BBA - Molecular Basis of Disease 1865 (2019) 749–758

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

A pilot study of exercise-induced changes in mitochondrial oxygen metabolism measured by a cellular oxygen metabolism monitor (PICOMET)☆

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ARTICLE INFO

Keywords:
Mitochondrial oxygen tension
Mitochondrial oxygen consumption
Mitochondrial oxygen delivery
Bioimpedance analysis
Cellular oxygen metabolism
COMET

ABSTRACT

Impaired tissue oxygenation is the key pathomechanism in the development of organ dysfunction in shock; mitochondrial impairment can aggravate the condition. However, measuring tissue oxygenation directly and non-invasively still poses a clinical challenge. A novel device (COMET) allows the assessment of mitochondrial oxygen metabolism using the Protoporphyrin IX Triplet State Lifetime Technique (PpIX-TSLT). Critically ill patients, especially in sepsis, often exhibit oedema which may interfere with the COMET measurement. Furthermore, patients' physical activity level differs significantly before and during hospitalisation. Thus, the aim of this study was to identify the effects of physical activity and body composition on mitochondrial oxygen tension ($mitoPO_2$) and consumption ($mitoVO_2$) in healthy controls (N = 40). Furthermore, the study tested the repeatability of the COMET variables and identified covariates. Multiple COMET measurements were performed before (T_1, T_2), during and after (T_3, T_4) ergometry. Body composition was assessed by bioimpedance analysis. Physiological variables (blood pressure, heart rate, oxygen saturation) were recorded. In the analytical sample (n = 26), physical activity significantly decreased $mitoVO_2$; other COMET variables remained unchanged between T_2 and T_3. During ergometry, $mitoPO_2$ increased significantly. The distribution of body water significantly influenced $mitoVO_2$. In our setting, the method demonstrated moderate repeatability. Variables of fitness (heart rate recovery, phase angle and physical activity level), signal quality and duration of exposure to 5-aminolevulinic acid (obligatory for PpIX-TSLT) were identified as significant covariates of $mitoVO_2$. Mitochondrial oxygen delivery ($mitoDO_2$) was established as a new variable of COMET analysis. Results of this pilot study should be validated in future studies.

1. Introduction

Impaired tissue oxygenation plays a significant role in the development of organ dysfunction in shock. For septic shock in particular, key mechanisms for the propagation of multiple organ dysfunction are a disturbance of the macro-circulation, micro-circulation and mitochondrial dysfunction [1]. The hitherto employed surrogate parameters of impaired circulation such as central venous oxygen saturation, cardiac output, mean arterial pressure and lactate are of limited prognostic value [1]. To this day, clinicians still lack surrogate parameters of mitochondrial dysfunction. Therefore, new methods of measuring tissue and especially mitochondrial oxygenation may prove vital for the monitoring of critically ill patients.

Recently, Mik et al. developed a new method to non-invasively measure mitochondrial oxygen tension ($mitoPO_2$) and consumption ($mitoVO_2$) [2–4]. Briefly, the technique is based on the measurement of

☆ This article is part of a Special Issue entitled: The power of metabolism: Linking energy supply and demand to contractile function edited by Torsten Doenst, Michael Schwarzer and Christine Des Rosiers.

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https://doi.org/10.1016/j.bbadis.2018.12.003
Received 25 July 2018; Received in revised form 29 November 2018; Accepted 3 December 2018
Available online 26 December 2018

the delayed fluorescence of protoporphyrin IX, the final precursor of heme synthesis in mitochondria [2]. Applying 5-aminolevulinic acid (5-ALA, a precursor of protoporphyrin IX) to the skin allows the non-invasive measurement: excitation by a pulse of green light causes a measurable fluorescence which is directly associated with the mitoPO$_2$. Harms et al. published a pilot study on the feasibility of this technique in healthy volunteers using a prototype device [5]. The technique has been developed into a CE-certified device, the monitor for cellular oxygen and metabolism (COMET) [6]. This has already been used in a pharmacological study [7].

Before conducting further studies in complex medical conditions, we saw the necessity to further test the COMET system in healthy subjects. Hereby we sought to identify potential influence factors of the obtained COMET variables, such as physical activity or the body composition of individuals under physiological conditions. Furthermore, studies on the repeatability of COMET variables are not available to date. Taking all of these factors into consideration, this study sought to achieve three main objectives: 1) to test whether physical activity influences mitochondrial oxygen metabolism 2) to analyse the repeatability of COMET variables and 3) to elucidate the influence of body composition as measured by bioimpedance analysis (BIA) on COMET-acquired variables in healthy individuals. This last objective is of particular interest, as critically ill patients frequently develop oedema due to endothelial barrier dysfunction and this may affect COMET measurement.

The primary endpoint of this study was the difference in COMET variables before and after physical activity. With the development of an alternative fitting method for COMET analysis we additionally introduced the novel variable mitochondrial oxygen delivery (mitoDO$_2$).

2. Methods

2.1. Recruitment

Healthy adults were recruited via the Jena University Hospital notice boards and social media. Subjects were required to be at least 18 years old, provide written informed consent and show readiness for physical activity as assessed by the Physical Activity Readiness Questionnaire [8] and confirmed by the study team. The following exclusion criteria prevented enrolment: significant cardiac, pulmonary or musculoskeletal conditions, allergies to contents of the Alacare® plaster (photonamic, Wedel, Germany), porphyria, skin conditions aggravated by sunlight or increased sensitivity to light, pregnancy or breastfeeding, participation in another interventional study, or prior participation in this study.

2.2. Experimental setup

Fig. 1 shows an overview of the experimental setup. First, BIA was performed after a resting period of 10 min. Subsequently, dynamic COMET measurements were performed 5 min apart as baseline analyses (T_1 and T_2). Then, the subjects performed submaximal cycle ergometry. Immediately after the physical activity (T_3), three dynamic COMET measurements were performed. After 10 min of recuperation (T_4), the measurements were repeated. Oxygen saturation (SpO_2) and heart rate (HR) were measured continuously during the entire experiment using standard pulse oximetry. Arterial blood pressure was measured non-invasively once for every time point (T_1–T_4) and every 2 min during the ergometry.

2.3. COMET measurement

Individuals were instructed to apply an approximately 2 cm^2 large 5-ALA containing plaster (Alacare®, photonamic, Wedel, Germany) to a patch of shaved, washed and dried skin in the middle of their thorax at least 6 h before measurement. All measurements were performed in the supine position using COMET measurement system (Photonics Healthcare, Utrecht, Netherlands). Dynamic continuous measurements took 105 s consisting of three steps: positioning the sensor and measuring a baseline for 30 s, applying pressure for 45 s to inhibit the microcirculation of that part of the skin to measure oxygen consumption and lastly releasing pressure and concluding the measurement after another 30 s. One such measurement was performed three times for each time point of the experiment. Between measurements, the plaster was reapplied. During physical activity, 10 single measurements were performed for each intensity level.

2.4. Exercise regimen

As the COMET measurement demanded the subjects to remain supine, physical activity was performed using a cycle ergometer from the supine position. The exercise regimen was adapted from a study by Finger et al. [9]. The intensity was initially set to 50 W and subsequently increased by 25 W every 2 min. Subjects were instructed to aim for a pedal frequency of 60 rotations per min receiving feedback when deviating from this target frequency. Physical activity was terminated once the target HR was reached for 30 s, the subject reported exhaustion or a medical reason necessitated termination. The target HR was:

$$\text{target HR} = (208 - 0.7 \times \text{age} - \text{resting HR}) \times 0.6 + \text{resting HR} \quad (1)$$

resulting in a moderate intensity target [10,11]. The Borg scale was used for the self-assessment of exertion (6 = no exertion at all to 20 = maximum exertion) and breathlessness (6 = breathlessness to 20 = too high, I have to stop) at the end of the exercise [12,13]. Heart rate recovery was determined here as the percental change between the maximum heart rate during and 4 min after ergometry (before third dynamic measurement at T_3).

2.5. Bioimpedance analysis

BIA was performed using a SECA medical Body Composition Analyzer 525 (seca Germany, Hamburg, Germany). Analysis yielded

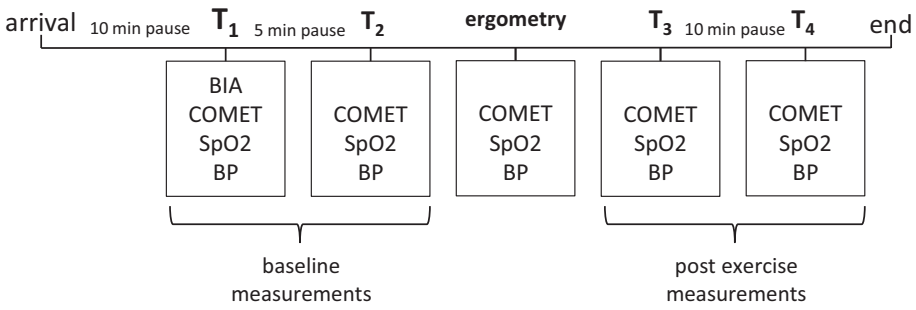

Fig. 1. Experimental setup. COMET measurements, blood pressure monitoring (BP) and pulse oximetry (SpO_2) were performed at 4 points in time (T_1–T_4) and every 2min during ergometry. Bioimpedance analysis (BIA) was performed at T_1.

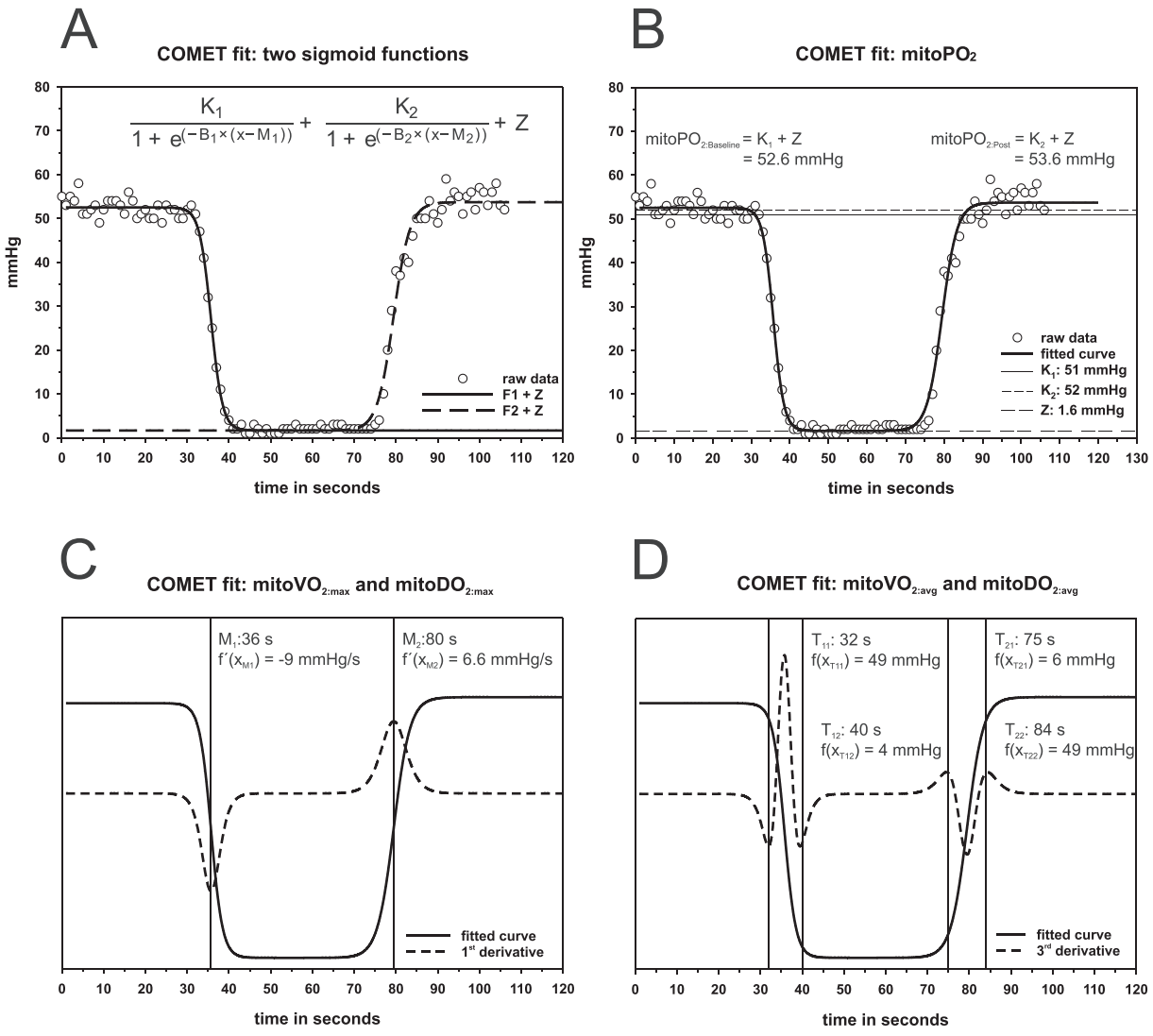

Fig. 2. COMET fit functions. (A) Raw data and sigmoid fit functions (F1 and F2). (B) Estimation of baseline mitochondrial oxygen tension ($mitoPO_{2:\ Baseline}$) and mitochondrial oxygen tension after reoxygenation ($mitoPO_{2:\ Post}$) (C) Estimation of the maximum oxygen consumption ($mitoVO_{2:\ max}$) and maximum oxygen delivery ($mitoDO_{2:\ max}$) using the slope (1st derivative) at the fitted M_1 parameter and M_2 parameter respectively. (D) Estimation of the average oxygen consumption ($mitoVO_{2:\ avg}$) and average oxygen delivery ($mitoDO_{2:\ avg}$). After identification of the inflection points (T_{11}, T_{12}, T_{21}, T_{22}) of the fitted curve using the 3rd derivative, the velocity of oxygen consumption and oxygen delivery can be derived. E.g. the first two inflection points (T_{11} = 32 s, T_{12} = 40 s) differ 8 s in time and 45 mm Hg in $mitoPO_2$ ($mitoPO_{2:\ T11}$ = 49 mm Hg, $mitoPO_{2:\ T21}$ = 4 mm Hg). This results in $mitoPO_{2:\ avg}$ of 5.625 mm Hg/s. For better visual clarity, the $mitoPO_2$ values of the fitted curves and derivatives were standardized in sections C and D.

following variables: fat mass (FM, %), fat free mass (FFM, %), skeletal muscle mass (SMM, %), total body water (TBW, %), extracellular water (ECW, %) and ECW/TBW ratio (%). In addition, the raw and standardized values [14] of resistance (Ω), reactance (Ω) and the corresponding phase angle (°) were analysed. The Physical Activity Level (PAL), as a subjective rating of daily activity (1.2 = extremely inactive to 2 = vigorously active), was assessed [15].

2.6. Statistical analysis

2.6.1. Estimation of the COMET variables

To fit the COMET signal we used two complementary sigmoid functions (Fig. 2A):

$$\frac{K_1}{1 + e^{(-B_1 \times (x - M_1))}} + \frac{K_2}{1 + e^{(-B_2 \times (x - M_2))}} + Z \quad (2)$$

The mean of baseline $mitoPO_2$ is the sum of K_1 and Z, where Z is the steady state of $mitoPO_2$ during inhibition of the microcirculation (Fig. 2B). Accordingly, $mitoPO_2$ after refilling is the sum of K_2 and Z. B_1 and B_2 modulate the steepness of oxygen consumption and reoxygenation, respectively. M_1 and M_2 indicate the time of the maximum slope of oxygen consumption during inhibition of microcirculation and the maximum slope of reoxygenation after the release of pressure, respectively (Fig. 2C). Therefore, the maximum oxygen consumption ($mitoVO_{2:\ max}$) was determined via the first derivative of the fitted curve at M_1 (Fig. 2C). The maximum reoxygenation ($mitoDO_{2:\ max}$) – a newly introduced variable for tissue reoxygenation – was determined via the first derivative of the fitted curve at M_2 (Fig. 2C). The duration of the ascending and descending part of the fit function was calculated in a standardized way using the inflection points (using the third derivative, Fig. 2D). The average oxygen consumption ($mitoVO_{2:\ avg}$) and average oxygen delivery ($mitoDO_{2:\ avg}$) were determined as difference in $mitoPO_2$ between inflection points divided by difference in time between inflection points (Fig. 2D). A more comprehensive overview of the fitted parameters is displayed in Supplementary material A. We used the *fit* function in MATLAB (MATLAB and Statistics Toolbox Release 2017a, The MathWorks, Inc., Natick, Massachusetts, United

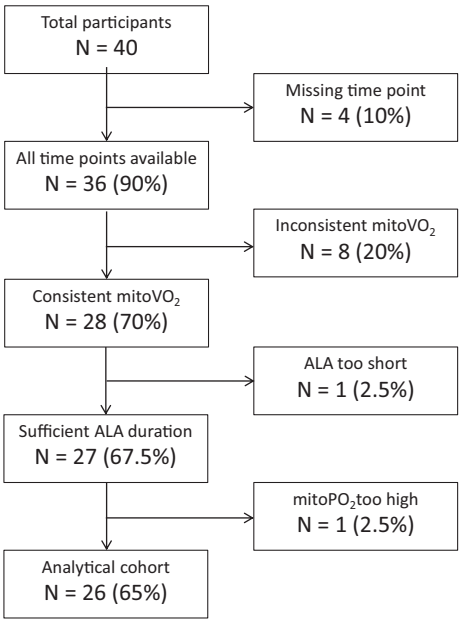

Fig. 3. Overview of the subject inclusion and final analytical cohort. STROBE diagram.

States) to estimate the parameters of the fit function. The model specifications are listed in Supplementary material A.

2.6.2. Descriptive analysis of COMET variables

We report means, standard deviations (SD), medians, and first and third quartiles (Q_1/Q_3) of the COMET variables for all time points (T_1–T_4). Upper and lower threshold were defined as Q_3 plus and Q_1 minus 1.5 times the interquartile range, respectively.

Variable distributions were inspected using histograms, Q-Q-plots, Shapiro-Wilk-Tests and estimation of kurtosis and skewness with corresponding standard errors (SE).

2.6.3. Activity-induced changes

To analyse activity-induced differences, we compared COMET variables between T_2 and T_3 using paired samples *t*-tests and Wilcoxon signed-rank tests. We used generalized estimating equations (GEE) to analyse differences between all time points (T_1–T_4). GEE are appropriate for repeated measurements and produce robust parameter estimates and standard errors [16]. The factor *time* was modelled as within-subject variable and within-subject dependencies were modelled first order autoregressive. The model type was set to linear (distribution: normal, link function: identity). Estimated marginal means for time points (T_1–T_4) were compared pairwise.

During the ergometry, mitoPO$_2$ was averaged for every intensity level. For comparability between subjects, data from the first, median and last intensity level were analysed. Mean differences in mitoPO$_2$ and physiological data were also analysed using GEE.

2.6.4. Repeatability of COMET variables

2.6.4.1. Intra-session analysis. After exclusion of outliers, all available measurement pairs of COMET variables within T_1 (e.g. 1st vs. 2nd, 1st vs. 3rd and 2nd vs. 3rd measurement of T_1), T_2 and T_4 were included in the analysis. First, we report descriptive mean differences between measurement pairs and p values of the paired samples *t*-tests. Second, we report Pearson correlation coefficients of all available measurement pairs. Coefficients were interpreted as follows: r < 0.10 negligible, r 0.1–0.39 weak, 0.40–0.69 moderate, 0.70–0.89 strong, 0.90–1.00 very strong [17]. Third, we estimated intra-class correlation coefficients (ICC) for all available measurements pairs using the two-way mixed effects analysis of variance (ANOVA) for single measures with absolute agreement [18]. ICCs and corresponding confidence intervals were interpreted as follows: ICC < 0.5 poor, 0.5 < ICC < 0.75 moderate, 0.75 < ICC < 0.90 good and ICC > 0.90 excellent reliability [18]. Fourth we used Bland-Altman plots to assess limits of agreement (LOA) [19,20]. On population level, 95% of COMET variable differences within one session with several measurements according to our protocol should lie within these LOA. In these plots, the mean of a measurement pair is plotted against the difference between both measurements (LOA = mean of the differences ± 1.96 × SD of the differences). The corresponding 95% confidence intervals (95%CI) for LOA were estimated based on two-sided tolerance factors [21]. Finally, we calculated the standard error of measurement (SEM) as the square root of the mean square error term from repeated-measures ANOVA for all available measurement pairs [22]. The corresponding Minimum Detectable Difference (MDD) was calculated as SEM × 1.96 × $2^{1/2}$.

2.6.4.2. Inter-session analysis. All available measurements from T_1 and T_2 were averaged separately for every participant and similar analyses as described above were performed. Because of the averaging we used ANOVA for average measures with absolute agreement to determine ICCs [18].

2.6.5. Exploratory analysis of covariates

For correlative analysis, COMET variables were averaged for T_1 and T_2. Using simple linear regression models and non-parametric Spearman's rank correlation coefficients associations between COMET variables, participant data (age, sex, height, weight, BMI, PAL and heart rate recovery), physiological data (heart rate, SpO_2, systolic and diastolic blood pressure) and BIA variables (see Section 2.3) were analysed.

Statistical analysis was performed with SPSS Statistics 24 (IBM Corporation, Armonk, NY, USA). A significance level of 5% was applied and two-sided p values are reported. When comparing multiple time points, Bonferroni-Holm correction of p values was performed.

3. Results and discussion

3.1. Sample description

In total, 40 subjects were enrolled in this study. Of these, 14 were excluded from final analysis (Fig. 3). Table 1 shows the characteristics of the sample population.

Several reasons led to the relatively high exclusion rate in this study. Correct skin preparation and application of the 5-ALA plaster by the subjects is essential for COMET measurement. However, this was assessed by questionnaire but not ultimately verifiable outside of the clinical setting. Furthermore, sweating and movement artefacts, which were unavoidable in this study, may have influenced some

Table 1
Characteristics of the analytical sample. Mean, standard deviation (SD) and 95% confidence interval (95%CI) for age, height, weight, body mass index (BMI) und exposure time to ALA are displayed for the analytical sample of 26 individuals.

Variable		Number	Percent
Sex	Female	16	61.5
	Male	10	38.5

Variable	[Unit]	Mean	SD	95%CI	
Age	[years]	27	7	24	29
Height	[cm]	174	8	171	177
Weight	[kg]	70	9	67	74
BMI	[kg/m^2]	23	2	22	24
ALA	[hours]	9.32	2.46	8.38	10.27

measurements. As the study design required that only subjects with complete datasets were included in the final analysis, missing data at any of the time points led to exclusion. In the clinical setting – where these factors can be controlled – we experienced a very low rate of invalid measurements. Additionally, the positioning of the COMET sensor and its shielding from other light sources were improved, hereby greatly enhancing the quality of the later measurements and yielding reliable values.

As many of our subjects were young and slim, our values may differ from those measured in critically ill patients, which may be significantly older or more obese.

In some participants, no reduction in $mitoPO_2$ was seen during the dynamic measurement and hence no $mitoVO_2$ was determinable. These were excluded from later analysis. Post hoc analysis yielded no significant differences in covariates between the affected cohort and the analytical cohort. The cause of this effect remains unclear and requires further methodical investigation.

3.2. Variable distribution

Measures of central tendency and variation of the COMET variables are displayed in Table 2, Fig. 4 and Supplementary material B. $MitoPO_2$ values were distributed near to normal (skewness: 0.20, SE: 0.15; kurtosis: 0.21, SE: 0.30), however the p value of the Shapiro-Wilk test was significant. $MitoVO_{2:\ max}$ (skewness: 1.15, SE: 0.15; kurtosis: 3.22, SE: 0.30), $mitoVO_{2:\ avg}$ (skewness: 0.88, SE: 0.15; kurtosis: 1.80, SE: 0.30), $mitoDO_{2:\ max}$ (skewness: 2.00, SE: 0.15; kurtosis: 6.31, SE: 0.30) and $mitoDO_{2:\ avg}$ (skewness: 0.52, SE: 0.15; kurtosis: 0.36, SE: 0.30) values were positively skewed. The p values for all Shapiro-Wilk tests reached significance. Especially values of kurtosis were highly influenced by outliers. After removing outliers, all variables were distributed near to normal.

To the best of our knowledge, no group has published $mitoPO_2$ and $mitoDO_2$ data of healthy controls using the COMET system. Compared to the study of Harms et al. [5] using a prototype of the system, our baseline $mitoPO_2$ values were about 20 mm Hg higher but the variation of $mitoPO_2$ variables was similar. Technical differences, especially in the sensor design, may account for these diverging results. In contrast, our determined $mitoVO_{2:\ max}$ showed a very similar mean and standard deviation compared to the study of Harms et al. [5]. $MitoVO_2$ seems to be independent or at least only moderately correlated with $mitoPO_2$. Van Diemen et al. [7] reported a baseline $mitoVO_2$ of 8.88 mm Hg/s, which was higher than our findings. This difference may be attributed to different fitting procedures of the dynamic COMET measurements. We used a new fitting approach to estimate the COMET variables. Hereby, we can model the whole dynamic measurement including the reoxygenation phase. Most notably, this yielded the new variable termed oxygen delivery ($mitoDO_2$). The proposed procedure is highly flexible for different measurement protocols. As displayed in Table 2, our fitting procedure could explain in median 99% of the variance of a

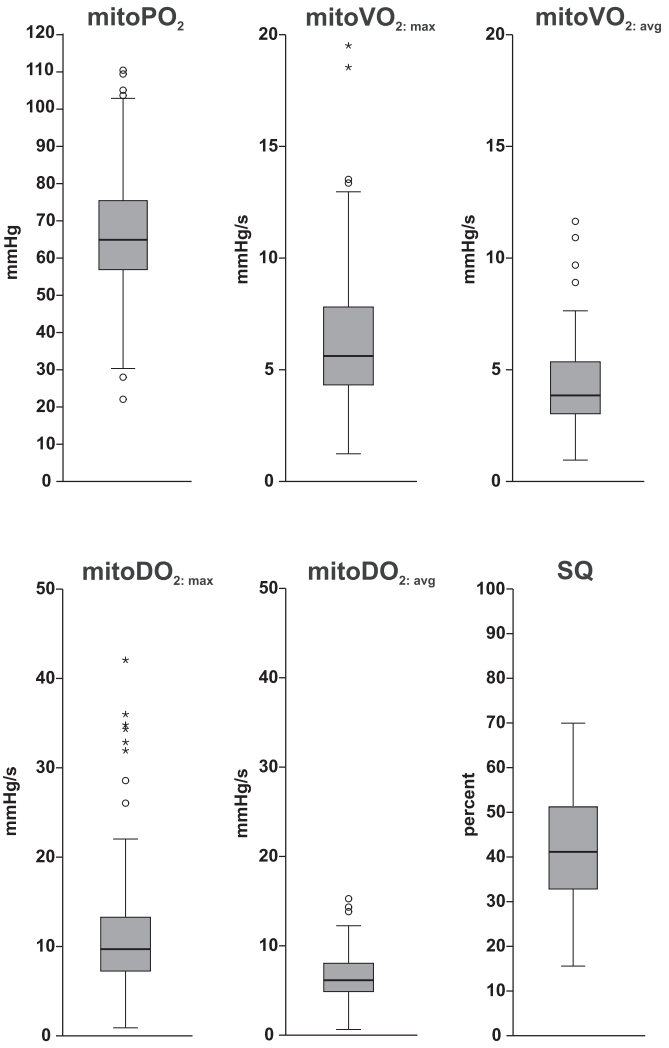

Fig. 4. Boxplots of COMET variables and signal quality (SQ) for all time points. Outliers ($Q_{1/3}$ ± 2 interquartile range) are marked with white circles and extreme outliers ($Q_{1/3}$ ± 3 interquartile range) are marked with asterisks. [$MitoPO_2$: mitochondrial oxygen tension|$MitoVO_2$: mitochondrial oxygen consumption|$MitoDO_2$: mitochondrial oxygen delivery].

Table 2
Descriptive statistics for COMET variables. All time points (T_1, T_2, T_3, T_4) are included.

Variable	Unit	N	Mean	SD	95%CI		Median	Q_1	Q_3	Threshold	
										Lower	Upper
$MitoPO_2$	[mm Hg]	261	66.31	15.97	64.38	68.25	64.91	56.82	75.55	28.71	103.65
$MitoVO_{2:\ max}$	[mm Hg/s]	259	6.15	2.67	5.83	6.48	5.62	4.31	7.83	0.00	13.10
$MitoVO_{2:\ avg}$	[mm Hg/s]	258	4.19	1.67	3.99	4.39	3.85	3.03	5.36	0.00	8.87
$MitoDO_{2:\ max}$	[mm Hg/s]	259	10.83	5.87	10.11	11.54	9.70	7.22	13.28	0.00	22.36
$MitoDO_{2:\ avg}$	[mm Hg/s]	259	6.52	2.49	6.22	6.83	6.15	4.86	8.05	0.07	12.84
Sensor temperature	[°C]	261	31.33	1.44	31.15	31.50	31.21	30.31	32.26	27.37	35.19
Signal quality	[percent]	261	41.91	12.19	40.43	43.39	41.15	32.83	51.23	5.24	78.83
R^2	[0–1]	261	0.98	0.07	0.97	0.99	0.99	0.98	0.99	0.96	1.00

avg: average|max: maximum|$MitoPO_2$: mitochondrial oxygen tension|$MitoVO_2$: mitochondrial oxygen consumption|$MitoDO_2$: mitochondrial oxygen delivery|$Q_{1/3}$: first and third quartile|SD: standard deviation|95%CI: 95% confidence interval.
Threshold is defined as $Q_1 - 1.5 \times$ interquartile range and $Q_3 + 1.5 \times$ interquartile range.
$MitoVO_{2:\ max}$ and $mitoVO_{2:\ avg}$ showed a Pearson correlation coefficient of $r = 0.990$ ($p < 0.001$) and Spearman correlation coefficient of $\rho = 0.995$ ($p < 0.001$).
$MitoDO_{2:\ max}$ and $mitoDO_{2:\ avg}$ showed a Pearson correlation coefficient of $r = 0.928$ ($p < 0.001$) and Spearman correlation coefficient of $\rho = 0.982$ ($p < 0.001$).

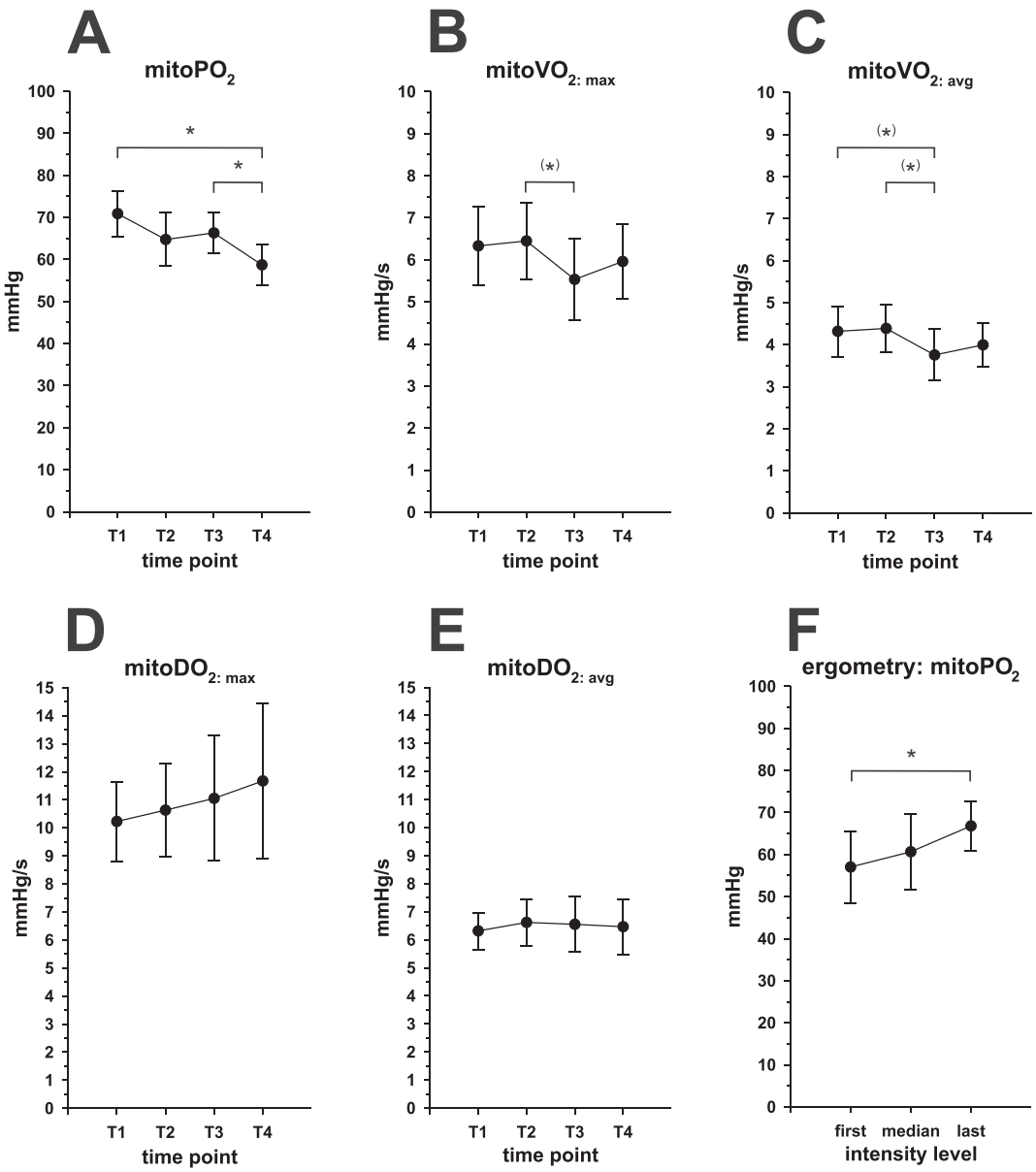

Fig. 5. COMET variables over the course of the experiment. (A–E) Marginal means and 95% confidence intervals (error bars) resulting from generalized estimating equation models (GEE) of the COMET variables for all time points. (F) Marginal means and 95% intervals (error bars) resulting from GEE of mitoPO$_2$ during ergometry. Data is displayed for the first, median and last intensity level of ergometry. All p-values were adjusted for multiple comparisons using Bonferroni-Holm correction. Significant differences are marked with * and significant differences before correction are marked with $^{(*)}$ [MitoPO$_2$: mitochondrial oxygen tension|MitoVO$_2$: mitochondrial oxygen consumption|MitoDO$_2$: mitochondrial oxygen delivery|T$_1$: baseline 1|T$_2$: baseline 2|T$_3$: first time point after ergometry|T$_4$: second time point after ergometry].

dynamic COMET measurement. Diffuse oxygen influx, however, as considered in other fitting procedures [3], is not part of our model estimation.

The p values of the Shapiro-Wilk tests for all COMET variables were significant. Nonetheless, visual inspection, QQ-plots and outlier analyses revealed that variables were distributed close to normal. In the study of Harms et al. [5] mitoPO$_2$ and mitoVO$_2$ followed the normal distribution according to Shapiro-Wilk tests. This difference may be due to different sample sizes, since in larger samples even small deviations from the normal distribution can reach significance.

Finally, we reported lower and upper thresholds for COMET variables. These may be of importance for future studies in healthy controls. Nonetheless, larger studies for establishing norm values are needed.

3.3. Primary endpoint: activity-induced changes in COMET variables

3.3.1. Comparison of T_2 and T_3

MitoPO$_2$ values did not differ significantly between T$_2$ (M = 64.72, SD = 16.83) and T$_3$ (M = 66.47, SD = 13.00, t(25) = −0.684, p = 0.500; Fig. 5A). MitoVO$_{2:\ max}$ showed a significant decrease between T$_2$ (M = 6.45, SD = 2.43) and T$_3$ (M = 5.53, SD = 2.56, t(25) = 2.29, p = 0.031, Fig. 5B). Accordingly, mitoVO$_{2:\ avg}$ decreased significantly between T$_2$ (M = 4.38, SD = 1.51) and T$_3$ (M = 3.75, SD = 1.63, t(25) = 2.41, p = 0.024; Fig. 5C). Neither did mitoDO$_{2:\ max}$ show significant differences between T$_2$ (M = 10.63, SD = 4.38) and T$_3$ (M = 11.05, SD = 5.93, t(25) = −0.385, p = 0.703; Fig. 5D) nor did mitoDO$_{2:\ avg}$ (T$_2$: M = 6.62, SD = 2.21 vs. T$_2$: M = 6.55, SD = 2.63, t(25) = 0.147, p = 0.884; Fig. 5E). Non-parametric testing revealed similar results.

MitoPO$_2$ values showed a general decline for the time points T$_1$, T$_2$ and T$_4$ (Fig. 5A). In these models, mitoVO$_{2:\ max}$ and mitoVO$_{2:\ avg}$ also differed significantly between T$_2$ and T$_3$. After Bonferroni-Holm correction the p values did not reach significance. Both mitoDO$_2$ variables showed no significant changes between time points.

We found no significant differences in mitoPO$_2$ between T$_2$ and T$_3$ (Fig. 5A). However, mitoPO$_2$ levels decreased over time except between T$_2$ and T$_3$ (Fig. 5A). Thus, the general decrease in mitoPO$_2$ may have masked smaller activity-induced changes. It is known, that with every measurement a small part of mitochondrial oxygen is consumed via photo-consumption [3], the decrease in mitoPO$_2$ may be explained by this effect.

The mitochondrial oxygen consumption was decreased after physical activity (Fig. 5B, C). Though the effect was small, it is surprising, as oxygen consumption should be physiologically increased after exercise, a phenomenon referred to as excess post-exercise oxygen consumption [23]. A decrease in oxygen consumption in skin cells as response to increased muscular oxygen demand may explain the observed effect. However, due to the methodology applied, the mitoVO$_2$ – at least in part – might also be influenced by an increased cardiac output, indicated by a significant rise in heart rate and blood pressure (Table 3) – leading to an improved microcirculation and subsequently to a potential higher diffuse oxygen influx [3]. However, this speculation needs to be validated in further studies.

3.3.2. Changes in mitoPO$_2$ during ergometry

Table 3 summarises the findings for the first, median and last level of the ergometry. As expected, exercise caused significant changes in heart rate, blood pressure and, albeit minor ones, in SpO$_2$. At the end of the ergometry, subjects reported a subjective exertion of 15.96 ('hard', SD = 1.04) and a subjective breathlessness of 13.42 ('quite strong', SD = 2.35) on the Borg scale. There was a significant increase in mitoPO$_2$ between first and last level of ergometry (Fig. 5F). Additional information on the ergometry can be found in Supplementary material C.

The increase of mitoPO$_2$ during the physical activity could indicate the physiological adaption to increased oxygen demands during the exercise.

3.4. Secondary endpoint: stability of COMET variables

3.4.1. Intra-session stability

Table 4 summarises the findings for intra-session stability including LOA, SEM and MDD. Single measurements showed moderate to strong Pearson correlation coefficients (0.627 to 0.738, all p values < 0.001). Mean differences and paired sample *t*-tests revealed significant decreases in mitoPO$_2$ and significant increases in mitoDO$_2$ variables between subsequent measurements. ICCs showed significant p values (p < 0.01) for all COMET variables and can be interpreted as moderate (0.583 to 0.733). E.g. the LOA for mitoPO$_2$ of 20.76 mm Hg and −25.13 mm Hg as well as the MDD of 22.95 mm Hg may suggest that changes in mitoPO$_2$ of about ± 20 mm Hg during one session may indicate an error of measurement, i.e. sensor displacement.

Like Harms et al. [5], we also recommend attaching the sensor to the skin to reduce movement artefacts and intra-individual variation. In addition, we recommend the use of a sufficiently large ALA plaster (4 cm^2) to increase the sensitized area for COMET measurement.

3.4.2. Inter-session stability

Table 5 summarises the findings for inter-session stability including LOA, SEM and MDD. Both averaged measurements (T$_1$ and T$_2$) showed moderate Pearson correlation coefficients (0.472 to 0.541, all p values < 0.05). Mean differences (M = −6.14, SD = 15.07) and paired sample *t*-tests revealed significant decreases in mitoPO$_2$ between T$_1$ and T$_2$. ICCs showed significant p values (p < 0.05) for all COMET variables and can be interpreted as moderate (0.62 to 0.71).

The LOA for mitoPO$_2$ of 23.4 mm Hg and −35.68 mm Hg as well as the MDD of 29.54 mm Hg may suggest that changes in average mitoPO$_2$ of about 30 mm Hg between two sessions may indicate a true change. This may be of interest for future (clinical) studies to evaluate changes in patient variables or to evaluate the effect of therapeutic interventions.

3.5. Analysis of covariates

A comprehensive overview of the covariate analyses is shown in Supplementary material D. The major findings are summarised in Table 6: At baseline (T$_1$ and T$_2$), mitoPO$_2$ and mitoDO$_2$ showed no correlative association with patient data, physiological variables and BIA results. MitoVO$_{2:\ max}$ and mitoVO$_{2:\ avg}$ were positively associated with heart rate recovery. In addition, mitoVO$_{2:\ max}$ and mitoVO$_{2:\ avg}$ showed positive associations with PAL. A higher signal quality was associated with higher levels of mitoVO$_{2:\ max}$ and mitoVO$_{2:\ avg}$. The duration of ALA application was only significantly correlated with mitoVO$_{2:\ max}$ and tended to correlate with mitoVO$_{2:\ avg}$. The ECW/TBW ratio was significantly, negatively associated with mitoVO$_{2:\ max}$ and by trend with mitoVO$_{2:\ avg}$. Finally, the raw phase angle showed significantly positive associations with mitoVO$_{2:\ max}$ and mitoVO$_{2:\ avg}$. Non-parametric analyses yielded similar findings. Visual inspection of scatter plots and non-linear fit procedures revealed no significant associations between COMET variables and patient data, physiological variables and BIA results (data not shown) with very few exceptions: after removing the only subject aged above 40 years, mitoPO$_2$ showed a significant negative correlation with age. In addition, the room temperature and ECW/TBW showed a quadratic relation to mitoPO$_2$.

A higher heart rate recovery after exercise, raw phase angle and PAL correlated with a higher mitoVO$_2$. Fitter people usually exhibit a higher heart rate recovery after exercise [24] and phase angle has been correlated with fitness [25] and muscle mass [26]. Systemic oxygen consumption capacity VO$_{2max}$ is also increased in fitter people [27].

Table 3
MitoPO$_2$ and physiological variables during ergometry. Marginal means and 95% confidence intervals (95%CI) of the generalized estimating equation (GEE) models for mitoPO$_2$ and physiological variables during ergometry are displayed. Variables are shown for the first, median and last intensity level. p values (Bonferroni-Holm corrected) result from the pairwise contrasts for the first, median and last intensity level. Significant p values are printed in bold.

Variable	[Unit]	First			Median				Last				
		Mean	95%CI		Mean	95%CI		$p_{first\ vs.\ median}$	Mean	95%CI		$p_{first\ vs.\ last}$	$p_{median\ vs.\ last}$
MitoPO$_2$	[mm Hg]	57.01	48.47	65.55	60.63	51.56	69.70	0.338	66.80	60.86	72.73	**0.030**	0.338
Intensity level	[Watt]	50.00	50.00	50.00	103.85	96.95	110.74	< **0.001**	165.38	152.31	178.46	< **0.001**	< **0.001**
Heart rate	[bpm]	106.38	102.10	110.67	126.58	123.49	129.66	< **0.001**	157.07	153.86	160.28	< **0.001**	< **0.001**
SpO$_2$	[%]	98.58	97.93	99.23	98.46	97.89	99.04	0.763	97.44	96.79	98.09	**0.037**	**0.001**
BP: sys	[mm Hg]	140.88	134.27	147.49	156.56	149.06	164.06	< **0.001**	177.41	168.07	186.76	< **0.001**	< **0.001**
BP: dia	[mm Hg]	83.00	78.17	87.83	87.16	82.34	91.98	0.238	94.13	85.11	103.14	0.070	0.238

BP: dia = blood pressure systolic|BP: sys = blood pressure diastolic|SpO$_2$ = saturation of peripheral oxygen (pulse oximetry).

Table 4
Intra-session stability of the COMET variables. The mean difference, standard deviation and 95% confidence interval (95%CI) for COMET measurements in one session (T_1, T_2, T_4) are shown. In addition, Pearson correlation coefficients (r), corresponding p-values (p_r) and p-values of the paired *t*-tests (p_{ttest}) for COMET measurements in one session (T_1, T_2, T_4) are displayed. Finally the limits of agreement (LOA), intraclass correlation coefficients (ICC), standard error of the measurement (SEM) and minimum detectable difference (MDD) are presented. Significant p-values are printed in bold.

Variable	[Unit]	n	Δ			r	p_r
			Mean ± SD	[95%CI]	p_{ttest}		
$mitoPO_2$	[mm Hg]	159	−2.19 ± 11.71	[−4.01, −0.37]	**0.020**	0.699	< **0.001**
$mitoVO_{2:\ max}$	[mm Hg/s]	161	0.15 ± 1.61	[−0.10, 0.40]	0.230	0.738	< **0.001**
$mitoVO_{2:\ avg}$	[mm Hg/s]	161	0.07 ± 1.04	[−0.09, 0.23]	0.386	0.734	< **0.001**
$mitoDO_{2:\ max}$	[mm Hg/s]	158	1.27 ± 3.23	[0.77, 1.78]	< **0.001**	0.627	< **0.001**
$mitoDO_{2:\ avg}$	[mm Hg/s]	160	0.58 ± 1.67	[0.32, 0.84]	< **0.001**	0.658	< **0.001**

Variable	[Unit]	n	Limits of agreement (LOA)			
			Upper	[95%CI]	Lower	[95%CI]
$mitoPO_2$	[mm Hg]	159	20.76	[18.54, 23.69]	−25.13	[−22.92, −28.07]
$mitoVO_{2:\ max}$	[mm Hg/s]	161	3.30	[3.00, 3.70]	−3.00	[−2.69, −3.40]
$mitoVO_{2:\ avg}$	[mm Hg/s]	161	2.10	[1.91, 2.36]	−1.96	[−1.76, −2.22]
$mitoDO_{2:\ max}$	[mm Hg/s]	158	7.60	[6.99, 8.41]	−5.05	[−4.44, −5.86]
$mitoDO_{2:\ avg}$	[mm Hg/s]	160	3.86	[3.54, 4.27]	−2.70	[−2.38, −3.11]

Variable	[Unit]	n	Intraclass correlation coefficients			SEM	MDD
			ICC	[95%CI]	p		
$mitoPO_2$	[mm Hg]	159	0.69	[0.59, 0.76]	< **0.001**	8.28	22.95
$mitoVO_{2:\ max}$	[mm Hg/s]	161	0.73	[0.65, 0.80]	< **0.001**	1.14	3.15
$mitoVO_{2:\ avg}$	[mm Hg/s]	161	0.73	[0.65, 0.80]	< **0.001**	0.73	2.03
$mitoDO_{2:\ max}$	[mm Hg/s]	158	0.58	[0.43, 0.70]	< **0.001**	2.28	6.33
$mitoDO_{2:\ avg}$	[mm Hg/s]	160	0.63	[0.50, 0.73]	< **0.001**	1.18	3.28

$MitoPO_2$: mitochondrial oxygen tension|$MitoVO_2$: mitochondrial oxygen consumption|$MitoDO_2$: mitochondrial oxygen delivery.

Table 5
Inter-session stability of the COMET variables. The mean difference, standard deviation and 95% confidence interval (95%CI) for mean COMET measurements at T_1 and T_2 are shown. In addition, Pearson correlation coefficients (r), corresponding p-values (p_r) and p-values of the paired *t*-tests (p_{ttest}) for mean COMET measurements at T_1 and T_2 are displayed. Finally the limits of agreement (LOA), intraclass correlation coefficients (ICC), standard error of the measurement (SEM) and minimum detectable difference (MDD) are presented. Significant p-values are printed in bold.

Variable	[Unit]	n	Δ			r	p_r
			Mean ± SD	[95%CI]	p_{ttest}		
$mitoPO_2$	[mm Hg]	26	−6.14 ± 15.07	[−11.93, −0.34]	**0.048**	0.541	**0.004**
$mitoVO_{2:\ max}$	[mm Hg/s]	25	−0.02 ± 2.25	[−0.90, 0.86]	0.969	0.448	**0.025**
$mitoVO_{2:\ avg}$	[mm Hg/s]	26	0.07 ± 1.50	[−0.51, 0.65]	0.815	0.537	**0.005**
$mitoDO_{2:\ max}$	[mm Hg/s]	25	−0.20 ± 3.65	[−1.63, 1.23]	0.790	0.503	**0.010**
$mitoDO_{2:\ avg}$	[mm Hg/s]	26	0.31 ± 2.07	[−0.49, 1.10]	0.458	0.472	**0.015**

Variable	[Unit]	n	Limits of agreement (LOA)			
			Upper	[95%CI]	Lower	[95%CI]
$mitoPO_2$	[mm Hg]	26	23.40	[17.36, 35.54]	−35.68	[−29.64, −47.81]
$mitoVO_{2:\ max}$	[mm Hg/s]	25	4.39	[3.48, 6.26]	−4.43	[−3.51, −6.29]
$mitoVO_{2:\ avg}$	[mm Hg/s]	26	3.01	[2.41, 4.22]	−2.87	[−2.27, −4.08]
$mitoDO_{2:\ max}$	[mm Hg/s]	25	6.95	[5.47, 9.98]	−7.34	[−5.86, −10.37]
$mitoDO_{2:\ avg}$	[mm Hg/s]	26	4.36	[3.53, 6.03]	−3.75	[−2.92, −5.42]

Variable	[Unit]	n	Intraclass correlation coefficients			SEM	MDD
			ICC	[95%CI]	p		
$mitoPO_2$	[mm Hg]	26	0.67	[0.28, 0.85]	**0.002**	10.66	29.54
$mitoVO_{2:\ max}$	[mm Hg/s]	25	0.62	[0.12, 0.83]	**0.013**	1.59	4.41
$mitoVO_{2:\ avg}$	[mm Hg/s]	26	0.71	[0.33, 0.87]	**0.002**	1.06	2.94
$mitoDO_{2:\ max}$	[mm Hg/s]	25	0.68	[0.25, 0.86]	**0.005**	2.58	7.15
$mitoDO_{2:\ avg}$	[mm Hg/s]	26	0.63	[0.18, 0.84]	**0.008**	1.46	4.06

$MitoPO_2$: mitochondrial oxygen tension|$MitoVO_2$: mitochondrial oxygen consumption|$MitoDO_2$: mitochondrial oxygen delivery.

Table 6
Results of the univariate linear regression models and non-parametric correlation analysis for COMET variables. Data was pooled for T_1 and T_2. The non-standardized regression coefficients (β), corresponding 95% confidence intervals (95%CI), Pearson correlation coefficients ($r_{Pearson}$) and corresponding p-values are displayed. In addition, the Spearman correlation coefficients ($\rho_{Spearman}$) and the corresponding p-values are displayed. Significant p-values are printed in bold.

Covariate	COMET variable	Linear regression					Non-parametric	
		β	95%CI		$r_{Pearson}$	p	$\rho_{Spearman}$	p
Heart rate recovery	$mitoVO_{2:\ max}$	0.18	0.07	0.29	0.56	**0.003**	0.41	**0.039**
	$mitoVO_{2:\ avg}$	0.12	0.04	0.20	0.55	**0.004**	0.42	**0.032**
Physical activity level	$mitoVO_{2:\ max}$	7.98	2.30	13.66	0.51	**0.008**	0.42	**0.035**
	$mitoVO_{2:\ avg}$	5.32	1.36	9.28	0.49	**0.011**	0.44	**0.025**
Signal quality	$mitoVO_{2:\ max}$	0.08	0.01	0.14	0.46	**0.019**	0.49	**0.012**
	$mitoVO_{2:\ avg}$	0.05	0.01	0.09	0.44	**0.024**	0.50	**0.010**
Duration of ALA application	$mitoVO_{2:\ max}$	0.32	0.00	0.63	0.39	**0.048**	0.35	0.083
	$mitoVO_{2:\ avg}$	0.21	0.00	0.43	0.38	0.054	0.36	0.070
ECW/TBW	$mitoVO_{2:\ max}$	−0.35	−0.69	0.00	−0.39	**0.049**	−0.40	**0.045**
	$mitoVO_{2:\ avg}$	−0.22	−0.46	0.02	−0.36	0.070	−0.35	0.076
Phase angle	$mitoVO_{2:\ max}$	0.91	0.11	1.70	0.43	**0.028**	0.36	0.074
	$mitoVO_{2:\ avg}$	0.59	0.04	1.15	0.41	**0.037**	0.33	0.097

ECW/TBW: ratio of extracellular water and total body water.

Therefore, the findings from this study could confirm on a cellular level what other studies have found systemically.

A higher signal quality was associated with a higher $mitoVO_2$. Attention should be paid to this effect in future studies.

The duration of the ALA patch application correlated positively with $mitoVO_2$. Though the effect was small, this finding stresses the importance of controlling for this confounder in future measurements.

The ECW/TBW ratio showed a negative association with $mitoVO_2$. This effect may be of greater significance in patients with a higher ECW/TBW ratio, i.e. oedema. Patients in shock often exhibit oedema due to endothelial barrier dysfunction. For this reason, we suggest that – at least in critically ill patients with a disturbed endothelial barrier function – bioimpedance analysis could be performed together with COMET measurement to control for this potential influence factor.

4. Conclusion

In this study, we found that exercise had little effect on the mitochondrial oxygen metabolism measured with the COMET system in our setting. Physical activity neither influenced $mitoPO_2$ nor $mitoDO_2$. Though significant changes were found in $mitoVO_2$, these changes were small. Nevertheless, in future studies, $mitoDO_2$ may prove an interesting variable of reoxygenation capacity as a surrogate marker of impaired microcirculation. Furthermore, the measurements showed moderate repeatability. Body composition as determined by bio-impedance analysis only showed relevant correlation for the ECW/TBW index. Variables of fitness (PAL, heart rate recovery, phase angle) were positively associated with $mitoVO_2$. Covariates that should be controlled for in future studies include duration of ALA application and the presence of oedema. Taken together, these findings suggest that the COMET system is a reliable method to monitor patient oxygen metabolism. As the number of participants in the final analysis was 26, the results will have to be validated in future studies.

Ethics statement

The study was approved by the local ethics committee (5400-01/18) and was registered at the German clinical trials register (DRKS00014033).

Conflict of interest statement

The authors declare that they do not have any competing or financial interests.

Funding

This study was funded by the Federal Ministry of Education and Research within the Centre for Innovation Competence Septomics (Research Group Translational Septomics, Grant 03Z22JN12 to SMC). The funding source had no involvement in study design, the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

Author contribution statement

Conceptualisation and design of the study: SMC, PB. Conduction of the study and data acquisition: PB, CN, SMC, SD, MN, AB. Statistical analysis: PB. Drafting the manuscript for important intellectual content: SMC, PB, CN. Revising the manuscript prior to submission for intellectual content: SMC, PB, CN, SD, MB, MN, AB. Funding acquisition: SMC. All authors agree to be accountable for all aspects of the work related to the accuracy or integrity of any part of the work. All authors carefully reviewed and approved the manuscript.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

We would like to thank Alina Ploiij, Rebecca Schild and Florian Schwarz for their assistance during the measurements as well as Prof. Dr. med. Ulrich C. Smolenski from the Institute of Physiotherapy, Jena University Hospital, for providing the ergometer and the room to conduct this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2018.12.003.

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