *via* reduced mouthpiece diameters [51]. The recent MIES hypothesis [15] incorporates 28 different parameters (from 44 research studies) out of which S_{pO_2} , P_{ETCO_2} , humidity, f_R , blood pressure and pulse rate are in common with our study. f_R and pulse rate only increased in older adults and were more pronounced with the use of FFP2. Other common physiological parameters changed in agreement with MIES by underlining the hypoxaemia- and hypercapnia-like effects, especially in older adults under FFP2

TABLE 2 Correlation (obtained *via* factor analysis) between physiological parameters and selected volatile organic compounds (VOCs) of interest

	FFP2 mask						Surgical mask					
	Cardiac output	Stroke volume	Pulse rate	MAP	f_R	S_{pO_2}	Cardiac output	Stroke volume	Pulse rate	MAP	f_R	S_{pO_2}
Isoprene												
R-value	0.11	-0.15	0.33	-0.02	0.38	0.18	-0.39	-0.39	0.14	-0.34	-0.23	0.08
p-value	0.099	0.037	0.000	0.395	0.001	0.018	0.000	0.000	0.064	0.000	0.006	0.184
Acetone												
R-value	0.03	-0.25	0.31	0.12	0.27	0.10	-0.28	-0.3	0.1	-0.46	-0.04	-0.09
p-value	0.380	0.002	0.000	0.085	0.001	0.126	0.001	0.000	0.139	0.000	0.337	0.172
Acetaldehyde												
R-value	0.05	-0.14	0.28	-0.25	-0.20	-0.05	-0.16	-0.11	0.03	0.19	0.02	0.07
p-value	0.288	0.047	0.000	0.002	0.009	0.268	0.044	0.111	0.373	0.020	0.395	0.212
Butyraldehyde												
R-value	-0.04	-0.15	0.15	-0.15	-0.10	-0.01	-0.24	-0.23	0.16	0.2	-0.05	0.13
p-value	0.312	0.037	0.042	0.045	0.115	0.461	0.005	0.005	0.045	0.016	0.288	0.078
Dimethyl sulfide												
R-value	0.17	-0.14	0.38	-0.03	0.33	0.15	-0.29	-0.34	0.16	-0.38	-0.25	0.07
p-value	0.027	0.051	0.000	0.373	0.000	0.043	0.001	0.000	0.045	0.000	0.003	0.229
Butanethiol												
R-value	-0.10	-0.13	0.07	-0.02	-0.13	0.33	-0.32	-0.11	-0.23	-0.26	-0.05	0.14
p-value	0.116	0.059	0.222	0.409	0.072	0.000	0.000	0.108	0.006	0.002	0.279	0.059
Acetic acid												
R-value	-0.14	-0.03	-0.15	-0.02	0.08	0.41	-0.16	-0.09	-0.09	-0.13	-0.2	0.2
p-value	0.051	0.355	0.039	0.390	0.190	0.000	0.041	0.171	0.162	0.074	0.016	0.015
Butyric acid												
R-value	0.11	0.01	0.14	0.02	0.12	0.20	-0.14	-0.09	-0.07	-0.15	-0.31	0.12
p-value	0.093	0.437	0.055	0.393	0.090	0.009	0.068	0.177	0.217	0.053	0.000	0.101
Ethanol												
R-value	0.082	-0.18	0.35	-0.17	-0.11	-0.04	-0.30	-0.27	0.09	0.05	0.03	-0.08
p-value	0.171	0.019	0.000	0.023	0.107	0.313	0.000	0.002	0.166	0.298	0.375	0.204
Limonene												
R-value	-0.04	-0.21	0.20	0.15	0.41	0.00	-0.02	-0.16	-0.02	-0.46	-0.01	-0.21
p-value	0.326	0.007	0.011	0.045	0.000	0.488	0.013	0.043	0.411	0.000	0.465	0.011
Furan												
R-value	0.09	-0.20	0.38	-0.03	0.27	0.13	-0.40	-0.40	0.16	-0.28	-0.27	0.06
p-value	0.139	0.009	0.000	0.381	0.001	0.067	0.000	0.000	0.044	0.001	0.001	0.245
Exhaled O₂												
R-value	0.10	-0.22	0.33	0.12	0.09	0.08	-0.62	-0.83	0.59	-0.62	-0.13	0.11
p-value	0.132	0.005	0.000	0.089	0.145	0.173	0.000	0.000	0.000	0.000	0.083	0.127
Exhaled H₂O												
R-value	-0.01	-0.15	0.13	0.31	0.28	-0.17	-0.11	-0.21	0.19	-0.02	-0.25	-0.29
p-value	0.448	0.041	0.065	0.000	0.000	0.023	0.122	0.010	0.021	0.428	0.003	0.001

Correlation coefficients (R-value) along with corresponding p-values are listed. Statistically significant ($p \leq 0.005$) correlations are assigned in bold. Values "0.000" represent $p < 0.001$. MAP: mean arterial pressure; f_R : respiratory frequency; S_{pO_2} : peripheral oxygen saturation; O₂: oxygen; H₂O: water vapour.

conditions. Observed short-term effects gave rise to substantial concern about immediate and long-term clinical significance. Our study has not only provided support for MIES, but has also advanced the existing hypothesis by extending the side-effects onto exhaled metabolites. Irrespective of the origins, significant and substance-specific changes in exhalation of many blood-borne volatile metabolites took place within minutes. Some changes mirrored the profiles of oxygen saturation, haemodynamics and respiratory parameters. Exhaled oxygen decreased while breath humidity increased over time. Exhalation profiles of potentially endogenous VOCs (aldehydes, hemiterpene, organosulfur, alcohols, ketones and short-chain fatty acids; related to certain metabolic pathways and/or physiological processes) have indicated *in vivo* physiometabolic cross-talk (*i.e.* complementary and/or counter-overlaps) between transient hypoxaemia and oxidative stress, deteriorating ventilation and compartmental vasoconstriction, altered systemic bacterial activity and energy homeostasis. Exhalation of exogenous aromatics, nitriles and monoterpenes were mainly related to pre-exposures and lifestyle.

TABLE 3 Correlation (obtained *via* factor analysis) between physiological parameters of interest

	FFP2 mask						Surgical mask					
	Cardiac output	Stroke volume	Pulse rate	MAP	f_R	S_{pO_2}	Cardiac output	Stroke volume	Pulse rate	MAP	f_R	S_{pO_2}
Cardiac output												
R-value	NA	0.71	0.45	-0.03	-0.02	-0.05	NA	0.84	-0.16	0.53	-0.04	0.007
p-value	NA	0.000	0.000	0.377	0.396	0.290	NA	0.000	0.043	0.000	0.317	0.472
Stroke volume												
R-value	0.71	NA	-0.38	0.03	0.13	-0.05	0.84	NA	-0.66	0.52	0.05	0.01
p-value	0.000	NA	0.000	0.363	0.067	0.295	0.001	NA	0.000	0.000	0.277	0.453
Pulse rate												
R-value	0.45	-0.38	NA	-0.07	-0.19	-0.01	-0.16	-0.66	NA	-0.13	-0.14	0.005
p-value	0.000	0.000	NA	0.202	0.015	0.452	0.043	0.000	NA	0.074	0.071	0.480
MAP												
R-value	-0.03	0.03	-0.07	NA	0.22	-0.38	0.53	0.52	-0.13	NA	0.05	-0.12
p-value	0.377	0.363	0.202	NA	0.006	0.000	0.000	0.000	0.074	NA	0.288	0.095
f_R												
R-value	-0.02	0.13	-0.19	0.22	NA	-0.09	-0.04	0.05	-0.14	0.05	NA	-0.16
p-value	0.396	0.067	0.015	0.006	NA	0.150	0.317	0.277	0.071	0.288	NA	0.047
S_{pO_2}												
R-value	-0.05	-0.05	-0.01	-0.38	-0.09	NA	0.007	0.01	0.005	-0.12	-0.15	NA
p-value	0.290	0.295	0.452	0.000	0.150	NA	0.472	0.453	0.480	0.095	0.047	NA

Correlation coefficients (R-value) along with corresponding p-values are listed. Statistically significant ($p \leq 0.005$) correlations are assigned in bold. Values "0.000" denote $p < 0.001$. MAP: mean arterial pressure; f_R : respiratory frequency; S_{pO_2} : peripheral oxygen saturation; NA: not applicable.

CO₂ toxicity may arise due to rebreathing. Studies have indicated linear changes in circulatory, cardiovascular and autonomic physiology due to inspiratory CO₂ exposure at concentrations between 500 and 5000 ppm [52]. Clinical evidence has depicted that short-term CO₂ exposure, *i.e.* beginning at 1000 ppm, affects cognitive attributes including decision-making and problem-solving. While considering the rebreathing of mask dead space, side-effects of low-level CO₂ exposure on physiometabolic processes is perceivable. While looking at the CO₂ exhalation and accumulation of breath humidity over time, a systematic effect of rebreathing to increase most of the VOCs (with high aqueous solubility or high volatility) could be assumed. Nevertheless, endogenous VOCs with similar physiochemical properties behaved in contrast by clearly indicating more systemic effects on their putative *in vivo*/metabolic origins.

Hypoxia facilitates the production of reactive oxygen species (ROS) and thereby promotes acute oxidative stress [53, 54]. This further leads to lipid peroxidation and production of α, β -unsaturated aldehydes [55]. In our setup, the instant and gradual increase in endogenous aldehyde exhalations along with the decreasing S_{pO_2} in case of FFP2 masks indicates an early onset and progression of oxidative stress. Such oxidative stress was insignificant in case of surgical masks, even in older adults. Oxidative stress-driven imbalance in O₂ and ROS interplay may lead to acute cardiac dysfunction [56], DNA damage [57], oncogene activation and cancers [58]. Oxidative stress (*e.g.* under resistive breathing) is a major stimulus to cytokine induction [17] and the regulation of immune checkpoint mechanisms are O₂-dependent [18]. Thus, significant deoxygenation and hypoxic conditions deprive O₂ supply to immune cells that hinder optimal responses and gradually lead to immunosuppression. Considering the well-known effects of transient hypoxaemia-driven risk of cardiac arrhythmias [59] and/or cerebral dysfunction, the observed transient, but profound, lowering of S_{pO_2} under FFP2 masks in older adults in our study rises significant concern about the choice of mask depending on age. Crotonaldehyde is formed *via* condensation of acetaldehyde molecules in alkaline medium and thereby cannot be attributed directly to oxidative stress. Acrolein behaved differently, due to its exogenous origin from diet, smoking and/or environment [60].

While looking at the exhalation profiles of organosulfur such as dimethylsulfide, allyl methyl sulfide and butanethiol, the effects of both face mask types are reflected in the systemic origin of these substances from microbial anaerobic methylation [61] in the lower gut. Studies have shown that effects of hypoxia act as an "invisible pusher" of gut microbiota [62]. Gut flora maintain the hypoxic balance of the intestinal environment in order to regulate nutrient absorption, gut permeability and immune response [63]. As all

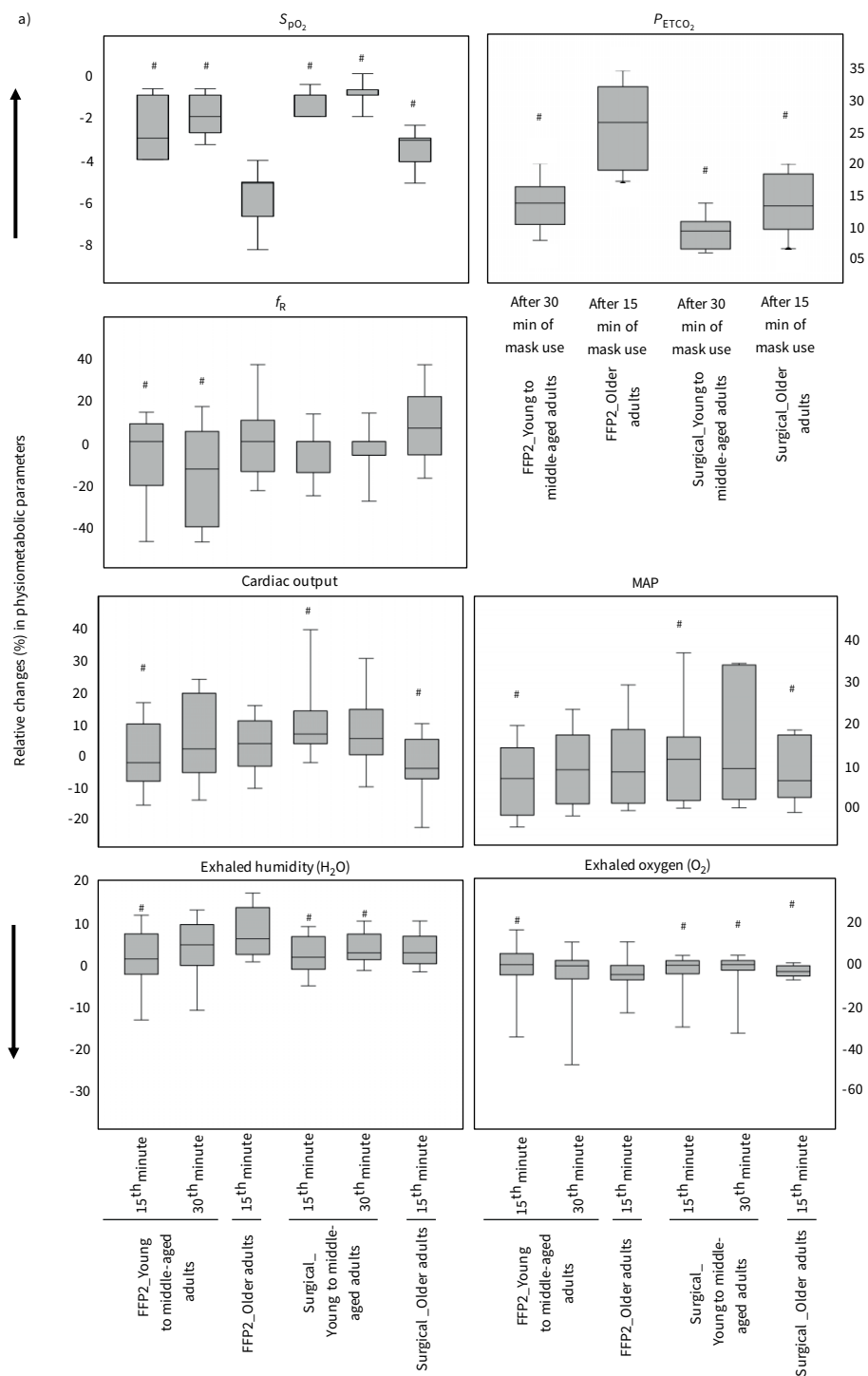


FIGURE 4 Comparison of relative changes (in %) in physiological parameters and in exhaled metabolic markers during the use of coronavirus disease 2019-protective face masks by young to middle-aged and older adults. **a)** Physiometabolic parameters and **b)** exhaled alveolar volatile organic metabolites. The x-axes present measurement time in four groups: two mask types (FFP2 and surgical) used by two age cohorts (young to middle-aged and older adults). The y-axes represent percentage changes (with respect to initial values) in measured parameters at 15th and/or 30th minutes. For both mask types, young to middle-aged and older adults were measured for 30 min and 15 min, respectively. Thus, for both mask types, changes in young to middle-aged adults at the 15th and 30th minutes are presented and the same is presented in older adults at the 15th minute. End-tidal carbon dioxide tension (P_{ETCO_2}) values are measured immediately before and after the use of masks. Measured values within each group were compared. Statistical significances were tested by means of repeated measurement-ANOVA on ranks ($p \leq 0.005$). Results from all pairwise multiple comparisons are listed in table 3. #: statistically significant differences with respect to changes caused by FFP2 mask on older adults from all pairwise multiple comparisons.

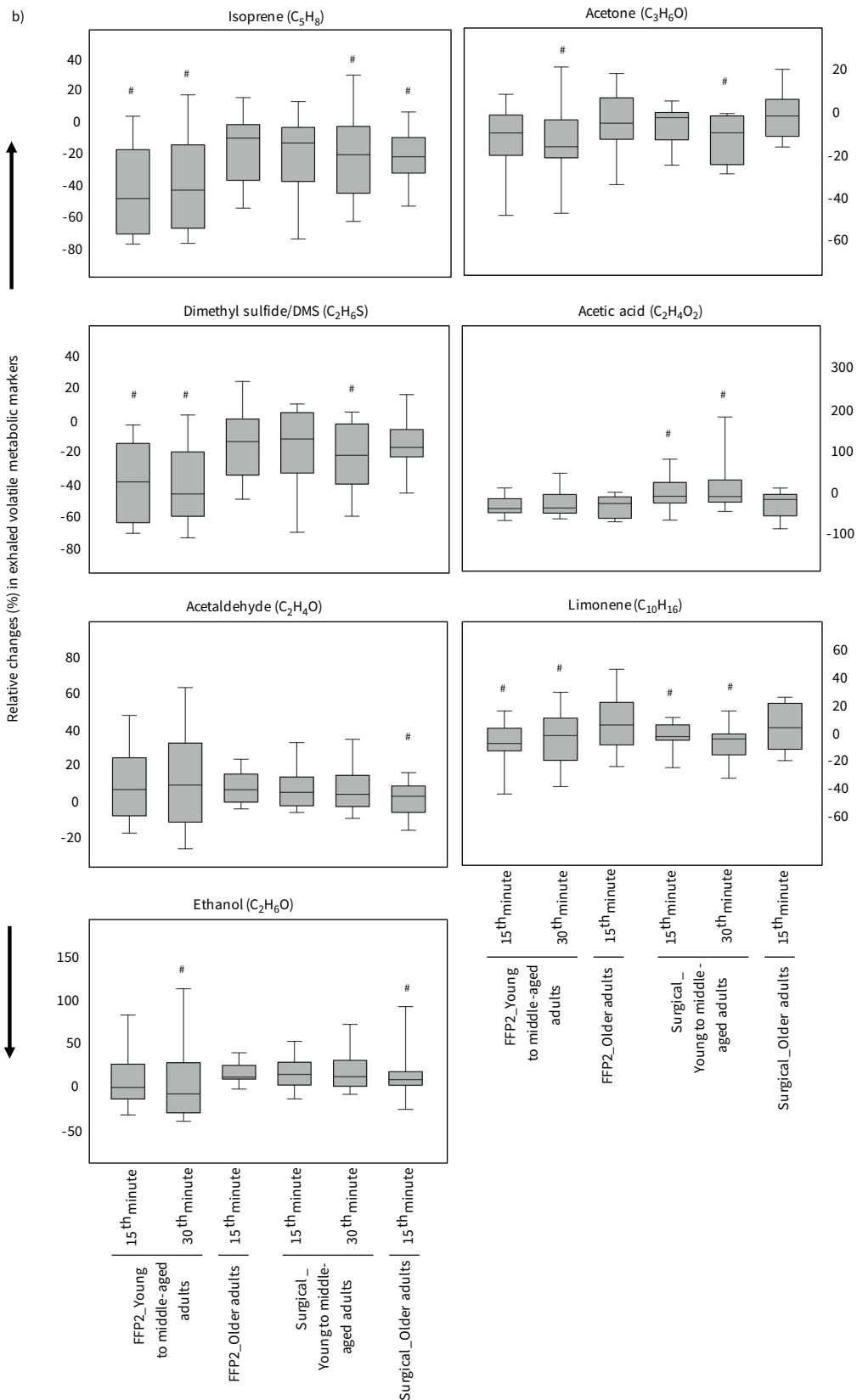


FIGURE 4 continued

types of face mask externally induce deoxygenation and are able to lead to hypoxia, the normal gut microbial activity is likely to reduce gradually, resulting in decreasing production of those organosulfurs in the colon region. Despite an increase in cardiac output, exhaled abundances of these substances decreased significantly with use of both mask types in both age groups. This could be due to the fact that the perfusion was distributed to active compartments (tissue/organs) with increased O_2 demand rather than in the gut. Due to its origin from the oral cavity bacteria [42], no systemic effects were observed in the exhalation of hydrogen sulfide.

Short-chain fatty acids (SCFAs), *e.g.* acetic acid and butyric acid, are produced by lower gut bacteria during the anaerobic lysis of primarily undigested dietary fibres and/or starch [64, 65]. Due to their origin from the large-intestine environment, exhaled profiles of SCFAs behaved as the gut originated sulphides. Further to that SCFAs plays important role in energy metabolism, plasma acid–base homeostasis and blood pressure regulation [66, 67]. As prolonged hypoxia may eventually lead to anaerobiosis and metabolic acidosis (lowering of plasma pH) [68], SCFA production is very likely to be reduced. Crotonic acid and formic acid are potentially sourced from cosmetics and disinfectants/sanitiser, and similar to our previous observations [35, 51], those VOCs reflected washout behaviours.

Despite its origin from carbohydrate-metabolising bacteria of the intestine [69, 70], ethanol did not follow the behaviours of organosulfur or SCFAs. In contrast, ethanol exhalations tend to increase and rose most significantly in older adults with FFP2 masks by mirroring the profiles of cardiac output and MAP. Evidence has indicated hypoxic switching of metabolic routes that produce more ethanol than lactates in order to regulate blood pH levels [68]. Endogenous ethanol increases the permeability of small-intestinal epithelium and colon in order to increase glucose transport towards hepatic and cellular glycolysis [71]. A consecutive decrease in endogenous breath acetone (*i.e.* the by-product of glycolysis) [72] indicates a decline in carbohydrate metabolism and an increased demand in glucose uptake for energy metabolism. An elevated MAP and cardiac output denominate increased perfusion [73] of vital organs to aid the primary source of energy from the compartments such as the small intestine. Therefore, the increase in ethanol exhalation may be due to its signalling [74] between intestinal permeability and glucose transport to blood for maintaining energy homeostasis. The observed tendencies of various gut-originated VOCs reflect the regional diversity of systemic microflora within same organ. Phenol and isopropanol behaved differently from ethanol due to their exogenous origin from dietary intake, beverages and uptake from the ambient environment, disinfectants or sanitiser.

During spontaneous breathing in a normal sitting position, exhalation of CO_2 and endogenous isoprene [75] exhalation remain closely related to each other and they positively mirror cardiac output and negatively mirror ventilation [35, 41]. Pronounced increase in P_{ETCO_2} values from before to after use of both mask types probably occurred due to partial rebreathing from the mask dead space and changes in spontaneous respiration (*e.g.* changes in inspiratory/expiratory time) that may alter alveolar slope [76]. Those effects are expected to elevate breath isoprene, as was observed during exhalation of expiratory reserve volume by healthy subjects [35]. Within this setup, isoprene exhalation remained independent of cardiac output and CO_2 exhalation. Previously, we have observed that breath-holding manoeuvres [36, 77] and externally applied upper-airway resistances against respiratory flow [51] significantly increased breath isoprene concentrations. Although respiratory rate tends to decrease in young to middle-aged adults and increase in older adults, the changes remained within the normal physiological range. In all cases, isoprene concentrations significantly decreased throughout the experiments; most likely due to sympathetic vasoconstriction (deoxygenation-induced) in muscle compartments [78], which are the potential storage of this VOC, but which stayed inactive while sitting. Previously, we witnessed such decline in breath isoprene (in contrast to cardiac output) in healthy adults during the second minute in a standing posture [41]. At that point, cardiac output started to increase, but isoprene still decreased as sympatho-adrenergic vasoconstrictions took place in the lower extremities of the body to push up (against gravitation) the peripheral blood volume towards thoracic compartments. This was in order to counter the falling blood pressure and cardiac output while standing. As distribution of blood flow is crucial under hypoxia [79], in the present setup, the same phenomena might have helped to redistribute the available perfusion within active compartments in order to aid the rising O_2 demand. Due to having both haemoglobin and plasma bicarbonate buffer, such effects were not observed in the case of P_{ETCO_2} . Surprisingly, we observed hyperventilation ($P_{ETCO_2} < 35$ mmHg) in most of the older adults, even before wearing the masks for our experiment. This could occur in order to compensate/eliminate the elevated P_{ETCO_2} from precedent mask wearing (while travelling to our setup) phase. Although we let all subjects sit without any mask for 15 min within our setup before starting experiments, this seem to be not enough to compensate the precedent effects in all subjects aged ≥ 60 years.

Exogenous monoterpene, like limonene, is sourced to the breath from the clinical environment or *via* recently consumed fruit juice or similar. Acetonitrile and aromatics such as furan, benzene and toluene are exposed from the environment and/or smoking habits [42, 80]. These substances are lipophilic in nature and are stored in the fatty tissues. They mimic isoprene exhalation, mainly due to having similar physiochemical properties (*e.g.* low aqueous solubility, high volatility, *etc.*) and compartmental storage.

Considering limitations, our pilot study is conducted on a limited sample size and only on young to middle-aged and older adults. Within this study we have not included children, adolescents and patients suffering from restrictive or obstructive lung conditions and cardiac dysfunction. Therefore, our findings cannot be generalised to all age groups, ethnicities or health conditions. Assuming this setup in a large population of age, body mass index and gender matched healthy and sick subjects (including children) could enhance our clinical understanding on the observed side-effects beyond the everlasting physiological (intra- and inter-individual) variations in patient suffering from obstructive and restrictive lung conditions and other respiratory diseases. In order to increase the compliance of older adults towards voluntary participation and to minimise close interactions (*i.e.* current mandatory distancing measures for clinical studies at the University Medicine Rostock) between the investigators and participants, we had to exclude the arterialised blood-gas analysis from our setup. In older adults, we had to limit measurements within 15 min as, by then, most of them reached a S_{pO_2} level <94% under FFP2 masks. We think it is likely that more profound outcomes would be observed if measurements were continued for >30 min in individuals ≥ 60 years of age. Such outcomes would be crucial for healthcare workers aged 60–68 years and/or for retired healthcare workers brought back to the workforce and are obliged to wear FFP2 continuously in COVID-19 wards for longer periods of time. Therefore, the effects of longer (*e.g.* 30–75 min) use of FFP2 masks in older adults raise further research questions. Besides our presented immediate/short-term effects, longitudinal investigations (*via* follow-up measurements) of long-term consequences of masks along with compensations after mask removal would be of potential research interest and relevance. In our setup, we did not measure the fraction of exhaled nitric oxide (F_{eNO}), which is known to regulate vasomotor tone, blood pressure and is regarded as a marker for oxidative stress [81]. The F_{eNO} in breath increases under acute exogenous hypoxia [82] and pilot findings have indicated inverse relationship between isoprene and nitric oxide [83]. Thus, future examination of breath nitric oxide under the same/improved experimental conditions may reveal its clearer relationship to isoprene and other endogenous VOCs that are associated with oxidative stress and systemic microbial activity. While considering additional research gaps, effects of masks on high-altitude inhabitants (those are naturally adapted live in low inspiratory O_2 fraction and low aerial viscosity) are yet to be investigated.

Real-time breathomics has revealed a deeper insight into the physiometabolic side-effects of face mask wearing. Based on more recent pilot observations of cardiopulmonary parameters during exercise and rest in 12 healthy adults [11], researchers have generally recommended the continuous mask use. Within our setup, we have investigated the respiratory, haemodynamic and downstream metabolic changes in young to middle-aged and older adults for a longer period of time. Although we observed significant side-effects and good compensation/adaptation trends in healthy adults aged <60 years, those side-effects emerged profoundly towards substantial risk in subjects aged ≥ 60 years within 15 min. Mask-induced profound arterial oxygen decline, hypercarbia, deteriorating ventilation, compartmental vasoconstrictions and blood pressure alterations in older adults turned out to be concerning upon the general health/clinical status under prolonged use of FFP2 face masks at rest. According to the recent observations from a randomised controlled trial, COPD patients (outpatient-diagnosed and nonsevere/moderate) wearing surgical masks during a 6-min walk test tend to develop dyspnoea (without having any decrease in 6-min walk distance or O_2 saturation) compared to those without masks [84]. Thus, HIRAI *et al.* [84] have recommended surgical mask as safe for COPD patients. In our study, the side-effects of surgical mask were examined for 15 min at rest in older adults (including those with the history of mild COPD and chronic bronchitis). In line with these observations, our findings also demonstrated mild to moderate physiometabolic effects of surgical masks on adults aged ≥ 60 years.

We observed immediate side-effects of medical masks on arterial oxygen saturation, respiratory–haemodynamic parameters and exhalation of volatile metabolites in adults at rest. Such effects are pronounced under FFP2 condition and especially in subjects aged ≥ 60 years. Surgical masks do not cause as many side-effects, even in older adults. Therefore, the use of surgical masks could be reasonable, considering their anticipated benefits (protection against SARS-CoV-2 infection). Our pilot findings underline the importance of further large-scale clinical investigations of face mask driven risk factors (*i.e.* those that are clinically relevant) in patients with various cardiorespiratory diseases/conditions and in children. These results could help to remodel COVID-19 pandemic health policies globally.

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Data availability: All raw data collected for the study will be made available to others after reasonable request. Data will be stored in anonymised form at online repository when the paper is published.

Author contributions: P. Sukul and W. Miekisch conceived the idea and, along with J.K. Schubert, planned the study. P. Sukul, J. Bartels, P. Fuchs, R. Remy and L. Rührmund recruited volunteers and performed experiments. P. Sukul and P. Trefz analysed data. P. Sukul prepared the results and performed statistical analysis. P. Sukul, J.K. Schubert and S. Kamysek contributed to clinical interpretation and discussion. W. Miekisch contributed to analytical interpretations. P. Sukul wrote the manuscript, which was reviewed and edited by all authors. Correspondence and requests for materials should be addressed to P. Sukul.

Conflict of interest: The authors have nothing to disclose.

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