PAPER

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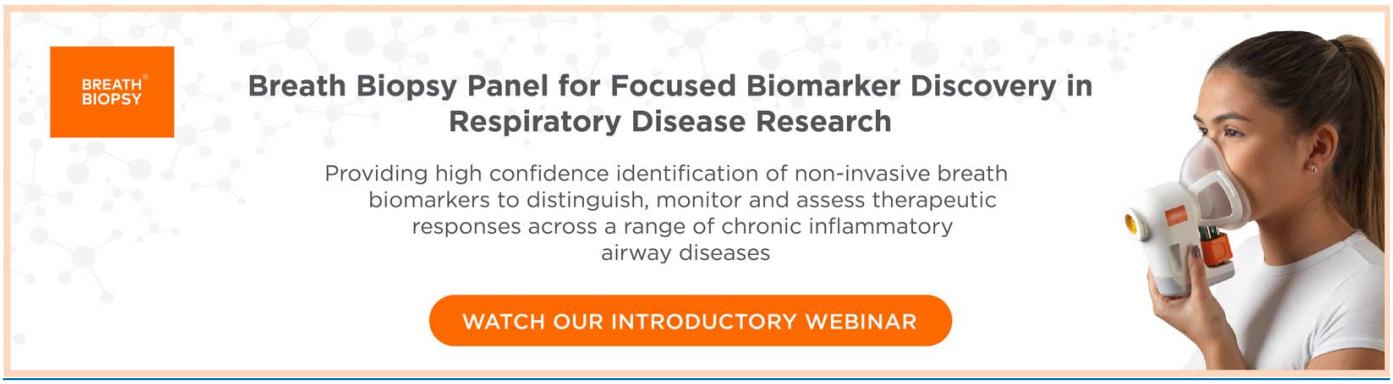

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Breath acetone change during aerobic exercise is moderated by cardiorespiratory fitness

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Abstract

Exhaled breath acetone (BrAce) was investigated during and after submaximal aerobic exercise as a volatile biomarker for metabolic responsiveness in high and lower-fit individuals in a prospective cohort pilot-study. Twenty healthy adults (19–39 years) with different levels of cardiorespiratory fitness (VO_{2peak}), determined by spiroergometry, were recruited. BrAce was repeatedly measured by proton-transfer-reaction time-of-flight mass spectrometry (PTR-TOF-MS) during 40–55 min submaximal cycling exercise and a post-exercise period of 180 min. Activity of ketone and fat metabolism during and after exercise were assessed by indirect calorimetric calculation of fat oxidation rate and by measurement of venous β-hydroxybutyrate (βHB). Maximum BrAce ratios were significantly higher during exercise in the high-fit individuals compared to the lower-fit group (t-test; $p = 0.03$). Multivariate regression showed 0.4% (95%-CI = −0.2%–0.9%, $p = 0.155$) higher BrAce change during exercise for every ml kg^{-1} min^{-1} higher VO_{2peak}. Differences of BrAce ratios during exercise were similar to fat oxidation rate changes, but without association to respiratory minute volume. Furthermore, the high-fit group showed higher maximum BrAce increase rates (46% h^{-1}) in the late post-exercise phase compared to the lower-fit group (29% h^{-1}). As a result, high-fit young, healthy individuals have a higher increase in BrAce concentrations related to submaximal exercise than lower-fit subjects, indicating a stronger exercise-related activation of fat metabolism.

1. Introduction

Regular exercise reduces the risk of all-cause mortality [1] and is particularly important for personalized prevention and treatment of metabolic disorders, such as obesity [2], diabetes [3], dyslipidemia [4], non-alcoholic fatty liver disease [5] and metabolic syndrome. High cardiorespiratory fitness (CRF) may improve metabolic responsiveness towards exercise stimuli [6]. Therefore, simple and accurate non-invasive monitoring of the individual metabolism is desired to personalize exercise modality, intensity and duration for optimal metabolic adaptations [7].

Endogenous volatile organic compounds (VOCs) in exhaled breath are promising for real-time monitoring of metabolic processes [8]. In particular, breath acetone (BrAce) is closely related to fat metabolism [9], thus reflecting metabolic responsiveness. Specifically, hepatic β-oxidation of fatty acids leads to an accumulation of acetyl coenzyme A, which further divides into the ketone body acetoacetate [10]. The latter undergoes decarboxylation and enzymatic degradation to acetone and β-hydroxybutyrate (βHB), respectively [10]. In contrast to βHB and acetoacetate, acetone is highly volatile and, hence, measurable non-invasively in exhaled breath [11] rendering its detection attractive for

routine metabolic assessment with compact sensors [12] that were integrated already into industrial prototype devices [13]. Note that there are other sources of endogenous BrAce including the dehydrogenation of isopropanol and amino acid degradation [9], but the first is relevant only in case of toxic isopropanol ingestion [14].

BrAce increases during both constant [15] and graded exercise [16], showing a peak at about 45% of the total workload during an individualized graded exhaustive exercise bout [17]. However, exercise protocols in previous studies [15–17] were not standardized for the individual CRF, expressed by maximum oxygen consumption (VO_{2peak}), that affects fat metabolism during exercise [6, 18–21]. Furthermore, BrAce is known to increase during post-exercise rest [7] that can be particularly important for exercise-associated weight reduction and mitigation of metabolic risk [22], but its relation to CRF has not been assessed yet.

This study investigates BrAce changes in high- and lower-fit individuals during and after CRF-standardized submaximal cycling. Therein, BrAce concentrations were monitored online by proton-transfer-reaction time-of-flight mass spectrometry (PTR-TOF-MS) connected to a tailor-made buffered [23] end-tidal breath sampler [24]. Simultaneous measurement of the fat and ketone body metabolism through indirect calorimetry and venous β-hydroxybutyrate (βHB) enabled a comparison to BrAce.

2. Methods

2.1. Study design and population characteristics

This pilot-study has been approved by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ 2018–00525) and is in accordance with the declaration of Helsinki. Informed consent from all study participants was obtained in written form and volunteers received no expense allowance. We calculated a sample size of twenty participants to reach a statistical power of at least 80% to detect relevant differences in BrAce concentrations between high- and lower-fit individuals. Twenty healthy volunteers with different histories of exercise habits (12 women, 8 men), aged between 19 and 39 years, participated in this study. None of them had relevant comorbidities or special dietary habits (e.g. low-carb). Each participant attended two appointments separated by, at least, five days (to avoid interfering effects of the previous workload), but no more than three weeks. At the first appointment, eligibility was assessed and exhaustive spiroergometry was conducted for determination of CRF as well as the individual workload at the second ventilatory threshold (VT_2). The VT_2 is the maximum exercise intensity at which endurance performance through aerobic metabolism is possible. At the second appointment, each participant underwent a submaximal exercise intervention. All participants were asked to abstain from alcohol, tobacco and intensive exercise 24 h prior to the appointments. Further, they were advised to consume a low-carb dinner at the evening before and avoid chemical mouthwash 2 h before the second appointment.

2.2. Baseline data collection and exercise intervention

At the first appointment, we conducted a medical interview and examination, a 12-lead resting ECG and blood pressure measurement. Body weight was measured to the nearest 0.1 kg (InBody720, InBody Co., Ltd., Seoul, South Korea). Afterwards, we determined VO_{2peak} and the VT_2 by spiroergometry (Ergoline ErgoSelect 200, Bitz, Germany equipped with MetaLyzer 3B-R2 spirometer, Cortex Biophysik GmbH, Leipzig, Germany). The ergometer incremental rate (10, 15 or 25 W per min) was chosen out of three protocols according to the expected maximum performance. The incremental rate was calculated following Hansen *et al* [25] with height, age and weight provided in the units of centimeter, year and kilogram, respectively:

$$incremental\,rate = (VO_{2peak} - VO_{2unload})/100 \quad (1)$$

$$\begin{aligned} VO_{2peak} &= (height - age) \cdot x, \; with \; x \\ &= 20 \; for \; men \; and \; 14 \; for \; women \end{aligned} \quad (2)$$

$$VO_{2unload} = 150 + (6 \cdot weight) \quad (3)$$

The protocol was chosen as the closest to the calculated incremental rate and the respective initial ergometer intensity was selected as 10, 20 or 50 W.

Standardization of the test included fulfillment of standard laboratory criteria [26], verbal encouragement and adherence to current recommendations for control of maximal participant effort [27]. The VO_{2peak} was accepted as the highest average of three values, which were consecutively measured in 10 s intervals. Two experienced examiners checked the automatically detected VT_2 and readjusted, if necessary. In agreement with literature [28], male participants with a $VO_{2peak} > 40$ ml kg^{-1} min^{-1} and female participants with a $VO_{2peak} > 34$ ml kg^{-1} min^{-1} were classified as high-fit (n = 13).

The second appointment started at 8 a.m. after an overnight fast (>8 h). An individualized submaximal aerobic exercise protocol was applied, representing a common type of exercise used by non-athletes to improve their fitness and general health [29]. We used a stepwise incremental protocol that started at 20% of the individual VT_2 (determined in the first exercise test) and increased by 10% every 5 min (figure 1). After 40 min, submaximal intensity of 100%

VT_2 was reached and the participants continued until exhaustion or for a maximum of 15 min. Participants were asked to maintain a cadence of 60 to 75 rpm during the whole exercise. Respiratory minute volume, VO_2 and VCO_2 were continuously monitored for indirect calorimetric measures (figure 1, blue line). We calculated fat oxidation rates for all VO_2 and VCO_2 values during exercise with the formula of Jeukendrup *et al* [30]. Calculated fat oxidation rates were then averaged for every intensity level. The post-exercise period lasted until 4 h after the start of exercise.

Blood samples were taken from an intravenous line that was installed prior to the measurements. A blood sample of 7.5 ml was drawn before and immediately after exercise, every 30 min during the first post-exercise hour and then every hour until the end of the post-exercise phase (figure 1, squares). Blood samples were centrifuged for 10 min (3000 rpm, 20 °C; Universal 320 R, Hettich Zentrifugen, Baech, Switzerland) immediately after they were obtained and only the serum was stored at −80 °C. After all samples of every participant had been collected, they were analyzed for βHB (Center of Laboratory Diagnostics, MVZ Clotten, Freiburg, Germany or Institute of Clinical Chemistry University Hospital Zurich, Switzerland). Note that some βHB levels were below 100 μM where the quantification is less accurate.

2.3. BrAce analysis

End-tidal BrAce concentrations were measured at the second appointment with a benchtop proton-transfer-reaction time-of-flight mass spectrometer (PTR-TOF 1000, Ionicon Analytik, Innsbruck, Austria) suitable to detect lowest BrAce concentrations [31]. Primary ions (H_3O^+) were generated from water vapor. The drift tube was operated at a voltage of 600 V, a pressure of 2.3 mbar and temperature of 60 °C. The value of the reduced electric field in the drift tube (E/N) was 130 Td. The BrAce (CAS 67–64-1) was determined at a mass-to-charge ratio of 59.050 ($C_3H_7O^+$) [32]. Four-point calibrations in the range of 500 to 1500 parts-per-billion (ppb) were carried out for absolute quantification of BrAce concentrations. For that, a certified acetone standard (10 parts-per-million (ppm) in synthetic air 6.0, PanGas, Switzerland) was dosed to synthetic air (C_nH_m and $NO_x \leq 0.1$ ppm, Pan Gas, Switzerland) using a similar mixing setup as described in literature [33]. Background acetone concentrations were typically below 100 ppb.

The baseline was obtained by averaging BrAce concentrations from three exhalations collected within 15 min just before exercise (figure 1, diamonds). During exercise, breath samples were collected 30 s before the end of each intensity level (every 5 min). For this, the spirometer mask was shortly removed while cycling was continued. After exercise, breath samples were collected every 30 min while the participants rested. Correct and standardized sampling of end-tidal breath is crucial for meaningful breath analysis [34]. Therefore, participants exhaled completely through a sterile and removable mouthpiece (EnviteC-Wismar GmbH, Wismar, Germany) into a tailor-made buffered end-tidal [23] breath sampler with tube length of 375 mm, as described [24] and validated [7] elsewhere. Note that the flow restrictor was removed to enable fast exhalations during exercise [24]. The breath sampler consisted of inert Teflon and all surfaces in contact with breath were heated (60 °C) to avoid analyte adsorption and/or water condensation. A pump (130 ml min^{-1}; Schwarzer Precision, Essen, Germany) guided the breath sample via a heated (60 °C) Teflon transfer line to the PTR-TOF-MS. A CO_2 sensor (Capnostat 5, Respironics, Murrysville, Pennsylvania, USA) placed in the transfer line was applied to evaluate if the participants reached the end-tidal breath portion ($CO_2 > 3\%$ [35]) at the end of their exhalations.

2.4. Data analysis

Data analysis was performed using SPSS version 25.0 for Windows (SPSS Inc. Chicago, Illinois, USA) and OriginPro 2018 G (OriginLab Corporation, Massachusetts, USA). Descriptive analysis included means and standard error of the mean (SEM). The level of significance was set at $p \leq 0.05$ and estimated effects were reported with 95% confidence intervals (95%-CI) in all tests. BrAce and βHB concentrations, fat oxidation rates and respiratory minute volumes were normalized to the individual baseline concentration to account primarily for exercise effects. Independent two-sampled t-tests were conducted for comparison of high- and lower-fit subjects regarding their maximum normalized BrAce (peak divided by baseline BrAce concentration), and similarly for fat oxidation rate, respiratory minute volume and βHB during exercise as well as the post-exercise period. Age- and sex-adjusted multivariate linear regression was used for analysis of the associations between maximum normalized BrAce during and after exercise with VO_{2peak}. Finally, the changes of normalized BrAce during and after exercise were compared against the change of fat oxidation rates and βHB concentrations.

3. Results

3.1. Baseline characteristics

Twelve women and eight men aged 19–39 years were included into statistical analysis. None of them was suffering from cardiovascular or respiratory diseases and all were non-smokers. Mean VO_{2peak} were 48.2 ± 2.0 ml kg^{-1} min^{-1} in the high- and 32.7 ± 2.0 ml kg^{-1} min^{-1} in the lower-fit group ($p < 0.001$, table 1). Mean BMI were

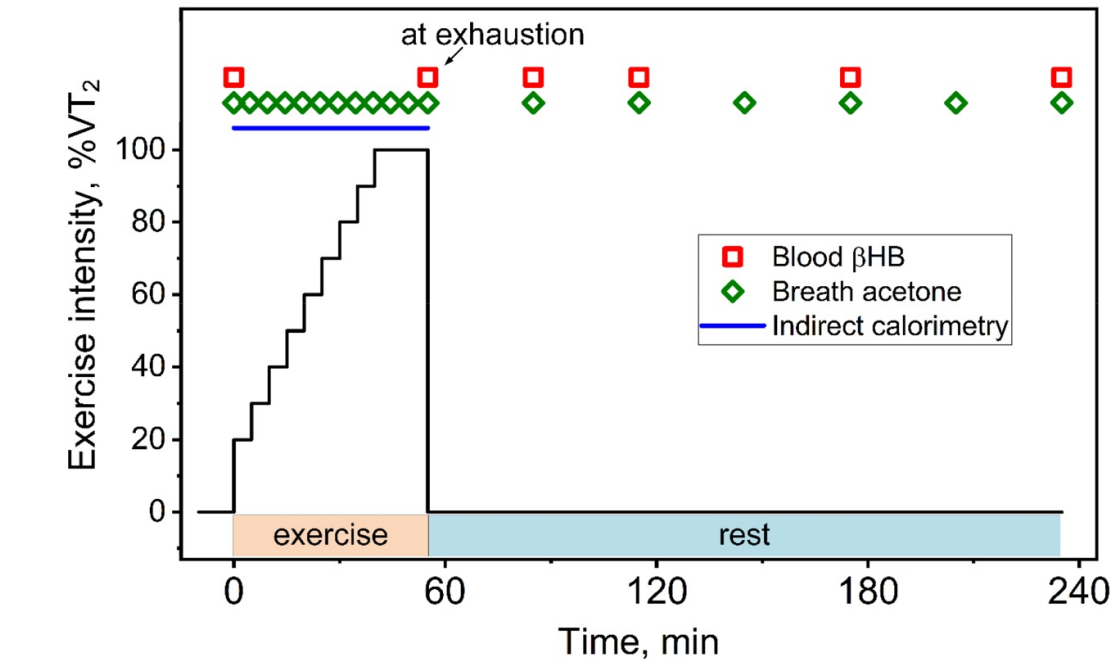

Figure 1. Intervention protocol. The exercise protocol was standardized on the individual VT_2. The graded exercise started at 20% of VT_2 and increased every 5 min by 10%. Submaximal intensity of 100% VT_2 was reached after 40 min and sustained until exhaustion or for a maximum of 15 min. Blood samples were taken at baseline (t = 0 min), at the end of the exercise bout and 85, 115, 175 and 235 min after the start of exercise. BrAce samples were taken at baseline and every 30 s before the end of an intensity level during exercise as well as every 30 min during the post-exercise period until 240 min after baseline. Indirect calorimetric measurements were obtained continuously during exercise.

21.4 ± 2.3 kg m^{-2} in the high- and 28.8 ± 7.1 kg m^{-2} in the lower-fit group ($p < 0.03$).

3.2. BrAce, fat oxidation and relation to CRF during aerobic exercise

Age- and sex-adjusted multivariate regression suggests a positive association between maximum normalized BrAce during exercise with weight-adjusted VO_{2peak} with 0.4% (95%-CI = −0.2%–0.9%; $p = 0.155$) higher BrAce for every ml kg^{-1} min^{-1} higher VO_{2peak}. No such association was found during the post-exercise period of 3 h.

Baseline BrAce concentrations were similar ($p = 0.93$, table 1) for the high- (960 ± 125 ppb) and lower-fit groups (942 ± 151 ppb), in agreement (mean: 951 ppb) to 67 healthy subjects after a comparable overnight fast [36]. However, maximum normalized BrAce during exercise was higher in the high-fit group compared to lower-fit individuals ($p = 0.03$; table 1). In the high-fit group, normalized BrAce constantly increased during exercise at a rate of 33% h^{-1} at low intensities (until 40% VT_2) and at a rate of 9% h^{-1} at higher intensities (figure 2(a)). In the lower-fit group, normalized BrAce hardly increased at low intensities (rate of 0.2% h^{-1}) and increased at 8% h^{-1} at higher intensities, comparable to the high-fit group. In other words, a normalized BrAce, for instance, of 1.035 was obtained for the high-fit individuals already at significantly lower VT_2 of 20% than for the lower-fit group (i.e. ∼90%), that corresponds also to a much lower workload of 37 vs. 166 W, respectively. Similarly to BrAce, the normalized fat oxidation rate increased until 20% VT_2 and reached a maximum of 2.27 ± 0.46 in the lower-fit and 3.17 ± 0.51 in the high-fit group (figure 2(b)). During the late exercise period, fat oxidation rate decreased rather linearly almost reaching zero at 100% VT_2 in both groups. There were no significant differences in maximum normalized minute volume between the groups ($p = 0.13$) which did not correlate with BrAce during exercise (figure S1 (https://stacks.iop.org/JBR/15/016006/mmedia)).

3.3. BrAce and ketone body metabolism post exercise

Maximum normalized BrAce during the post-exercise period showed no significant difference between the high-fit and the lower-fit group ($p = 0.5$; table 1). However, normalized BrAce only modestly increased at a rate of 4% h^{-1} during the first 75 min after termination of the exercise bout, whereas in the lower-fit group, normalized BrAce initially showed an increase of 19% h^{-1} rate (figure 3(a)). Interestingly, during the last post-exercise period (starting 75 min after exercise), normalized BrAce increased in the high-fit group at a rate of 46% h^{-1} and less (29% h^{-1}) in the lower-fit group. Also, venous βHB increased during the post-exercise period until 180 min in both groups and attenuated afterwards (figure 3(b)). The individual maximum normalized βHB was not

Table 1. Baseline characteristics. * Differences between high-fit and lower-fit groups were assessed by independent t-test with the level of significance set at $p \leq 0.05$; SEM = standard error of the mean; VO_{2peak} = maximum oxygen consumption; BrAce = breath acetone; βHB = β-Hydroxybutyrate.

Parameter	n = 13 high-fit: mean (±SEM)	n = 7 lower-fit: mean (±SEM)	p^*
Age [years]	25.1 (±1.2)	26.2 (±2.4)	0.68
Women [number]	8	4	–
Body mass index [kg m^{-2}]	21.4 (±2.3)	28.8 (±7.1)	**0.03**
Weight-adjusted VO_{2peak} [ml kg^{-1} min^{-1}]	48.2 (±2.0)	32.7 (±2.0)	**<0.001**
Weight-adjusted VO_2 at VT_2 [ml kg^{-1} min^{-1}]	39.0 (±1.9)	26.2 (±1.3)	**<0.001**
Max. norm. respiratory minute volume [-]	7.94 (±2.13)	6.25 (±2.29)	0.13
Baseline BrAce concentration [ppb]	960 (±125)	942 (±151)	0.93
Max. norm. BrAce during exercise [-]	1.16 (±0.03)	1.07 (±0.02)	**0.03**
Max. norm. BrAce during post-exercise period [-]	2.08 (±0.19)	1.77 (±0.42)	0.50
Max. norm. fat oxidation rate during exercise [-]	3.17 (±0.51)	2.27 (±0.46)	0.20
Max. norm. βHB [-]	5.61 (±1.25)	3.78 (±0.74)	0.23

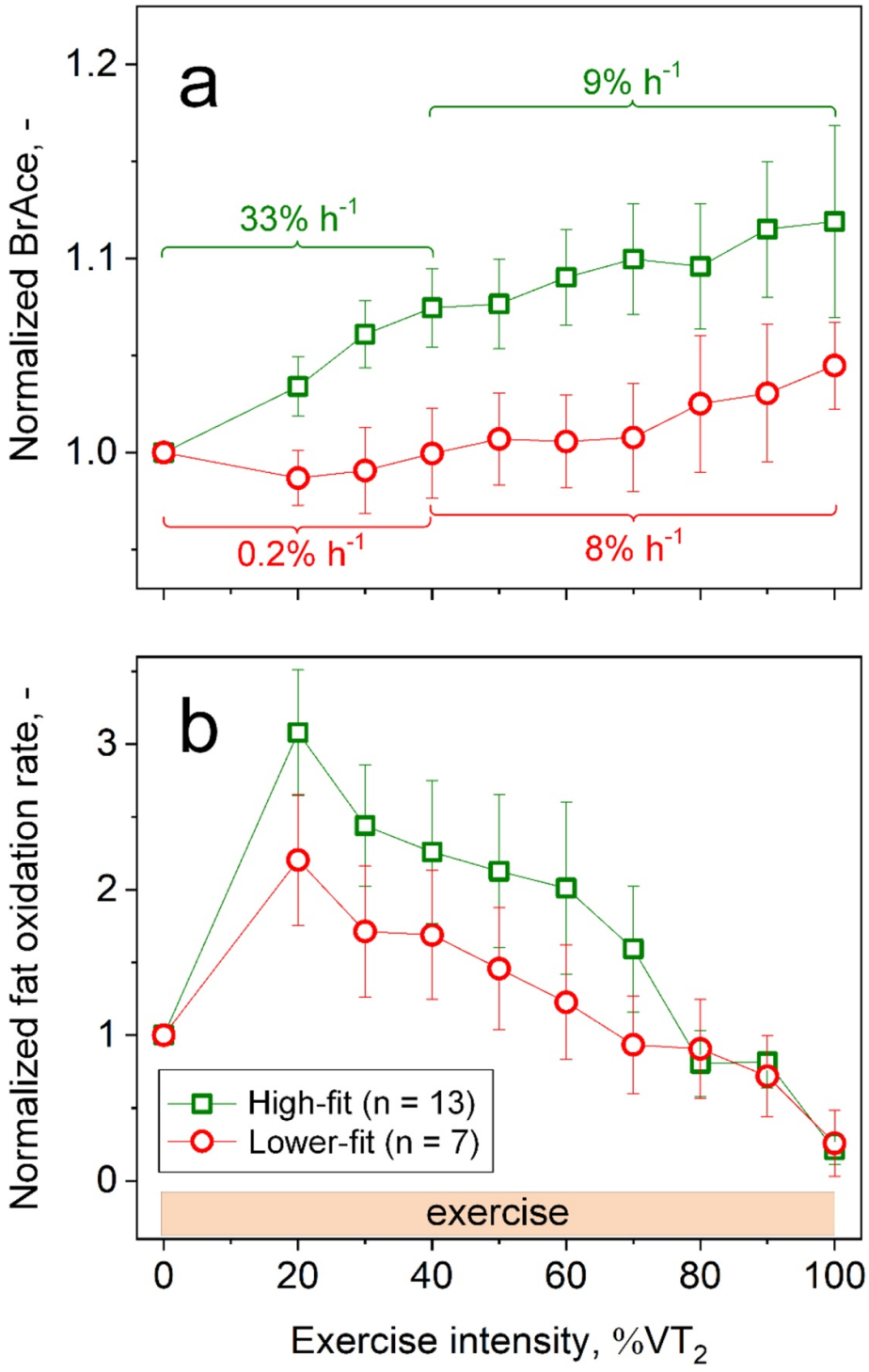

Figure 2. Comparison of BrAce and fat oxidation during exercise. Normalized (a) BrAce and (b) fat oxidation rates during exercise (0–55 min) in the high-fit (n = 13; squares) versus lower-fit (n = 7; circles) group. Values are normalized to their baseline value (t = 0 min). Normalization to the individual baseline concentration was done to account primarily for exercise effects. Error bars show the standard error of the mean (SEM).

significantly different in the high-fit (5.61 ± 1.25) group than in the lower-fit group (3.78 ± 0.74; $p = 0.23$; table 1).

4. Discussion

In agreement with previous studies, we found an enhanced increase of BrAce during [15–17] and after [7] submaximal aerobic exercise in comparison to resting subjects [7]. Most importantly, we revealed that BrAce rates were higher in high- compared to lower-fit individuals during exercise, similar as observed for fat oxidation. Therefore, BrAce seems to indicate VO_{2peak}-dependent responsiveness of fat metabolism. In fact, previous studies indicated higher exercise-related fat oxidation rates and ketone body metabolism in highly fit individuals as well, compared to those with lower fitness, as

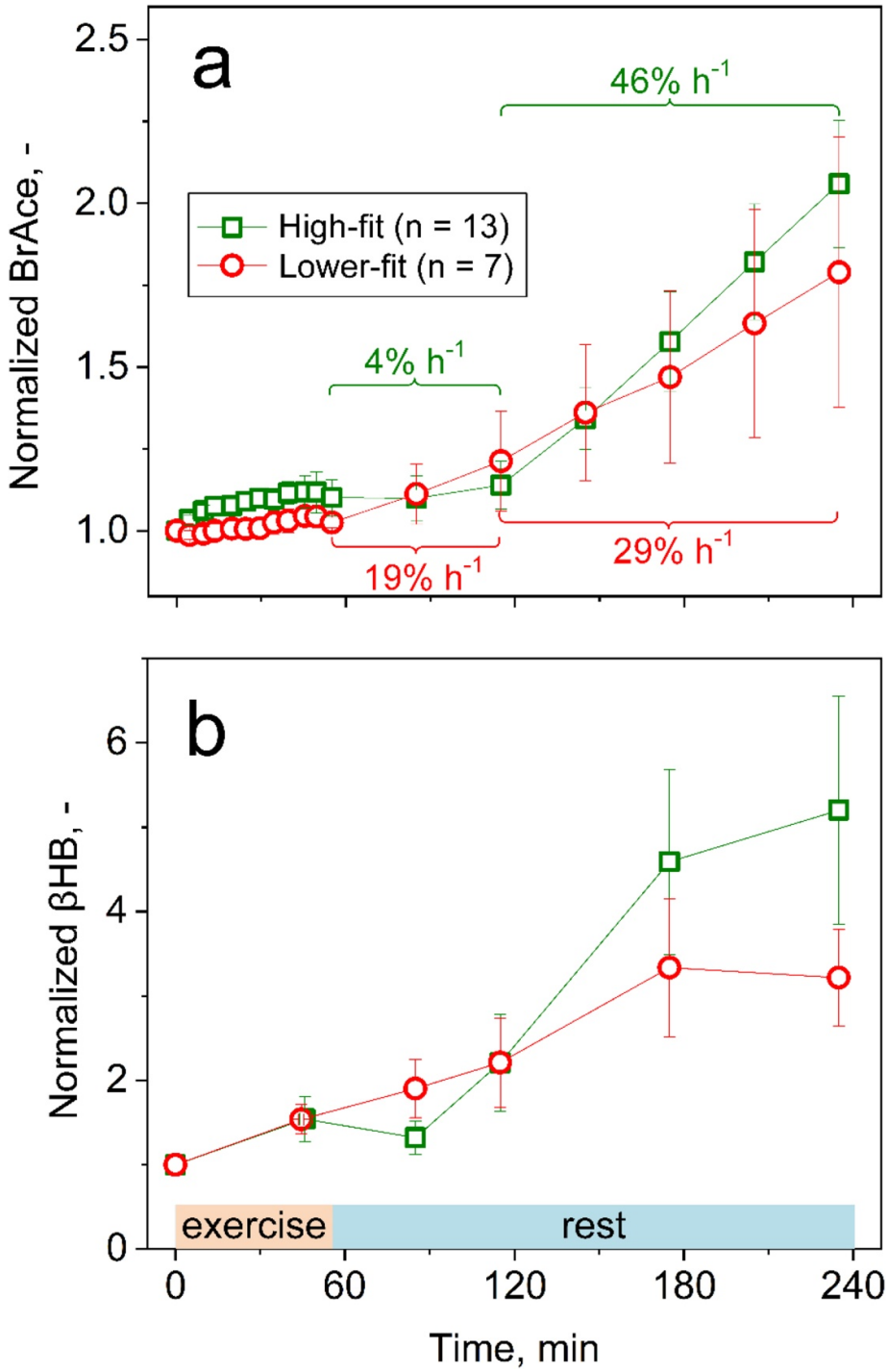

Figure 3. Comparison of BrAce and blood βHB during the post-exercise period. Normalized (a) BrAce and (b) venous βHB during a 3 h post-exercise period (0–240 min) in the high-fit (n = 13; squares) versus lower-fit (n = 7; circles) group. Values are normalized to their baseline value (t = 0 min). Error bars show the standard error of the mean (SEM).

determined by indirect calorimetry [6, 19] and blood assay [20].

There is a biochemical association between BrAce and fat metabolism, but the specific characteristics of this relationship and clinical utility are not yet fully understood [37]. This study reveals that the BrAce increase during submaximal aerobic exercise reflects the fitness-dependent different responsiveness of fat metabolism (as shown by fat oxidation measurement). This is an important finding underlining the potential of non-invasive real-time methods to monitor the metabolic response towards an exercise stimulus. Further studies need to confirm these observations in patients with obesity and metabolic disorders.

It is important that normalized BrAce acetone change was not correlated to respiratory minute volume during exercise. Hydrophilic gases like BrAce interact with the mucosa layers of the upper airways, thus are affected only moderately by respiratory

ventilation and coronary perfusion, as modelled by Anderson *et al.* [38] and King *et al.* [39] and demonstrated by forced expiratory [40] and breath holding [41] maneuvers.

During post-exercise rest, maximum BrAce was not significantly altered. However, the dynamics of the changes per time differed between high-fit and lower-fit subjects, in agreement with βHB. The later increase of BrAce in the high-fit group might reflect a delayed recovery of fat metabolism after longer suppression during performance at high intensity of 100% VT_2. In fact, the rate of increase was higher in the high-fit group during the later post-exercise period. Indeed, a stronger response of BrAce during the 180 min post-exercise period in the high-fit versus the lower-fit individuals is expected. This assumption was based on previous observations regarding the CRF-dependency of post-exercise concentrations of irisin, a marker of adipocyte metabolic activity [22].

Future studies during a longer post-exercise period (e.g. 6 or 12 h) may clarify whether the measurement of BrAce indicates different long-term metabolic responses towards a training intervention depending on an individual's level of CRF. Metabolic real-time monitoring during exercise complements other lifestyle applications [42], for instance, sleep [43], ketogenic diet [44, 45] and fasting [46] where BrAce and other VOC are promising as well. There are also efforts to engineer portable [47] detectors (e.g. chemoresistive sensors [48]/arrays [49] or ion mobility spectrometers [50]) to monitor acetone routinely.

4.1. Strengths and limitations

We applied an exhaustive and individually adapted exercise protocol and controlled for pretest dietary habits, smoking, alcohol consumption, diurnal variations of metabolic state and pretest physical activity. Pulmonary and metabolic alterations [51] as well as the female menstrual cycle [52] might be further confounders, potentially increasing variability in exercise-related dynamics in BrAce and fat metabolism. In this context, body composition should also be considered in upcoming studies, as fat metabolism is frequently altered in people with obesity [53]. As the positive association between BrAce and VO_{2peak} did not reach statistical significance, a small risk for statistical type 1 mistake remains. Therefore, the reproduction of our study with larger samples is needed for further clarification.

A strength of this study is the choice of gold standard measures for all parameters and especially the determination of CRF by strict adherence to highest-level guidelines for exercise testing [27]. Also, the comparison of BrAce with indirect calorimetric fat oxidation rates has rarely been done in literature. Finally, the choice of an individualized submaximal exercise intervention enabled physiological reactions near real-life situations in a laboratory setting.

5. Conclusions

This study demonstrates a higher respiration-independent increase of BrAce during and after exercise in high- than lower-fit subjects. The comparability of BrAce with rates of fat metabolism underlines the potential of BrAce analysis for metabolic monitoring during exercise training and lifestyle interventions. These results provide new insights for the interpretability and clinical utility of BrAce measurements during exercise training for therapeutic guidance during lifestyle interventions.

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Disclosure Statement

The authors declare no competing interests.

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