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

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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 mid-aged (age: 20–60 years) adults over the period of 15 and 30 min, respectively by high-resolution real-time mass-spectrometry (PTR-ToF-MS). Peripheral oxygen saturation, respiratory- and haemodynamic parameters were measured (non-invasively) simultaneously.

Results: Profound, consistent and significant (p-value≤0.001) changes in SpO_2 (Adults>60_FFP2-15min: 5.8±1.3%↓, Adults>60_surgical-15min: 3.6±0.9%↓, Adults<60_FFP2-30min: 1.9±1.0%↓, Adults<60_surgical-30min: 0.9±0.6%↓) and pET-CO_2 (Adults>60_FFP2-15min: 19.1±8.0%↑, Adults>60_surgical-15min: 11.6±7.6%↑, Adults<60_FFP2-30min: 12.1±4.5%↑, Adults<60_surgical-30min: 9.3±4.1%↑) indicate ascending deoxygenation and hypercarbia. Secondary changes (p-value≤0.005) to hemodynamic parameters (e.g. MAP: Adults>60_FFP2-15min: 9.8±10.4%↑) were found. Exhalation of blood-borne volatile metabolites e.g. aldehydes, hemiterpene, organosulfur, short-chain fatty acids, alcohols, ketone, aromatics, nitrile and monoterpene mirrored behaviour of cardiac output, MAP, SpO_2, respiratory rate and pET-CO_2. Exhaled humidity (e.g. Adults>60_FFP2-15min: 7.1±5.8%↑) and exhaled oxygen (e.g. Adults>60_FFP2-15min: 6.1±10.0%↓) changed significantly (p-value≤0.005) over time.

Conclusions: Breathomics allows unique physio-metabolic 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 hypoxemia, oxidative stress, hypercarbia, vasoconstriction, altered systemic microbial activity, energy homeostasis, compartmental storage and washout. FFP2 masks affected more pronouncedly 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.

Keywords: FFP2 and surgical mask, Breathomics, SARS-CoV-2, Respiratory and haemodynamic effects, side effects, Pandemic policy, PTR-ToF-MS, Volatile organic compounds (VOCs)

Take-home message: While assumed to protect against SARS-CoV-2 transmission, face-masks cause various physio-metabolic side effects and changes in exhaled VOC profiles. Effects are more pronounced in FFP2 masks and are profound in age >60.

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 pandemic[1, 2]. During the second wave of the pandemic, use of surgical and/or FFP2/N95/KN95 masks were recommended as strictly mandatory attributes while in public. National and/or global policy makers have recommended even adapting FFP3 masks for further protection considering the emerging CoV-2 variants[3, 4]. In Germany, government has recommended use of FFP2 masks up to 75 min at a stretch and use of surgical mask throughout one's presence in the public and the same guidelines are applied for school attending children as well as for individuals over age 60[5]. A recent meta-analysis demonstrated 72% reduction of infection risk in healthcare workers and an overall reduction of the same up to 62%[6]. Nevertheless, this systemic review was based on ununiform case control studies, which were not adjusted for bundled approach and aerosol generating procedures. On the other hand, a randomized controlled trial demonstrated non-occupational use (~ 3 hrs/day) 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 of Germany) mandated respirator masks (FFP2 or KN95) in stores and public transit. Although FFP2 masks were recommended to all members of the general public, there was no association with any preventive effect during explosive 3rd waves (and casualty) of COVID-19 in Austria and Bavaria during the spring of 2021. In line with that, a recent analysis of cases and fatalities from west European countries without mask mandate could not find increase in number of infection or death in other 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], broncho-pulmonary gas-exchange[12] and *in vivo* metabolic processes[13, 14]. Studies have shown effects of surgical masks on cardiopulmonary parameters, O_2 - CO_2 homeostasis, blood pH and thermoregulation [15]. Studies have also 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] as well as might lead to metabolic alkalosis[20].

Studies have indicated both complementary and/or conflicting results upon the side effects of different masks. Chan *et al* reported near-zero impact of nonmedical cloth masks and surgical masks on SpO_2 and CO_2 tension in 50 young adults during sitting and briskly walking for 10 min and minimal effects of nonmedical masks on SpO_2 (self-monitored by subjects) in 25 older adults (>65 years) for one hour at the community setup[21]. Rhee *et al* have demonstrated significant increase in CO_2 (measured by nasal canula) concentrations in 11 healthy subjects within 15 min under FFP2 conditions, which remained within the National Institute for Occupational Safety and Health (NIOSH) limits for short-term use - exceeding the long-term exposure threshold of 0.5% [22]. Blad *et al* 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[23] but they overall found a rise in CO_2 rebreathing of approximately 10000 ppm (1Vol % CO_2). On the other hand, a recent comprehensive review by Kisielinski *et al* has demonstrated significantly measurable ($p<0.05$) decrease in O_2 saturation under fabric, surgical and N95 masks in 17%, 22% and 44% of the studies, respectively [15]. Here, authors have hypothesized mask induced exhaustion syndrome (MIES) that 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 (with $p<0.05$ and $p<0.01$) below lower limits with values ranging from 92.1% to 93.2% SpO_2 in mask users while compared to values from individuals without mask, ranging from 95.8% to 97.6%, respectively[24–26].

Other human studies have demonstrated clinically concerning side effects of FFP2 masks on the risk populations suffering from COPD and other lung conditions, patients undergoing dialysis as well as significant physio-metabolic effects on pregnant women and/or health care workers (HCWs) 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 non-

athlete 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 four hours of haemodialysis. While dyspnoea was in common under N95 masks, during haemodialysis, 70% patients had significant reduction in arterial partial pressure of O_2 ($Pa O_2$), 19% had reached various degrees of hypoxemia, 11 experienced chest discomfort and 17 had respiratory distress[30]. Recent analysis on 4747 ESRD patients (1925 on haemodialysis) depicted that universal use of surgical mask in haemodialysis units along with other preventive measures were effective against SARS-CoV-2 transmission in the Republic of Ireland[31]. On the other hand, pregnancy driven physiological changes in normal breathing patterns (upliftment of diaphragm), increase in O_2, nutrient/energy demand (by the developing embryo) and efforts to eliminate additional CO_2 (from foetal respiration), mid – end phase of gestation often leads to 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 woman (during 37 – 41 weeks of gestation) have demonstrated moderate (up to 93%) and major (below 92%) decrease in SpO_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 HCWs (between 27 – 32 weeks' gestation) have shown significant reductions in tidal volume, minute ventilation (without significant change in RR), O_2 consumptions and CO_2 production under N95 masks[33]. Such effects were more pronounced under low intensity (3 metabolic equivalent) work, 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 down-stream level. In order to understand the immediate physio-metabolic 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 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 physio-metabolic attributes. Combining VOC profiling with simultaneous pulseoximetry, 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 – time-of-flight – mass-spectrometry / PTR-ToF-MS) based breathomics in parallel to non-invasive measurements of peripheral oxygen saturation (SpO_2), respiratory rate (RR), partial pressure of the end-tidal CO_2 ($pET-CO_2$), exhaled humidity and oxygen, cardiac output (CO), stroke volume (SV), pulse rate (PR) and blood pressure (BP). The physio-metabolic side effects of FFP2 and surgical masks over 15 – 30 min of use on healthy human subjects aged between 20 – 80 years will be addressed in detail. Effects from both masks will be compared upon wearing duration and age.

Methods

Human subjects:

All experiments of this pilot study were conducted according to the amended *Declaration of Helsinki* guidelines and signed informed consent from 30 subjects (aged between 20 – 80 years) were obtained (Approval number: *A2021-0012* – issued by the Institutional Ethics Committee of University Medicine Rostock, Germany) prior to inclusion. As inclusion criteria, subjects were adults (male and

female) until the age of 80 years. As exclusion criteria, included subjects were not suffering from any acute diseases/health condition (during the last 6 months from participation) and were not undertaking any special diet and/or medication during inclusion. Among the subjects above the age of 60, three had mild COPD (one male and two female) and two had chronic bronchitis (female) in the past. At the time of inclusion, these five participants had no symptoms, pathological findings or ongoing medications. They had described and confirmed about their good health condition (since a year or more).

Determination of sample size:

We applied the analysis of variance (ANOVA) test for calculation of sample size. For a minimum detectable difference in mean substance intensities of 450 cps, a standard deviation of 300 was estimated. To attain an alpha value of 0.005 and a test power of 0.99, 2 experimental groups, while considering a population of 100,000, the sample size resulted at 26 (with minimal group size of at least 10 each). In this study, we have included 30 subjects for analysis in order to detect even less than 5% differences in exhaled VOCs up to low parts per trillion by volume (pptV) levels.

Table 1: Anthropometric information of subjects.

	Demographic Data				**Lifestyle Habits / Life Events**					
Participant groups	**Gender**	**Number**	**Age range (Years)**	**BMI range (Kg/sq.m)**	**Smoker**	**Alcoholic**	**Special diet**	**Acute, chronic disease / medication / past history**	**Contraception**	**Pregnancy/ Expecting**
Young to mid-aged adults	M	8	20 to 59	(18 - 25)	Yes (n = 01)	No	No	No	N/A	N/A
	F	9	20 to 59	(19 - 29)	Yes (n = 03)	No	No	No	No	No
Older adults	M	7	60 to 80	(21 - 27)	Yes (n = 02)	No	No	Mild COPD (n =1) Chronic Bronchitis (n = 2)	N/A	N/A
	F	6	60 to 80	(23 - 29)	Yes (n = 02)	No	No	Mild COPD (n =2)	N/A	N/A

Number of subjects in each group, age range and body mass index (BMI) range are listed along with life style attributes e.g. cigarette smoking or alcohol drinking or any special dietary habits and clinically important parameters e.g. any health condition or medication etc.

Assignment of groups:

We have divided the study population in two groups; namely: young to mid-aged adults (<60 years of age) and older adults (>60 years of age). Anthropometric data were confirmed by participants during inclusion and are presented in Table 1.

Experimental setup:

Three devices were synchronized for real-time measurements of several parameters simultaneously (Fig. 1). Continuous monitoring of breath VOCs, O_2, CO_2 and humidity via PTR-ToF-MS, non-invasive measurements of haemodynamic parameters via volume clamp method, SpO_2 monitoring via

pulseoximetry. Main-stream capnography (for pET-CO_2) was performed immediately before and after the mask use. Data acquisition was initiated in parallel.

Breath sampling protocol:

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

The transfer-line of PTR-ToF-MS was connected (via PEEK finger-tight fittings) to a PEEK extension line (i.e. 30 cm long, with 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 (Fig. 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 till the front of subject's lips. The tip of this sampling line was cased within a conical PEEK ferrule in order to avoid any unwanted contact with mask surface or with subject's lips. These extension lines were sterilized for reuse.

In each volunteer, measurements with two different masks (viz. 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 for FFP2 mask experiment on the first day and others participated in surgical mask experiment. Young to mid-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 a SpO_2 level <94%.

PTR-ToF-MS measurements of breath VOCs:

Breath VOCs were measured continuously via a PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria) and with pre-optimized experimental conditions[36, 43], i.e. continuous side-stream mode of sampling via a 6m long heated (at 75°C) silico-steel transfer-line connected to a sterile mouthpiece. A continuous sampling flow of 20 ml/min 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 on 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 H_2O flow was set to 6 ml/min. Drift tube temperature was set to 75°C, voltage was 610 V and the pressure was 2.3 mbar. The resulting 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 (H_3O^+-Isotope), 29.9980 (NO^+) and 59.049 (C_3H_6O).

VOC data processing:

VOCs were measured in counts per seconds (cps) and corresponding intensities were normalised onto primary ion (H_3O^+) 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 in expiration than in inhalation. As the mass resolution of PTR-ToF-MS (4000–5000 Δm/m) 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 multi-component mixture of standard reference substances. Quantification process under adapted sample humidity (as in exhaled breath) using a liquid calibration unit (LCU, Ionicon Analytik GmbH, Innsbruck, Austria) is our pre-established state-of-the-art[44].

Selection of VOCs for analysis:

Here 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*:**

Non-invasive 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 volume clamp method (ClearSight system-EV1000, Edwards Lifesciences, California, USA)[35, 41].

Mainstream capnography:

Main stream capnography was performed just before and after each mask use via a small portable capnograph (EMMA™ PN 3639, Ref: 605102, Masimo® Sweden AB, Danderyd, Sweden) attached to a sterile breathing mouthpiece. pET-CO_2 values were recorded in mmHg unit. Absolute values are considered from the 3rd breath onward as first two to three breaths are used to calibrate the CO_2 and RR sensor.

Statistical analysis:

Analytical mean values (of measured parameters) from each participant were calculated over each minute of breath-resolved measurement. Data from every 5th minute were included for statistical analysis. In case of any non-parametric 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 groups (young to mid-aged and older adults).

As every group mean/median value are contributed by each volunteer (of that group), the relative standard deviations (RSDs) in VOC abundances from each group were also calculated for each substance. The RSDs were calculated (in %) by rating sample standard deviations (SDs) over corresponding sample means.

Statistically significant differences within groups were assessed via repeated measurement ANOVA on ranks (Friedman repeated measures analysis of variance on ranks, Shapiro-Wilk test for normal distribution and post hoc Student–Newman–Keuls method for pairwise multiple comparisons between all groups; p-value ≤ 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 1st minute of each mask 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 (in %) over time (with respect to initial values) were calculated for selected variables in each group. Here, we have selected the principal physio-metabolic denominators and candidate VOCs that are potentially originating from several *in vivo* metabolic processes. Relative changes were calculated at 15th and 30th min in young to mid-aged and at 15th min in older adults. The changes in pET-CO_2 values were calculated immediately before and after mask use. In case of inter-group 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 % of changes caused by FFP2 mask on older adults.

In order to understand the correlations between exhaled VOCs and physiological parameters within each mask type, dimension reduction factor analysis (Factor extraction via principal components method, factor scores via regression method and 1-tailed significance at p-value ≤ 0.005) were performed in SPSS.

Results

Figure 2 shows heatmaps of relative changes (normalised mean values) of physiological parameters such as, pET-CO_2, SpO_2, RR, CO, SV, PR, 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 mid-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 5th minute is presented in the heatmaps. pET-CO_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 via heatmaps in Supplementary Figure-S1.

Figure 3 (Boxplots) is depicting absolute or normalised values of physiological parameters and of alveolar concentrations of exhaled VOCs in every 5th minute (starting from the 1st min) in four groups. First two groups consist of data from FFP2 masks on young to mid-aged and older adults, respectively. Later two groups contain data from surgical masks on young to mid-aged and older adults, respectively. pET-CO_2 values are presented from immediately before and after the use of masks. Here, 3 (A) represents the physiological parameters viz. absolute values (with units) of SpO_2, pET-CO_2 and respiratory rate and normalised values of hemodynamics. 3 (B) represents aliphatic aldehydes and organosulfur, 3 (C) represents hemiterpene, ketone and smoking/environment related VOCs, exhaled humidity and oxygen, 3 (D) represents aliphatic acids, 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 (viz. statistically significant at p-value ≤ 0.005) in each variable within each group is indicated with respect to the corresponding '1st minute' of measurement.

The correlation coefficients and respective p-values between physiological parameters and VOCs of interest are presented in Table 2 and the same 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 Table-S1 and S2. Noteworthy, that CO showed strong positive correlation to SV under both masks whereas, PR remained only moderately related to CO under FFP2 conditions. CO, SV and PR showed relatively higher variations under the surgical masks, whereas PR remained completely unrelated to CO. While RR remained unrelated to other physiological parameters under both masks, MAP has shown significant and moderate negative correlations to SpO_2 only under FFP2 masks and good positive correlations to CO and SV under surgical masks.

Figure 4 (Boxplots) is depicting comparison of relative changes (in %) in physiological parameters and in selected metabolic breath markers within four groups. First two groups are of FFP2 masks on young to mid-aged and older adults, respectively and later two groups are of surgical masks on young to mid-aged and older adults, respectively. Relative changes (with respect to initial values) in young to mid-aged adults at the 15th and 30th min and in older adults at the 15th min is presented. For pET-CO_2, relative changes between measured values immediately before and after use of masks are presented. Here, 4 (A) represents physio-metabolic parameters and 4 (B) represents exhaled alveolar volatile organic metabolites. From all pairwise-multiple comparisons, statistically significant differences are indicated with respect to changes caused by FFP2 mask 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.

Table 2: Correlation (obtained via factor analysis) between physiological parameters and selected VOCs of interest.

	FFP2 mask							**Surgical mask**					
	CO	***SV***	***PR***	***MAP***	***RR***	***SpO_2***		***CO***	***SV***	***PR***	***MAP***	***RR***	***SpO_2***
Isoprene	0.11	-0.15	0.33	-0.02	0.38	0.18	***R value***	-0.39	-0.39	0.14	-0.34	-0.23	0.08
	0.099	0.037	**0.000**	0.395	**0.001**	0.018	***p-value***	**0.000**	**0.000**	0.064	**0.000**	0.006	0.184
Acetone	0.03	-0.25	0.31	0.12	0.27	0.10	***R value***	-0.28	-0.3	0.1	-0.46	-0.04	-0.09
	0.380	**0.002**	**0.000**	0.085	**0.001**	0.126	***p-value***	**0.001**	**0.000**	0.139	**0.000**	0.337	0.172
Acetaldehyde	0.05	-0.14	0.28	-0.25	-0.20	-0.05	***R value***	-0.16	-0.11	0.03	0.19	0.02	0.07
	0.288	0.047	**0.000**	**0.002**	0.009	0.268	***p-value***	0.044	0.111	0.373	0.020	0.395	0.212
Butyraldehyde	-0.04	-0.15	0.15	-0.15	-0.10	-0.01	***R value***	-0.24	-0.23	0.16	0.2	-0.05	0.13
	0.312	0.037	0.042	0.045	0.115	0.461	***p-value***	**0.005**	**0.005**	0.045	0.016	0.288	0.078
Dimethyl sulfide	0.17	-0.14	0.38	-0.03	0.33	0.15	***R value***	-0.29	-0.34	0.16	-0.38	-0.25	0.07
	0.027	0.051	**0.000**	0.373	**0.000**	0.043	***p-value***	**0.001**	**0.000**	0.045	**0.000**	**0.003**	0.229
Butanethiol	-0.10	-0.13	0.07	-0.02	-0.13	0.33	***R value***	-0.32	-0.11	-0.23	-0.26	-0.05	0.14
	0.116	0.059	0.222	0.409	0.072	**0.000**	***p-value***	**0.000**	0.108	0.006	**0.002**	0.279	0.059
Acetic acid	-0.14	-0.03	-0.15	-0.02	0.08	0.41	***R value***	-0.16	-0.09	-0.09	-0.13	-0.2	0.2
	0.051	0.355	0.039	0.390	0.190	**0.000**	***p-value***	0.041	0.171	0.162	0.074	0.016	0.015

Butyric acid	0.11	0.01	0.14	0.02	0.12	0.20	***R value***	-0.14	-0.09	-0.07	-0.15	-0.31	0.12
	0.093	0.437	0.055	0.393	0.090	0.009	***p-value***	0.068	0.177	0.217	0.053	**0.000**	0.101
Ethanol	0.082	-0.18	0.35	-0.17	-0.11	-0.04	***R value***	-0.30	-0.27	0.09	0.05	0.03	-0.08
	0.171	0.019	**0.000**	0.023	0.107	0.313	***p-value***	**0.000**	**0.002**	0.166	0.298	0.375	0.204
Limonene	-0.04	-0.21	0.20	0.15	0.41	0.00	***R value***	-0.02	-0.16	-0.02	-0.46	-0.01	-0.21
	0.326	0.007	0.011	0.045	**0.000**	0.488	***p-value***	0.013	0.043	0.411	**0.000**	0.465	0.011
Furan	0.09	-0.20	0.38	-0.03	0.27	0.13	***R value***	-0.40	-0.40	0.16	-0.28	-0.27	0.06
	0.139	0.009	**0.000**	0.381	**0.001**	0.067	***p-value***	**0.000**	**0.000**	0.044	**0.001**	**0.001**	0.245
Exhaled O_2	0.10	-0.22	0.33	0.12	0.09	0.08	***R value***	-0.62	-0.83	0.59	-0.62	-0.13	0.11
	0.132	**0.005**	**0.000**	0.089	0.145	0.173	***p-value***	**0.000**	**0.000**	**0.000**	**0.000**	0.083	0.127
Exhaled H_2O	-0.01	-0.15	0.13	0.31	0.28	-0.17	***R value***	-0.11	-0.21	0.19	-0.02	-0.25	-0.29
	0.448	0.041	0.065	**0.000**	**0.000**	0.023	***p-value***	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*-value ≤ 0.005) correlations are assigned in bold. The values '0.000' denominates *p*-values <0.001.

Table 3: Correlation (obtained via factor analysis) between physiological parameters of interest.

	FFP2 mask							**Surgical mask**					
	CO	***SV***	***PR***	***MAP***	***RR***	***SpO_2***		***CO***	***SV***	***PR***	***MAP***	***RR***	***SpO_2***
CO	*NA*	0.71	0.45	-0.03	-0.02	-0.05	***R value***	*NA*	0.84	-0.16	0.53	-0.04	0.007
	NA	**0.000**	**0.000**	0.377	0.396	0.290	***p-value***	*NA*	**0.000**	0.043	**0.000**	0.317	0.472
SV	0.71	*NA*	-0.38	0.03	0.13	-0.05	***R value***	0.84	*NA*	-0.66	0.52	0.05	0.01
	0.000	*NA*	**0.000**	0.363	0.067	0.295	***p-value***	**0.001**	*NA*	**0.000**	**0.000**	0.277	0.453
PR	0.45	-0.38	*NA*	-0.07	-0.19	-0.01	***R value***	-0.16	-0.66	*NA*	-0.13	-0.14	0.005
	0.000	**0.000**	*NA*	0.202	0.015	0.452	***p-value***	0.043	**0.000**	*NA*	0.074	0.071	0.480
MAP	-0.03	0.03	-0.07	*NA*	0.22	-0.38	***R value***	0.53	0.52	-0.13	*NA*	0.05	-0.12
	0.377	0.363	0.202	*NA*	0.006	**0.000**	***p-value***	**0.000**	**0.000**	0.074	*NA*	0.288	0.095
RR	-0.02	0.13	-0.19	0.22	*NA*	-0.09	***R value***	-0.04	0.05	-0.14	0.05	*NA*	-0.16
	0.396	0.067	0.015	0.006	*NA*	0.150	***p-value***	0.317	0.277	0.071	0.288	*NA*	0.047
SpO_2	-0.05	-0.05	-0.01	-0.38	-0.09	*NA*	***R value***	0.007	0.01	0.005	-0.12	-0.15	*NA*
	0.290	0.295	0.452	**0.000**	0.150	*NA*	***p-value***	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*-value ≤ 0.005) correlations are assigned in bold. The values '0.000' denominates *p*-values <0.001. '*NA*' denotes to not applicable test.

Discussion

FFP2 and surgical face-masks immediately affected physiological and metabolic attributes. Effects were progressed with the course of mask wearing time. Most pronounced effects were observed in case of FFP2 mask and especially in older adults. Profound and consistent decrease in SpO_2 and increase in pET-CO_2 have indicated hypercarbia 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 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 SpO_2 and/or most increased pET-CO_2. Despite significant increases in RR (as compensatory responses to increased $PaCO_2$) in older adults, pET-CO_2 increased most significantly after 15 min of FFP2 masks wearing in our setup – indicating compromised respiratory compensation. Notably, at the end of participation, our volunteers had explained altered breathing patterns and respiratory discomfort as their breathing experiences under both masks and more pronouncedly under FFP2 masks. Deeper and slow inhalation and exhalation patterns (slow breathing) were experienced by young to mid-aged adults, whereas the older adults explained 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 above-mentioned facts along with observed significant decrease in O_2 exhalation and constant increase in exhaled humidity suggest hypercapnia like effects (profound increase in pET-CO_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 physiological response to counterbalance the biological oxygen demand. Phillips *et. al* demonstrated dose-response manner of cardiac output

increase under acute isocapnic hypoxemia in healthy adults [50]. Correlations (Table 3) indicated that SV was the principal determinant of CO under both masks. During spontaneous breathing, changes in intra-thoracic 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-mask induces additional (and variable according to mask fittings and tightness) upper-airway resistance against breathing and change the normal breathing patterns, high SVVs is 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] has incorporated 28 different parameters (from 44 research studies) out of which, SpO_2, pET-CO_2, humidity, RR, BP and PR are in common with our study. RR and PR 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 hypoxemia and hypercapnia like effects especially in older adults under FFP2 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 the but also has 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, hemodynamics and respiratory parameters. Exhaled oxygen decreased while breath humidity increased over time. Exhalation profiles of potentially endogenous VOCs (related to certain metabolic pathways and/or physiological processes) viz. aldehydes, hemiterpene, organosulfur, alcohols, ketones and short-chain fatty acids have indicated *in vivo* physio-metabolic cross-talks (i.e. complementary and/or counter overlaps) between transient hypoxemia 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-exposers and lifestyle.

CO_2 toxicity may arise due to rebreathing. Studies have indicated linear changes in circulatory, cardiovascular and autonomic physiology due to inspiratory CO_2 exposure at concentrations between 500 – 5000 ppm[52]. Clinical evidence has depicted that short-term CO_2 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 CO_2 exposure on physio-metabolic processes is perceivable. While looking at the CO_2 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 SpO_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 disbalance in O_2 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 O_2 dependent [18]. Thus, significant deoxygenation and hypoxic condition deprives O_2 supply to immune cells that hinder optimal responses and gradually lead to immunosuppression. Considering the well-known effects of transient hypoxemia driven risk of cardiac arrhythmias[59] and/or cerebral disfunction, the observed transient but profound lowering of SpO_2 under FFP2 mask in older adults of our study rises significant concern upon 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 DMS, AMS and butanethiol, the effects of both face-masks are reflected on the systemic origin of these substances from microbial anaerobic methylation[61] in the lower gut. Studies have shown that effects of hypoxia acts as an

‘invisible pusher’ of gut microbiota[62]. Gut flora maintain the hypoxic balance of the intestinal environment in order to regulate the nutrient absorption, gut permeability and immune response[63]. As all 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 descending production of those organosulfur in colon region. Despite an increase in cardiac output, exhaled abundances of these substances decreased significantly in case of both masks in either 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 H_2S.

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 large-intestinal environment, exhaled profiles 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 eventually may leads to anaerobiosis and metabolic acidosis (lowering of plasma *p*H)[68], SCFAs production is very likely to be reduced. Crotonic acid and formic acid are potentially sourced from cosmetics and disinfectant/sanitizers and similar to our previous observations[35, 51], those VOCs reflected washout behaviours.

Despite its origin from carbohydrate metabolizing 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 mask by mirroring the profiles of cardiac output and MAP. Evidences have indicated hypoxic switching of metabolic routes that produce more ethanol than lactates in order to regulate blood *p*H 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 consecutives descent in endogenous breath acetone (i.e. the by-product of glycolysis)[72] indicate a decline in carbohydrate metabolism and a 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 act[74] between intestinal permeability and glucose transport to blood for maintaining the 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 than ethanol due to their exogenous origin from the dietary intake, beverages and uptake from the ambient environment, disinfectants or sanitizers.

During spontaneous breathing in 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 pET-CO_2 values from before to after use of both masks occurred most likely 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] had significantly increased breath isoprene concentrations. Although respiratory rate tends to decrease in young to mid-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 the sympathetic vasoconstriction (deoxygenation induced) in muscle compartments[78], which are the potential storage of this VOC but 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 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 case of pET-CO_2. Surprisingly, we have observed hyperventilation (pET-CO_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 pET-CO_2 from precedent mask wearing (while arriving from elsewhere 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 above the age of 60 years.
Exogenous monoterpene like limonene is sourced to breath from 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 mimicked the isoprene exhalation mainly due to having similar physio-chemical properties (e.g. low aqueous solubility, high volatility etc.) and compartmental storage.

While considering the limitations, our pilot study is conducted on a limited sample size and only on young to mid-aged and older adults. Within this study we have not included children, adolescents and patients suffering from restrictive or obstructive lung conditions and cardiac disfunctions. Therefore, our findings cannot be generalised upon all age groups, ethnicity or health conditions. Assuming this setup on a large population of age, gender and BMI 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 minimize close interactions (i.e. mandatory distancing measure at the University Medicine Rostock for clinical studies at the moment) between the investigators and participants, we had to exclude the arterialized blood-gas analysis from our setup. In older adults, we had to limit the measurements within 15 min as most of them reached a SpO_2 level <94 by then under FFP2 masks. We think it is likely 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 HCWs aged 60 – 68 years and/or for the retired HCWs, who are brought back to the workforce and are obelized to continuously wear FFP2 in COVID-19 wards for longer period of time. Therefore, the effects of longer (e.g. 30 – 75 min) use of FFP2 masks in older adults rise 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 (FeNO), which is known to regulate vasomotor tone, blood pressure and is regarded as a marker for oxidative stress[81]. The FeNO 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 NO 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 physio-metabolic 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-, hemodynamic- and down-stream metabolic changes in young to mid-aged and older adults for longer period of time. Although we observed significant side effects and good compensation/adaptation trends in healthy adults below the age 60 years, those side effects emerged profoundly towards substantial risk in subjects over the age of 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 non-severe/moderate) wearing surgical masks tend to develop dyspnoea during 6-min walk test without having any decrease in 6-min walk distance or O_2 saturation that those

without masks[84]. Thus, Hirai *et. al* 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 on older adults (including those with the history of mild COPD and chronic bronchitis). In line with the above-observation, our findings also demonstrated mild to moderate physio-metabolic effects of surgical masks on adults >60 years of age.

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

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Authorship

P.S. and W.M. conceived the idea and along with J.K.S. planned the study. P.S., J.B., P.F., R.R. and L.R. recruited volunteers and performed experiments. P.S. and P.T. analysed data. P.S. prepared the results and performed statistical analysis. P.S., J.K.S. and S.K. contributed to clinical interpretation and discussion. W.M. contributed to analytical interpretations. P.S. wrote the manuscript, which was reviewed and edited by all authors. Correspondence and requests for materials should be addressed to P.S. (email: *pritam.sukul@uni-rostock.de*).

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.

Conflict of interest

The authors have nothing to disclose.

Figure legends

Figure 1: Instrumentation and experimental setup of the study. Customised PEEK extension of the PTR transfer line for direct sampling from the mask dead space is presented at the top. Continuous real-time breathomics via PTR-ToF-MS (1) in parallel to continuous non-invasive monitoring of haemodynamic via ClearSight system (2) and pulseoximetry (3) is presented at the bottom.

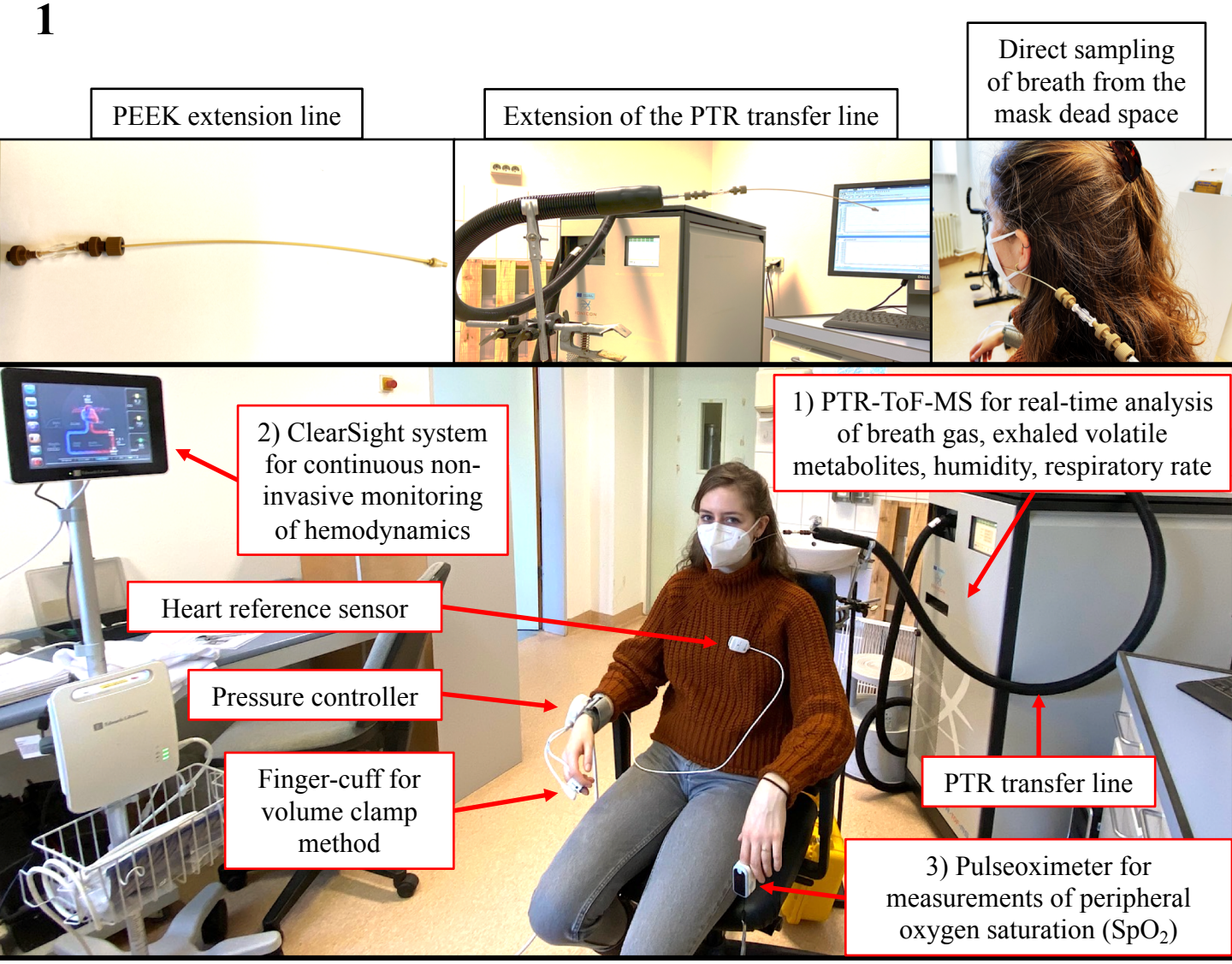
Figure 2: Relative changes in normalised mean values of physiological parameters and of exhaled alveolar VOC abundances during the use of COVID-protective face masks by young to mid-aged and older adults.
Y-axis represents the physiological parameters viz. pET-CO_2, SpO_2, respiratory rate, cardiac output, stroke volume, pulse rate, mean arterial pressure (MAP) values and the protonated/charged VOCs of interest. X-axis indicates time in minutes. 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. Respiratory-, haemodynamic parameters and SpO_2 were normalised likewise. The mean of those normalised values from every 5th minute is presented here. Only pET-CO_2 values are presented from immediately before and after mask use and are placed at the first and final minute of heatmaps. Red and blue colour 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 pET-CO_2, the points without any measurements are coloured in black.

Figure 3: Comparison of differences in physiological parameters and in exhaled alveolar VOC concentrations during the use of COVID-protective face masks by young to mid-aged and older adults. (A) physiological parameters, (B) aliphatic aldehydes and organosulfur, (C) hemiterpene, ketone, nitrile, aromatics, exhaled humidity and oxygen (D) aliphatic acids, alcohols and monoterpene. X-axis represents measurement time in four groups viz. two mask types (FFP2 and surgical) used by two age cohorts (young to mid-aged and older adults). Y-axis represents absolute values (with units) or normalised (onto corresponding

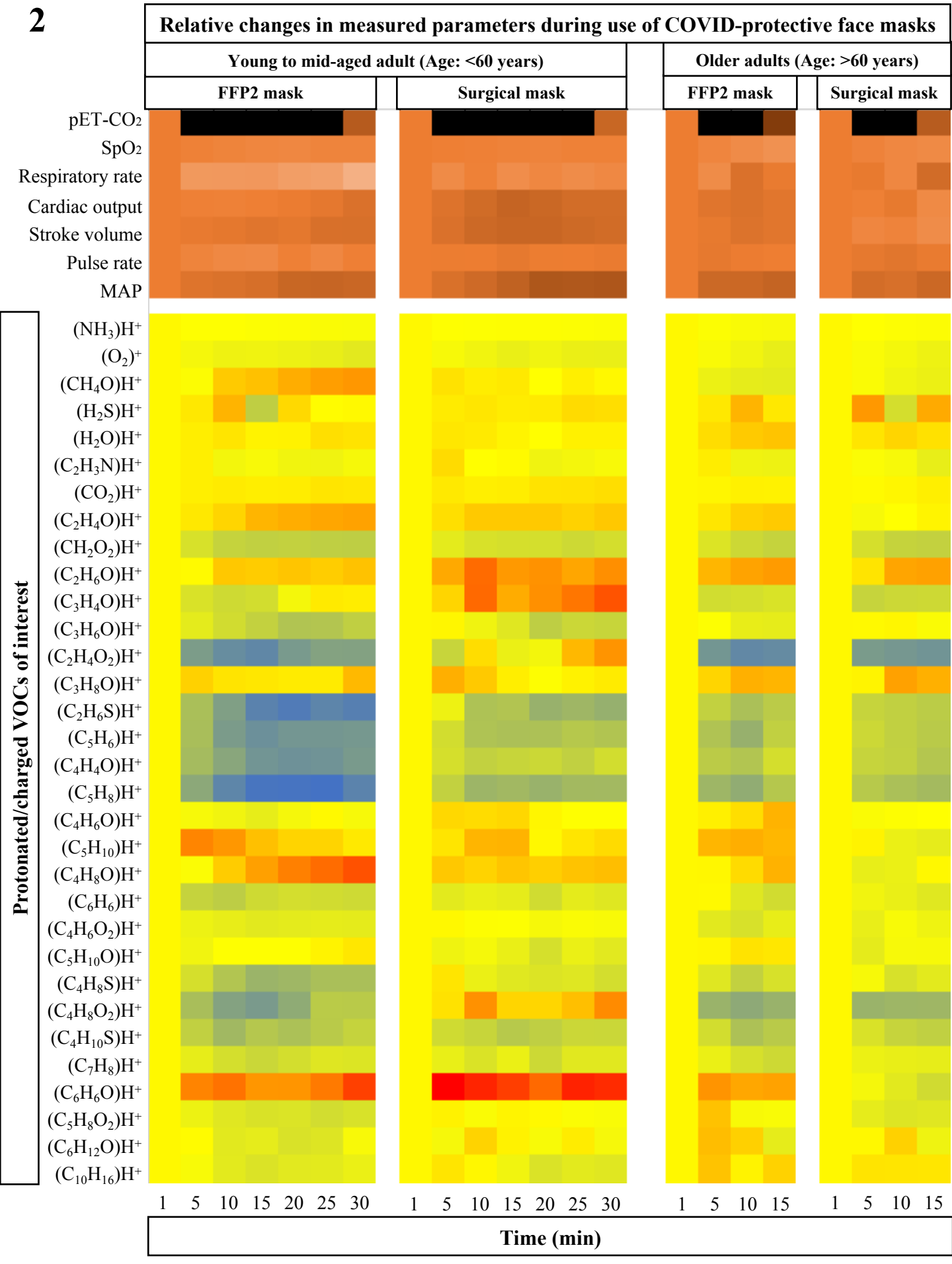
initial values) values of measured parameters from every 5th minute, starting from the 1st minute. For both mask types, young to mid-aged and older adults were measured for 30 min and 15 min, respectively. pET-CO_2 values are measured immediately before and after the use of masks and absolute values (with unit) are presented. Measured values within each group were compared. Statistical significances were tested by means of repeated measurement-ANOVA on ranks (p-value $\leq$ 0.005). From all pairwise-multiple comparisons, statistically significant differences with respect to the '1st minute' are indicated via coloured '**hash**' and '**star**'. Here, '**hash**' and '**star**' are assigned to FFP2 and surgical masks, respectively. Green and red colours denote young to mid-aged and older adults, respectively. Similarly, pET-CO_2 values before and after mask use are compared statistically.

Figure 4: Comparison of relative changes (in %) in physiological parameters and in exhaled metabolic markers during the use of COVID-protective face masks by young to mid-aged and older adults. (A) Physio-metabolic parameters and (B) Exhaled alveolar volatile organic metabolites. X-axis represents measurement time in four groups viz. two mask types (FFP2 and surgical) used by two age cohorts (young to mid-aged and older adults). Y-axis represents % of changes (with respect to initial values) in measured parameters at 15th and/or 30th minute. For both mask types, young to mid-aged and older adults were measured for 30 min and 15 min, respectively. Thus, for both mask types, changes in young to mid-aged adults at the 15th and 30th min are presented and the same is presented in older adults at the 15th min. pET-CO_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-value $\leq$ 0.005). Results from all pairwise-multiple comparisons are listed in Table 3. From all pairwise-multiple comparisons, statistically significant differences with respect to changes caused by FFP2 mask on older adults (**FFP2_Older adults**) are indicated via '**hash**'.

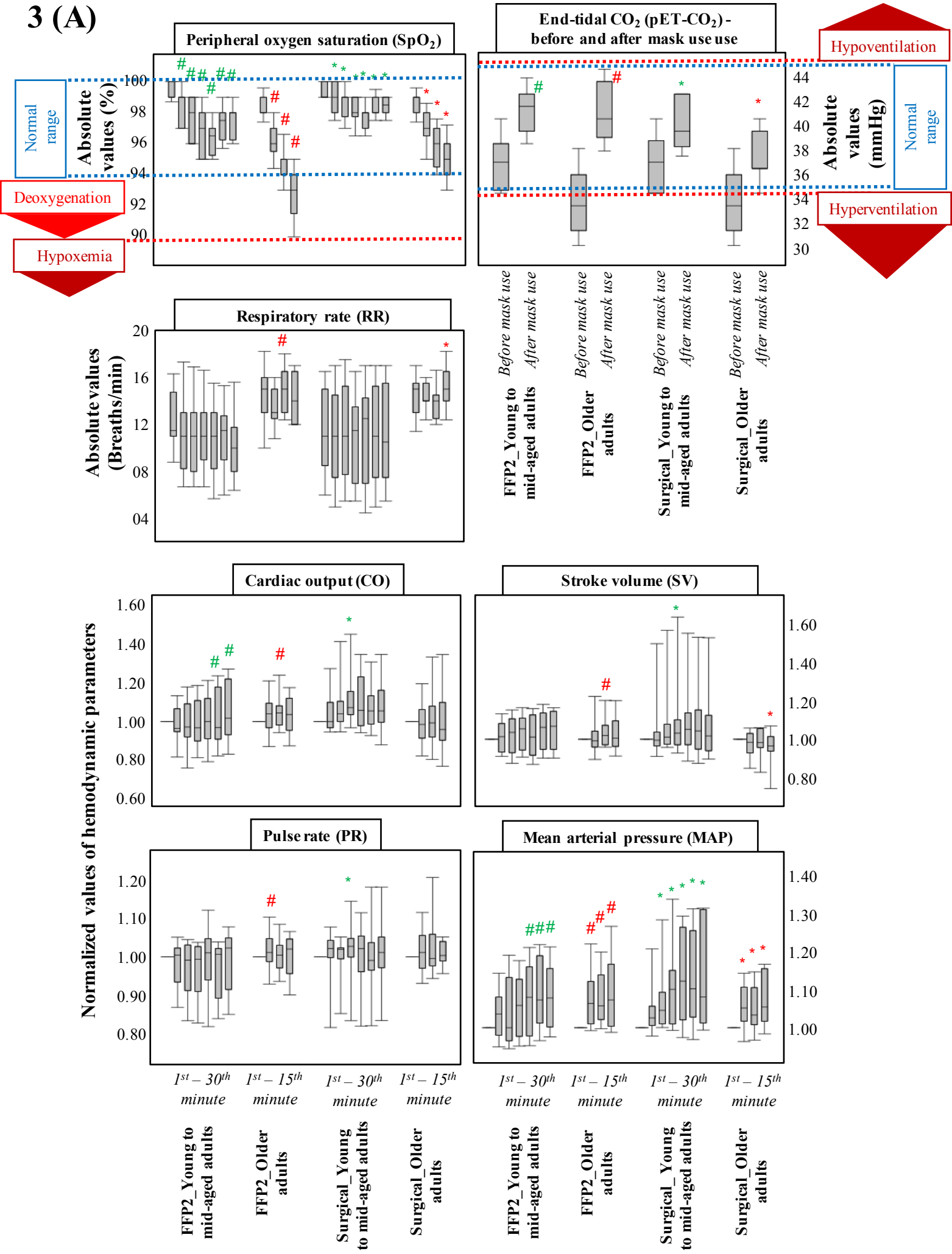
1

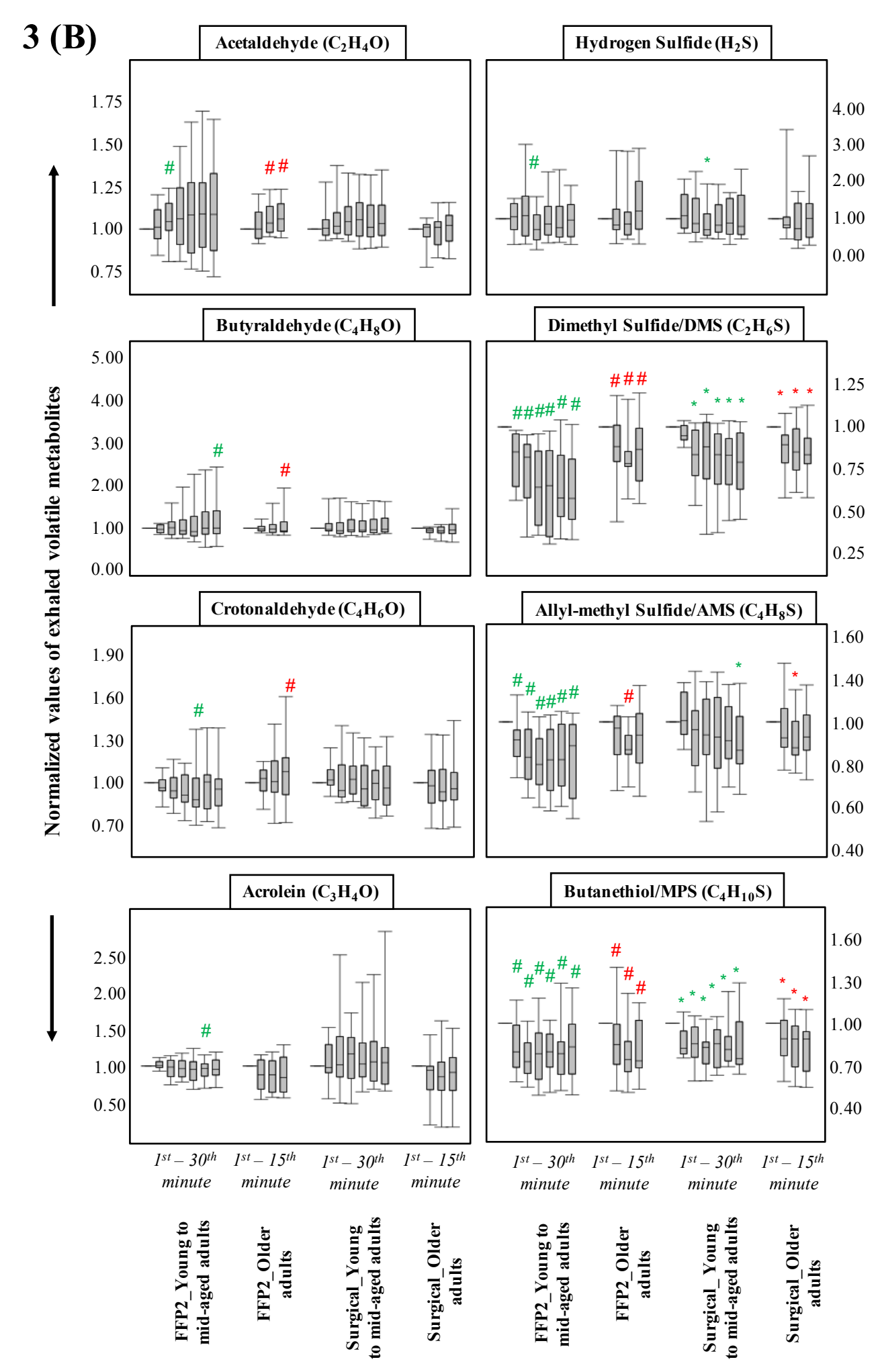

PEEK extension line
Extension of the PTR transfer line
Direct sampling of breath from the mask dead space
2) ClearSight system for continuous non-invasive monitoring of hemodynamics
1) PTR-ToF-MS for real-time analysis of breath gas, exhaled volatile metabolites, humidity, respiratory rate
Heart reference sensor
Pressure controller
Finger-cuff for volume clamp method
PTR transfer line
3) Pulseoximeter for measurements of peripheral oxygen saturation (SpO_2)

2

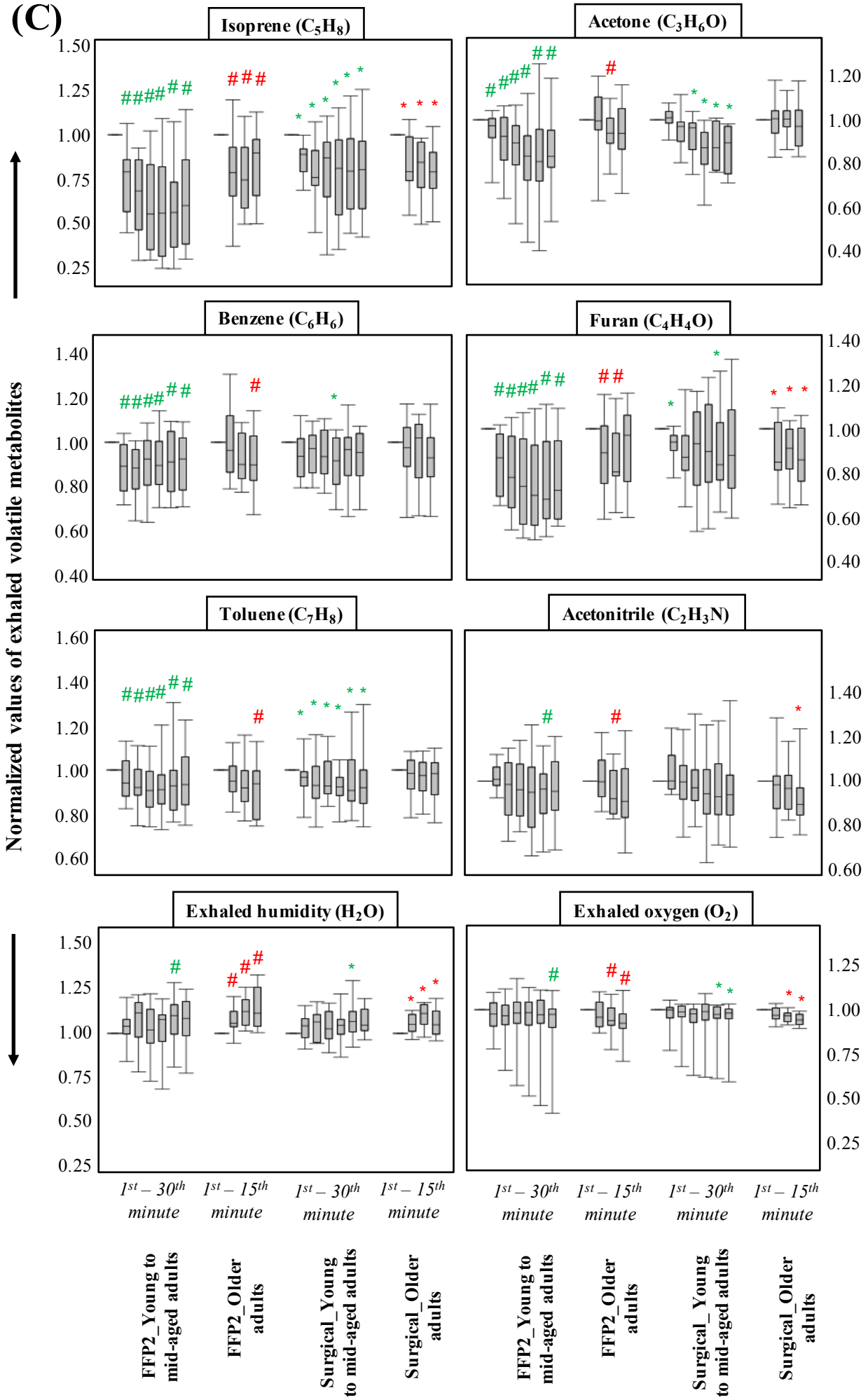

Relative changes in measured parameters during use of COVID-protective face masks
Young to mid-aged adult (Age: <60 years)
Older adults (Age: >60 years)
FFP2 mask
Surgical mask
FFP2 mask
Surgical mask
pET-CO2
SpO2
Respiratory rate
Cardiac output
Stroke volume
Pulse rate
MAP
Protonated/charged VOCs of interest
(NH3)H+
(O2)+
(CH4O)H+
(H2S)H+
(H2O)H+
(C2H3N)H+
(CO2)H+
(C2H4O)H+
(CH2O2)H+
(C2H6O)H+
(C3H4O)H+
(C3H6O)H+
(C2H4O2)H+
(C3H8O)H+
(C2H6S)H+
(C5H6)H+
(C4H4O)H+
(C5H8)H+
(C4H6O)H+
(C5H10)H+
(C4H8O)H+
(C6H6)H+
(C4H6O2)H+
(C5H10O)H+
(C4H8S)H+
(C4H8O2)H+
(C4H10S)H+
(C7H8)H+
(C6H6O)H+
(C5H8O2)H+
(C6H12O)H+
(C10H16)H+
1 5 10 15 20 25 30
1 5 10 15 20 25 30
1 5 10 15
1 5 10 15
Time (min)

3 (A)

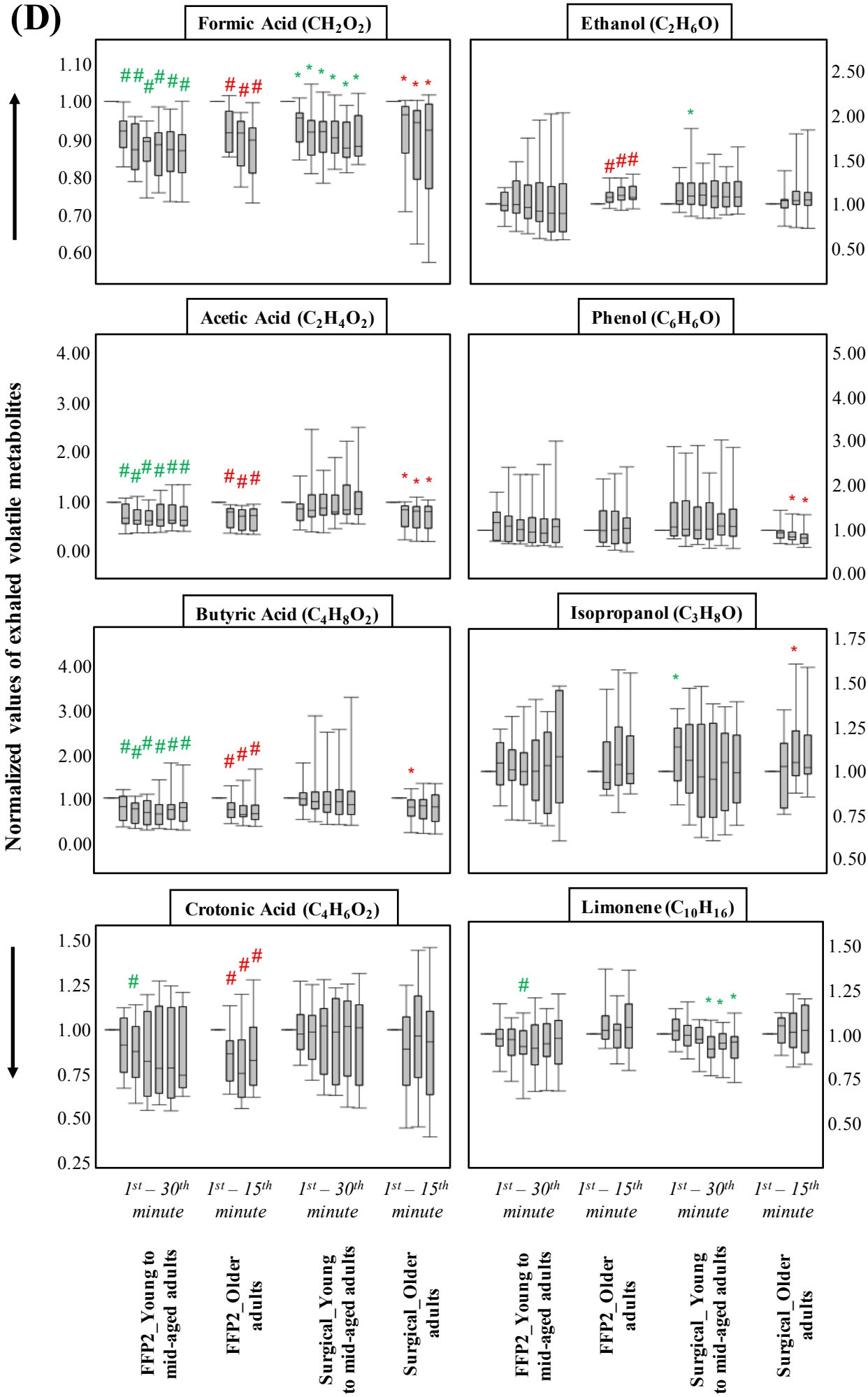

Peripheral oxygen saturation (SpO_2)
End-tidal CO_2 ($pET-CO_2$) - before and after mask use use
Normal range
Deoxygenation
Hypoxemia
Hypoventilation
Hyperventilation
Absolute values (%)
Absolute values (mmHg)
Respiratory rate (RR)
Absolute values (Breaths/min)
Before mask use
After mask use
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults
Cardiac output (CO)
Stroke volume (SV)
Pulse rate (PR)
Mean arterial pressure (MAP)
Normalized values of hemodynamic parameters
1st – 30th minute
1st – 15th minute

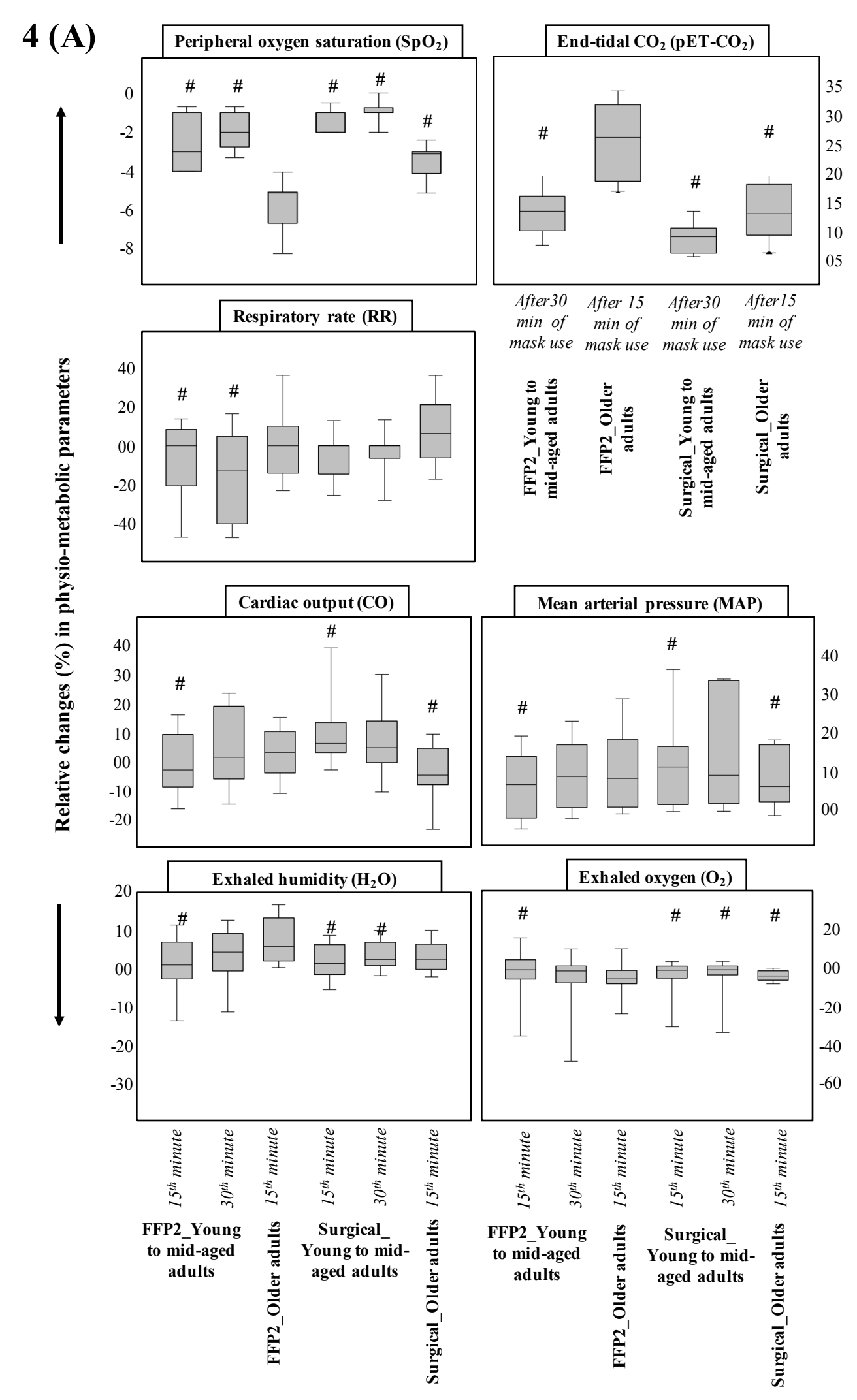

3 (B)
Acetaldehyde (C_2H_4O)
Hydrogen Sulfide (H_2S)
Butyraldehyde (C_4H_8O)
Dimethyl Sulfide/DMS (C_2H_6S)
Crotonaldehyde (C_4H_6O)
Allyl-methyl Sulfide/AMS (C_4H_8S)
Acrolein (C_3H_4O)
Butanethiol/MPS ($C_4H_{10}S$)
Normalized values of exhaled volatile metabolites
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults

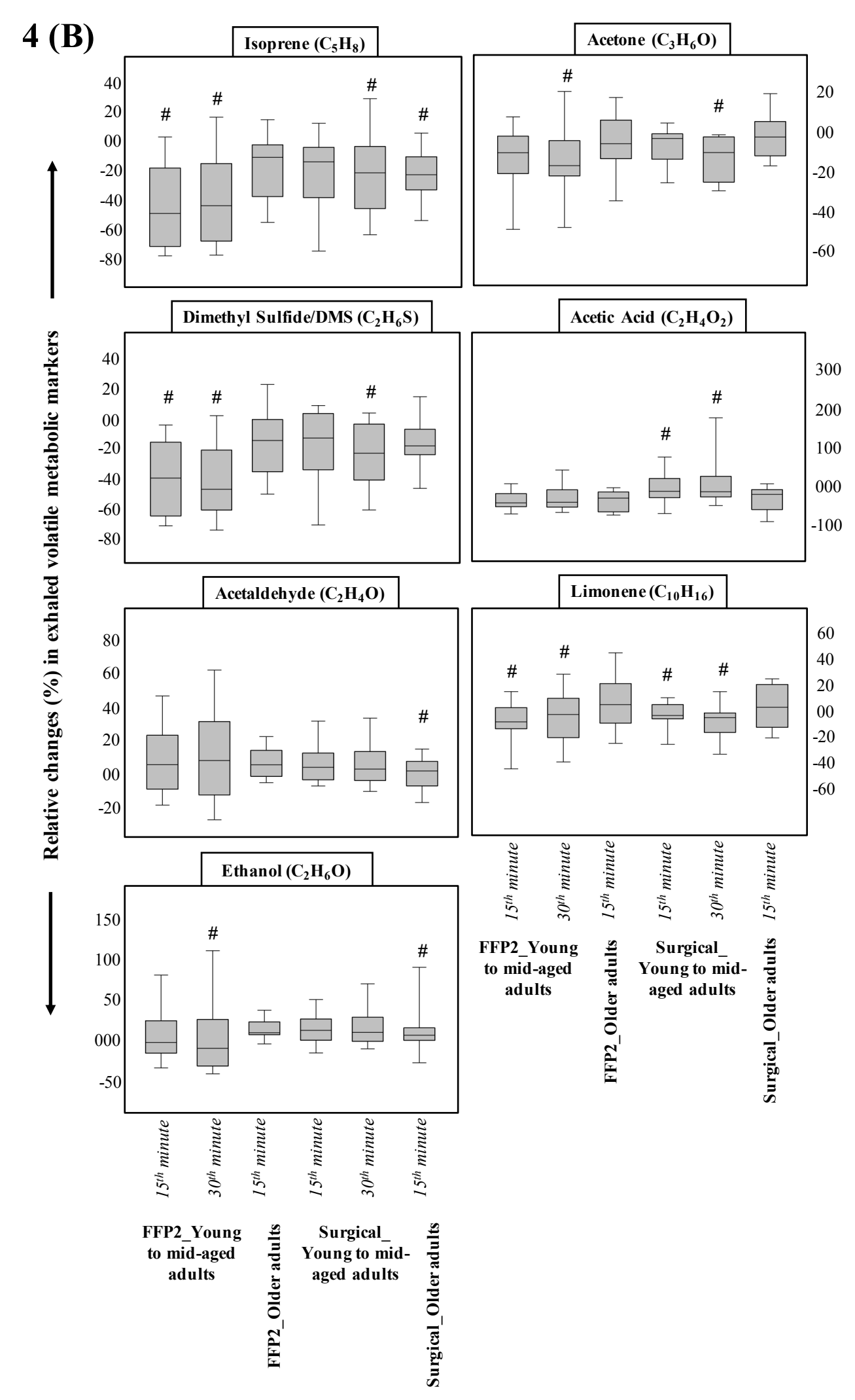
3 (C)


Isoprene (C_5H_8)
Acetone (C_3H_6O)
Benzene (C_6H_6)
Furan (C_4H_4O)
Toluene (C_7H_8)
Acetonitrile (C_2H_3N)
Exhaled humidity (H_2O)
Exhaled oxygen (O_2)
Normalized values of exhaled volatile metabolites
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults

3 (D)


Formic Acid (CH_2O_2)
Ethanol (C_2H_6O)
Acetic Acid ($C_2H_4O_2$)
Phenol (C_6H_6O)
Butyric Acid ($C_4H_8O_2$)
Isopropanol (C_3H_8O)
Crotonic Acid ($C_4H_6O_2$)
Limonene ($C_{10}H_{16}$)
Normalized values of exhaled volatile metabolites
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
1st – 30th minute
1st – 15th minute
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults


4 (A)
Peripheral oxygen saturation (SpO_2)
End-tidal CO_2 (pET-CO_2)
Respiratory rate (RR)
Cardiac output (CO)
Mean arterial pressure (MAP)
Exhaled humidity (H_2O)
Exhaled oxygen (O_2)
Relative changes (%) in physio-metabolic parameters
After30 min of mask use
After 15 min of mask use
After30 min of mask use
After15 min of mask use
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_Young to mid-aged adults
Surgical_Older adults
15th minute
30th minute
15th minute
15th minute
30th minute
15th minute
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_ Young to mid-aged adults
Surgical_Older adults


4 (B)
Isoprene (C_5H_8)
Acetone (C_3H_6O)
Dimethyl Sulfide/DMS (C_2H_6S)
Acetic Acid ($C_2H_4O_2$)
Acetaldehyde (C_2H_4O)
Limonene ($C_{10}H_{16}$)
Ethanol (C_2H_6O)
Relative changes (%) in exhaled volatile metabolic markers
15th minute
30th minute
15th minute
15th minute
30th minute
15th minute
FFP2_Young to mid-aged adults
FFP2_Older adults
Surgical_ Young to mid-aged adults
Surgical_Older adults

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

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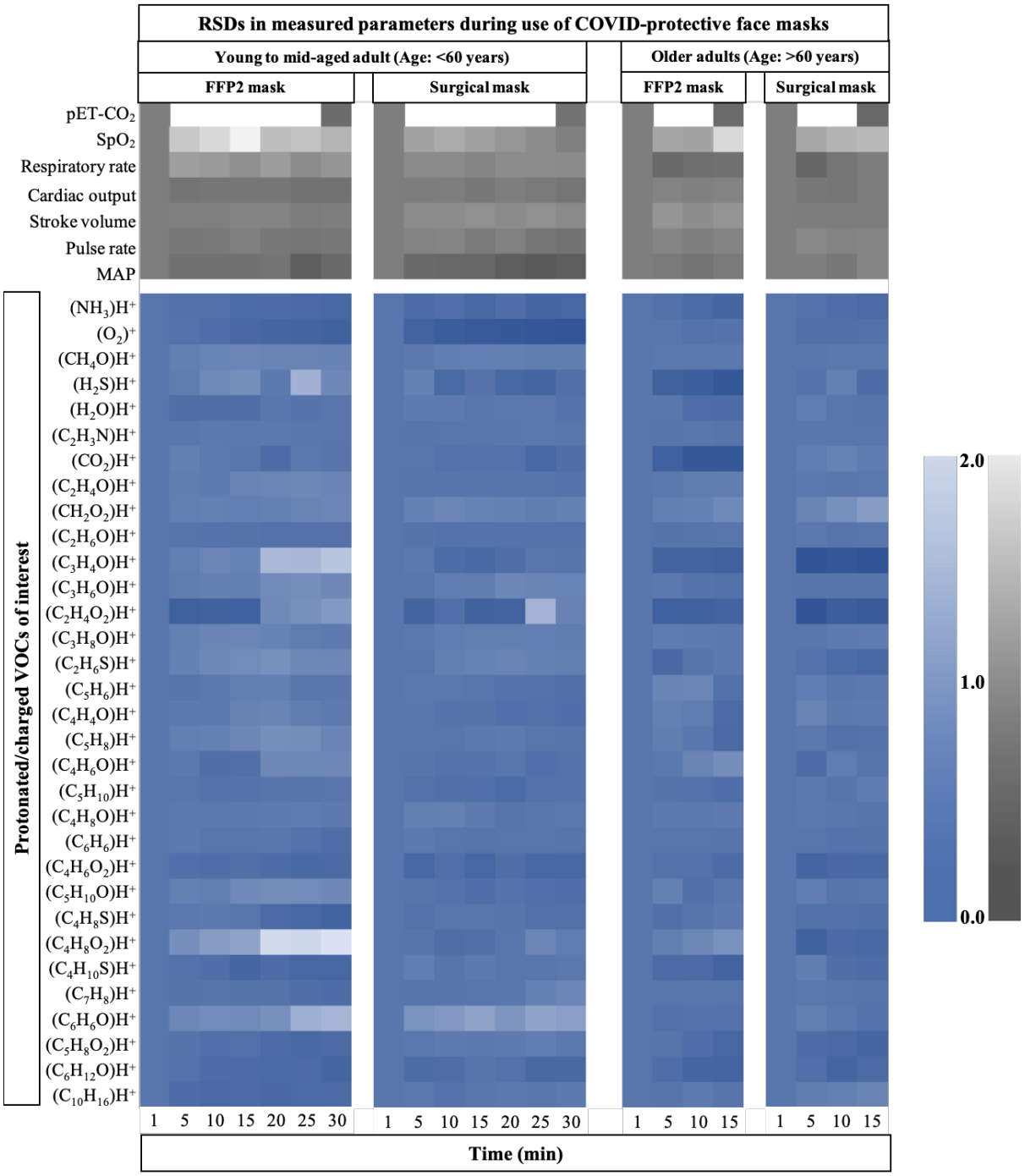
Supplementary information



Supplementary Figure S1: Relative changes in coefficient of variation (RSDs) of physiological parameters and of exhaled alveolar VOCs during the use of COVID-protective face masks by young to mid-aged and older adults. Y-axis represents the RSDs of physiological parameters viz. pET-CO_2, SpO_2, respiratory rate, cardiac output, stroke volume, pulse rate, mean arterial pressure (MAP) and the protonated/charged VOCs of interest. X-axis indicates time in minute. VOCs were tentatively identified according to their mass/charge ratio. For each individual, RSDs of VOCs were normalised onto corresponding values from the first minute. Respiratory-, hemodynamic parameters and SpO_2 are normalized likewise. The normalised RSDs from every 5th minute is presented here. The RSDs of pET-CO_2 are presented from immediately before and after mask use and are placed at the first and final minute of heatmaps. Light and dark colours symbolise relatively higher and lower RSDs of measured parameters, respectively.

Supplementary Table S1: FFP2 mask driven inter-VOC correlations (with respect to physiological parameters) along with corresponding *p*-values. Observed positive and negative correlation factors are presented. Statistically significant correlations (1 – tailed) are assigned with *p*-value ≤ 0.005.

Supplementary Table S2: Surgical mask driven inter-VOC correlations (with respect to physiological parameters) along with corresponding *p*-values. Observed positive and negative correlation factors are presented. Statistically significant correlations (1 – tailed) are assigned with *p*-value ≤ 0.005.

Supplementary Table S3: Results from pairwise-multiple comparisons of relative changes (in %) between groups. Statistical significances are tested by means of repeated measurement-ANOVA on ranks (p-value ≤ 0.005). From all pairwise-multiple comparisons, statistically significant difference along with corresponding p-values are listed with respect to % of changes caused by "FFP2 mask on older adults (FFP2_15min_O)". Statistically significant (p-value ≤ 0.005) correlations are assigned in bold.

SpO_2	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**<0.001**	**Yes**
	FFP2_30min_A	**<0.001**	**Yes**
	Surg_15min_A	**<0.001**	**Yes**
	Surg_30min_A	**<0.001**	**Yes**
	Surg_15min_O	**<0.001**	**Yes**

pET-CO2	Vs.	P-value	Sig.
FFP2_15min_O	Surg_30min_A	**<0.001**	**Yes**
	FFP2_30min_A	**<0.001**	**Yes**
	Surg_15min_O	**<0.001**	**Yes**

RR	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**<0.001**	**Yes**
	FFP2_30min_A	**0.003**	**Yes**
	Surg_15min_A	0.212	No
	Surg_30min_A	0.149	No
	Surg_15min_O	0.365	No

CO	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**0.002**	**Yes**
	FFP2_30min_A	0.35	No
	Surg_15min_A	**0.004**	**Yes**
	Surg_30min_A	1.471	No
	Surg_15min_O	**0.005**	**Yes**

MAP	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**0.003**	**Yes**
	FFP2_30min_A	1.25	No
	Surg_15min_A	**0.005**	**Yes**
	Surg_30min_A	0.007	No
	Surg_15min_O	**0.001**	**Yes**

Exhaled O_2	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**<0.001**	**Yes**
	FFP2_30min_A	0.266	No
	Surg_15min_A	**0.002**	**Yes**
	Surg_30min_A	**<0.001**	**Yes**
	Surg_15min_O	1.567	No

Exhaled H_2O	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**<0.001**	**Yes**
	FFP2_30min_A	0.37	No
	Surg_15min_A	**<0.001**	**Yes**
	Surg_30min_A	**0.005**	**Yes**
	Surg_15min_O	**0.001**	**Yes**

Isoprene	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**<0.001**	**Yes**
	FFP2_30min_A	**0.002**	**Yes**
	Surg_15min_A	1.453	No
	Surg_30min_A	**0.005**	**Yes**
	Surg_15min_O	**0.001**	**Yes**

Acetone	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	0.063	No
	FFP2_30min_A	**<0.001**	**Yes**
	Surg_15min_A	1.055	No
	Surg_30min_A	**<0.001**	**Yes**
	Surg_15min_O	1.121	No

DMS	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**0.003**	**Yes**
	FFP2_30min_A	**<0.001**	**Yes**
	Surg_15min_A	0.75	No
	Surg_30min_A	**0.004**	**Yes**
	Surg_15min_O	0.368	No

Acetic acid	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	2.003	No
	FFP2_30min_A	1.301	No
	Surg_15min_A	**0.001**	**Yes**
	FFP2_30min_A	**<0.001**	**Yes**
	Surg_15min_O	0.554	No

Acetaldehyde	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	1.81	No
	FFP2_30min_A	2.065	No
	Surg_15min_A	0.05	No
	Surg_30min_A	0.021	No
	Surg_15min_O	**0.001**	**Yes**

Limonene	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	**<0.001**	**Yes**
	FFP2_30min_A	**0.005**	**Yes**
	Surg_15min_A	**0.004**	**Yes**
	Surg_30min_A	**0.001**	**Yes**
	Surg_15min_O	1.331	No

Ethanol	Vs.	P-value	Sig.
FFP2_15min_O	FFP2_15min_A	0.006	No
	FFP2_30min_A	**0.001**	**Yes**
	Surg_15min_A	0.031	No
	Surg_30min_A	0.4	No
	Surg_15min_O	**0.003**	**Yes**