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Original study

Correlation properties and respiratory frequency of ECG-derived heart rate variability during multiple intervals of prolonged running in female and male long-distance runners

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None.

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Ethical approval for the present study was given by the local ethics committee of the MSH Medical School Hamburg (reference no.: MSH-2023/233). All participants gave written informed consent and all testing and measurements were conducted in accordance with the principles of the recent revision of the Declaration of Helsinki.

Availability of data and material:

Data are available from the corresponding author on reasonable request.

Authors' contributions:

KH, DF, MS, and TG conceived the study. TG, OH and KH designed the research question. MS and DF conducted the experiments and data processing. TG, MS and DF conducted data analysis and interpretation. TG drafted the manuscript. All authors provided critical comments on the manuscript, read, and approved the final version of the manuscript.

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Abstract

Aim: To evaluate alterations of the non-linear short-term scaling exponent α_1 of detrended fluctuation analysis (DFAa1) of heart rate (HR) variability (HRV) as a sensitive marker for assessing global physiological demands during prolonged running intervals. Furthermore, agreement of ECG-derived respiratory frequency (EDR) compared to gas exchange-derived respiratory frequency (RF) was evaluated with the same chest belt device. Methods: Fifteen trained female and male long-distance runners completed four running bouts over five minutes on a treadmill at marathon pace. During the last three minutes of each bout gas exchange data and a single-channel ECG for the determination of HR, DFAa1 of HRV, EDR and RF were analyzed. Additionally, blood lactate concentration (BLC) was determined and rating of perceived exertion (RPE) was requested. Results: DFAa1, oxygen consumption, BLC, and RPE showed stable behaviors comparing the running intervals. Only HR ($p < 0.001$, $d = 0.17$) and RF ($p = 0.012$, $d = 0.20$) indicated slight increases with small effect sizes. Additionally, results point towards remarkable inter-individual differences in all internal load metrics. The comparison of EDR with RF during running revealed high correlations ($r = 0.80$, $p < 0.001$, $ICC_{3,1} = 0.87$) and low mean differences (1.8 ± 4.4 breaths/min), but rather large limits of agreement with 10.4 to -6.8 breaths/min. Conclusions: Results show the necessity of EDR methodology improvement before being used in a wide range of individuals and sports applications. Relationship of DFAa1 to other internal load metrics, including RF, in quasi-steady-state conditions bears the potential for further evaluation of exercise prescription and may enlighten decoupling mechanisms in exercise bouts of different type and duration.

Key words: HRV, DFAa1, autonomic nervous system, running economy, exercise prescription

Introduction

Analyses of the non-linear characteristics of heart rate (HR) variability (HRV) indicate that the short-term scaling exponent α_1 of detrended fluctuation analysis (DFAa1) may be a sensitive marker for assessing global physiological demands during endurance exercise (Gronwald & Hoos, 2020; Gronwald et al., 2020; Rogers & Gronwald, 2022). DFAa1 quantifies the fractal scale and correlation properties of HR time series in cardiac beat-to-beat intervals and represents a rather qualitative marker of autonomic nervous system (ANS) regulation. Given these properties, and considering the corresponding signal-theory background, this metric may be used as a biomarker for exercise intensity domain delineation (Gronwald et al., 2020). For this purpose, it could be shown, that discrete numerical values of DFAa1 may demarcate the transition from moderate to heavy exercise intensity and from heavy to severe exercise intensity (3-zone-model), and may correspond to traditional threshold markers based on different physiological subsystem measures like blood lactate concentration (BLC) or gas exchange data with potential limitations and deviations on an individual level (Rogers et al., 2021a,b; Mateo-March et al., 2023, van Hooren et al., 2023b, Schaffarczyk et al., 2023; Sempere-Ruiz et al., 2024). Further, DFAa1 has been shown to be useful as a marker of acute fatigue in terms of systemic perturbation patterns in HR time series (Rogers et al., 2021c; Schaffarczyk et al., 2022; van Hooren et al., 2023a,b) or as a measure of fatigue resistance in studies with prolonged exercise (Gronwald et al., 2018, 2019, 2021a). Therefore, expanding these findings to future approaches of real-time monitoring of prolonged exercise seems to be promising, as the DFAa1 marker might bear the potential to mirror decoupling mechanisms as alterations of external-to-internal-load relationships (Mauder et al., 2021; Smyth et al., 2022). In this context, respiratory frequency (RF) was recently endorsed as a promising internal load marker for intensity monitoring during endurance exercise as well, with new possibilities for wearable analyses in research and practical settings (Nicolo et al., 2017; Tipton et al., 2017; Nicolo et al., 2020; Passfield et al., 2022; Nicolo & Sacchetti, 2023). Currently, there is large interest in exercise science and sports practice to analyse RF via wearable technology and remote devices (Vitazkova et al., 2024). Data of DFAa1 and estimated RF derived from an electrocardiogram (ECG-derived RF; EDR; Rogers et al., 2022a,b) bear the potential of a more comprehensive internal load assessment during endurance exercise with real-time applications recorded with a chest belt form factor complementary to established internal load indicators like HR and rating of perceived exertion (RPE). However, data of DFAa1 and estimated RF via EDR during steady-state exercise bouts are scarce and the true significance for exercise prescription remains to be elucidated. This applies especially for data during running exercise given the high risk of movement artefacts and signal distortion in ECG-waveform and HRV analysis. Therefore, the aim of the present report was to evaluate alterations of DFAa1 and EDR compared to further respiratory and metabolic measures, including actual measured RF via gas exchange, during multiple bouts of prolonged running at marathon race pace in a group of trained female and male long-distance runners.

Methods

Participants

Fifteen trained marathon (5m, 3w) and half-marathon (3m, 4w) runners (age: 32.6 ± 5.4 years, body height: 174.6 ± 7.6 cm, body weight: 64.5 ± 7.8 kg) were recruited from the German athletics federation, Hamburg athletics federation and from local clubs through personal contacts during September and December 2023. Inclusion criteria were race performance in the marathon and half-marathon corresponding to 400 points in the World Athletics “Scoring Table of Athletics” (Spiriev, 2022), age between 18 and 65 years, and absence of injuries > 3 months before measurements. Ethical approval for the present study was given by the local

ethics committee of the MSH Medical School Hamburg (reference no.: MSH-2023/233). All participants gave written informed consent and all testing and measurements were conducted in accordance with the principles of the recent revision of the Declaration of Helsinki.

Study design

The cross-sectional assessment was part of a larger study that aimed to investigate running economy and habituation with advanced footwear technology (Fohrmann et al., 2024; Schwalm et al., 2024). Based on the initial study setting, with a single laboratory session, participants completed four to six running bouts over five minutes at submaximal velocity based on individual running speed in marathon or half-marathon. Running speed was defined as the pace of the marathon season best (SB) or converting the SB race performance in the half-marathon into an estimated marathon time and corresponding race speed (multiplied by the factor of 2.11). The first four running bouts were used for the present study analysis.

Data recording

Body height and body weight of the participants were assessed using an analysis scale (655-US, seca GmbH & Co. KG., Hamburg, Deutschland). In addition, participants were asked for their maximum HR (HR_{MAX}) from a recent treadmill performance test or competition. In case of unknown maximum HR calculation according to Tanaka's formula was applied: $208 - 0.7 \times \text{age}$ (Tanaka et al., 2001). Afterwards, a general warm-up over ten minutes was conducted at preferred running speed prior to the running bouts at submaximal velocity on a motorized treadmill over five minutes (FDM-T, h/p/cosmos, Nussdorf-Traunstein, Germany); the first bout was designated as a specific warm-up at race speed (see Figure 1). Immediately after the running bouts BLC (in mmol/l) from the capillary blood of the earlobe (20 μ l) with the Biosen C-Line Clinic analyzer (EKF-diagnostic GmbH, Barleben, Germany) was determined and RPE was requested using the Borg scale (6-20; Borg, 1982). A passive break of five minutes was introduced in between the running bouts. Recordings of a single-channel ECG for the determination of HR (in beats per minute, bpm), RR-intervals (in ms) and EDR (in breaths per minute, breaths/min) were taken continuously with the Movesense Medical sensor (firmware version 2.1.2) implemented in a chest belt (Movesense, Vantaa, Finland) and the Movesense Showcase app via smartphone (sampling rate: 256 Hz; iOS: version 1.1.0; Rogers et al., 2022b, see Figure 1). Breath-by-breath pulmonary gas exchange data were recorded using a metabolic card (Quark CPET, module A-670-100-005, COSMED Deutschland GmbH, Fridolfing, Germany; Omnia version 2.2). Expired gas fractions were continuously measured to determine oxygen consumption (VO_2 , in ml/min/kg), and RF (in breaths per minute, breaths/min). Physiological measures were determined during the last three minutes of each running bout. Resting values were taken prior to the general warm-up period over two minutes.

HRV and EDR analysis

To analyze HR, RR-intervals and EDR data were exported from the Movesense Showcase app via .csv file and processed in Kubios HRV Premium (version 3.5.0, Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland). Preprocessing settings were set to the default values, including the RR detrending method, which was kept at "smoothness priors" ($\Lambda = 500$). The RR-interval series were then corrected using the Kubios HRV "automatic correction" method (Lipponen & Tarvainen, 2019). HR and DFAa1 were determined during the last three minutes of each running bout. To calculate DFAa1, the root mean square fluctuations of the integrated and detrended RR-intervals were analyzed in observation windows of different sizes and then further processed as the slope between the root mean square fluctuation data in relation to the different window sizes on a log-log scale (Peng et al., 1995). Window size was set to $4 \leq n \leq 16$ beats in the

software preferences. For EDR assessment Kubios HRV software RF estimation algorithm was used (Lipponen & Tarvainen, 2021). The algorithm combines the cyclic cardiac beat-to-beat time domain changes in RR-intervals associated with respiratory sinus arrhythmia and the single-channel ECG-associated R wave amplitude changes seen during the respiratory cycle. For EDR calculation, the window width was set to 30 s with a recalculation grid interval of 1 s based on recommendations from Lipponen and Tarvainen (2021). During the last three minutes of each running bout, maximum EDR was noted. Data sets with >5% artifacts were excluded from RR-interval and EDR analysis. Data were also scanned visually for artifacts and ECG tracing quality by an expert with experience in HRV-data analysis and removed manually if necessary.

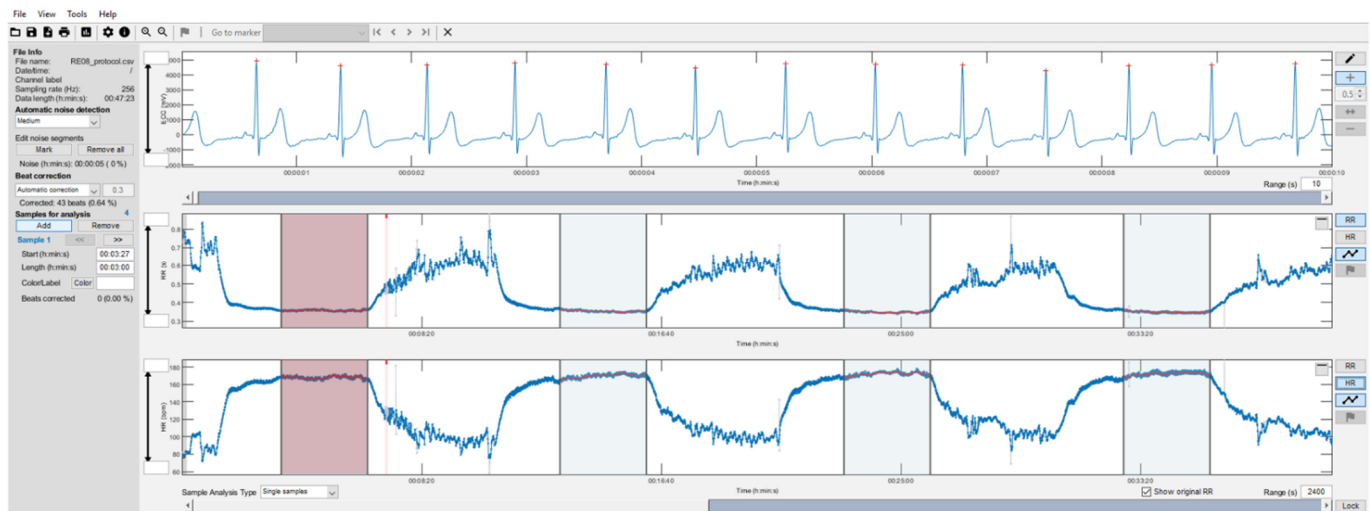


Figure 1. Course of an example of a single-channel ECG tracing and corresponding RR-intervals and HR of the running session with four running bouts over five minutes at submaximal velocity corresponding to individual running speed in marathon of one participant. The red shaded area indicates the analysis interval over 3 minutes of the first running bout designated as a specific warm-up at race speed; the blue shaded areas indicate the analysis intervals of the second, third and fourth running bout. Screenshot modified from Kubios HRV Premium (version 3.5.0).

Efficiency factor

For the analysis of internal-to-external-load relationship and a possible decoupling mechanism in comparison of the running bouts an efficiency factor (EF) was defined. This internal-to-external workload ratio was calculated using the ratio of the internal load indicators (VO_2 , RF, BLC, RPE, HR, $\% \text{HR}_{\text{MAX}}$, DFAa1, and EDR) and running pace (in km/h). The difference of the EF between the second and the fourth running bout was calculated and divided by the EF from the second running bout multiplied by 100 to get a percentage of alteration (%). Thus, a value of 10% indicates that internal-to-external ratio was 10% greater during the fourth running bout compared to that observed in the second running bout (Mauder et al., 2021; Smyth et al., 2022).

Statistical methods

The statistical analysis was performed using SPSS 27.0 (IBM Statistics, USA) for Windows (Microsoft, USA) and Microsoft Excel (Microsoft Corp, Redmond, USA). The Shapiro-Wilk test was applied to verify the Gaussian distribution of the data. The degree of variance homogeneity was verified by Levene test. Subsequently, a one-way ANOVA for repeated measurements was used to evaluate physiological changes over time (for data of second, third, and fourth running bout). Paired t-tests were applied to analyze differences between the

second and the fourth running bout. In addition, Cohen's d was calculated for effect size estimation (difference between mean values divided by the pooled standard deviation) with no effect ($d < 0.2$), small effect size ($d < 0.5$), moderate effect size ($d \geq 0.5$) and large effect size ($d > 0.8$) (Cohen, 1988). Further, interrelations and agreement between RF and EDR of all available data pairs of the rest condition, and all running bouts were evaluated using linear regression, Pearson's correlation coefficient (r), coefficient of determination (R^2), Intraclass Correlation Coefficient ($ICC_{3,1}$), and Bland-Altman plot with limits of agreement (LoA) (Bland & Altman, 1999). In addition, the mean absolute error (MAE) was calculated as the sum of absolute errors divided by the number of available data pairs of the rest condition, and all running bouts to add a quantification of the mean random scattering around the systematic bias (mean difference) and to account for different directions of this difference. If proportional bias was detected (change in the bias over the RF range), a regression-based calculation of mean differences was performed (Ludbrook, 2010). The size of Pearson's r correlation coefficient was evaluated as follows; low: $0.3 \leq r < 0.5$; moderate: $0.6 \leq r < 0.8$, high: $r \geq 0.8$ (Chan, 2003). Bland-Altman mean differences for data comparisons were expressed as absolute bias. The paired t -test was used for comparison of RF vs. EDR. Statistical tests were deemed to be significant at $p \leq 0.05$. All results are reported as means $\pm$ standard deviation (SD).

Results

The SB times corresponded to 795.7 ± 246.0 points of the World Athletics "Scoring Table of Athletics" (Spiriev, 2022), related to mean marathon times of 2:41:20 h:min:s and mean half-marathon times of 1:26:40 h:min:s. Consequently, mean running speed for the four submaximal running bouts was 15.3 ± 2.4 km/h (MIN: 11.7 km/h, MAX: 19.5 km/h). One-way ANOVA revealed significant main effects of time for RF, HR, and %HR_{MAX} (VO₂: $F=0.224$, $p=0.801$, $\eta^2=0.016$; RF: $F=6.818$, $p=0.004$, $\eta^2=0.327$; BLC: $F=0.279$, $p=0.759$, $\eta^2=0.020$; RPE: $F=0.596$, $p=0.506$, $\eta^2=0.041$; HR: $F=12.522$, $p<0.001$, $\eta^2=0.472$; %HR_{MAX}: $F=12.707$, $p<0.001$, $\eta^2=0.476$; DFAa1: $F=0.267$, $p=0.768$, $\eta^2=0.024$; EDR: $F=0.309$, $p=0.738$, $\eta^2=0.033$). In comparison of the second and fourth running bout both RF and HR showed statistically significant increases with small effect sizes. Furthermore, EF revealed values $< 5\%$ for all internal load indicators (see Table 1).

Table 1. Physiological measures during resting state before and during the four running bouts: mean $\pm$ SD (Range: MIN-MAX). VO₂: oxygen consumption, RF: respiratory frequency, BLC: blood lactate concentration, RPE: rating of perceived exertion, HR: heart rate, %HR_{MAX}: percentage of maximum heart rate, DFAa1: short-term scaling exponent alpha1 of detrended fluctuation analysis, EDR: ECG-derived estimated respiratory frequency, EF: Efficiency factor

Measure	Rest	First bout (specific) Warm-up	Second bout	Third bout	Fourth bout	Statistics*
VO ₂ [ml/min/kg], n=15	5.38 $\pm$ 0.92 (3.16-6.70)	49.34 $\pm$ 6.30 (39.30-61.87)	50.19 $\pm$ 6.53 (39.20-63.47)	50.04 $\pm$ 6.16 (40.10-64.51)	50.21 $\pm$ 6.59 (39.20-65.85)	$p=0.939$, $d=0.00$, EF=0.1%
RF [breaths/min], n=15	14.1 $\pm$ 3.7 (9.4-19.6)	41.5 $\pm$ 6.3 (33.3-56.1)	44.2 $\pm$ 6.5 (35.9-61.5)	44.5 $\pm$ 7.4 (34.2-63.0)	45.6 $\pm$ 7.4 (36.6-63.2)	$p=0.012$, $d=0.20$, EF=3.1%
BLC [mmol/l], n=15	1.27 $\pm$ 0.21 (1.02-1.76)	2.12 $\pm$ 0.78 (1.11-3.69)	2.09 $\pm$ 0.72 (1.29-3.82)	2.05 $\pm$ 0.85 (1.16-4.00)	2.11 $\pm$ 0.90 (1.26-4.07)	$p=0.862$, $d=0.02$, EF=-0.1%
RPE [6-20], n=15	-	12.8 $\pm$ 0.8 (11.0-14.0)	13.1 $\pm$ 0.9 (12.0-15.0)	12.8 $\pm$ 1.0 (11.0-14.0)	13.0 $\pm$ 1.3 (11.0-15.0)	$p=0.719$, $d=-0.15$, EF=-0.7%
HR [bpm], n=15	64.5 $\pm$ 9.7 (49.7-85.5)	163.0 $\pm$ 12.7 (145.5-190.5)	167.2 $\pm$ 12.5 (149.2-193.0)	168.4 $\pm$ 12.7 (151.0-195.5)	169.4 $\pm$ 13.0 (151.4-198.5)	$p<0.001$, $d=0.17$, EF=1.3%

%HR_{MAX}, n=15	34.2±5.1 (25.8-43.2)	86.5±6.8 (77.4-97.0)	88.8±6.5 (79.9-98.9)	89.4±6.8 (79.0-99.5)	89.9±6.7 (79.3-99.9)	p<0.001 , d=0.18, EF=1.3%
DFAa1, n=11-14	1.03±0.16 (0.70-1.21)	0.54±0.26 (0.25-0.99)	0.54±0.27 (0.26-0.93)	0.53±0.25 (0.25-0.90)	0.51±0.23 (0.18-0.87)	p=0.585, d=-0.14, EF=2.1%
EDR [breaths/min], n=10-12	15.0±4.1 (9.8-19.7)	39.0±7.6 (23.6-55.8)	42.3±7.0 (32.0-55.7)	42.6±5.6 (33.6-56.3)	42.6±4.0 (39.1-52.5)	p=0.443, d=0.04, EF=1.7%

*Comparison of the second and the fourth running bout.

Regarding the comparison of RF vs. EDR, 59 of 75 (79%) of all data pairs (resting condition, all four running exercise bouts) could be used. A strong linear relationship could be seen between the two measurement principles, with a high Pearson's r coefficient for the resting condition ($r=0.81$, $R^2=0.66$, $p<0.001$) and exercise bouts ($r=0.80$, $R^2=0.64$, $p<0.001$), and an intraclass correlation coefficient ICC_{3,1} of 0.90 for the resting condition and 0.87 for the exercise bouts. The comparison of RF vs. EDR revealed no significant difference for resting data ($p=0.435$, $d=-0.13$) but significant difference for the exercise data ($p=0.008$, $d=0.27$). Bland-Altman analysis showed a mean difference of 1.3 ± 4.1 breaths/min (resting values: -0.5 ± 2.4 ; exercise values: 1.8 ± 4.4) with limits of agreement of 9.3 to -6.8 breaths/min (resting values: 4.2 to -5.2 ; exercise values: 10.4 to -6.8), respectively (see Figure 2). The MAE indicated a value of 2.7 ± 3.3 breaths/min (resting values: 1.6 ± 1.8 ; exercise values: 3.1 ± 3.6).

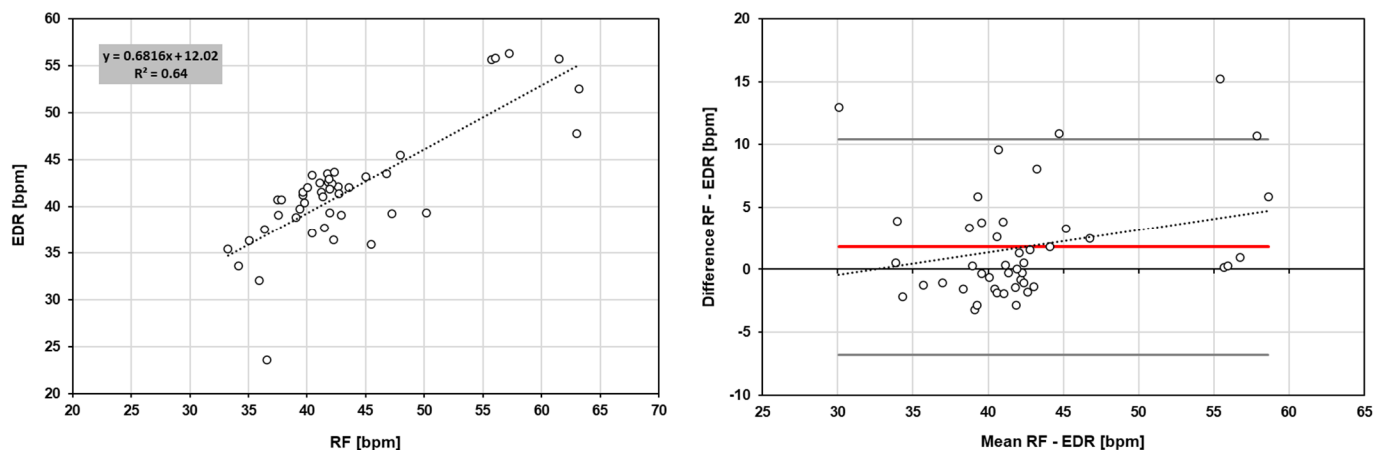


Figure 2. Regression plot for all data pairs of the exercise bouts of RF vs. EDR values (left). Bland-Altman plot for all data pairs of the exercise bouts of RF vs. EDR values (right); center line in red represents the mean difference between each paired value (dotted line: with regression-based calculation of mean differences), the top and bottom lines in grey display ± 1.96 standard deviations from the mean difference. R^2 : coefficient of determination.

Discussion

The aim of the present report was to evaluate alterations of DFAa1 and further respiratory and metabolic measures during multiple bouts of prolonged running at marathon pace in a group of trained female and male long-distance runners. Furthermore, agreement of EDR compared to gas exchange derived RF was evaluated during resting condition and the running bouts.

Results show that DFAa1 values decreased from ~ 1.0 at rest to ~ 0.5 at marathon running pace with no alterations when comparing the exercise bouts, which indicates a loss of fractal dynamics and a change towards uncorrelated and random behavior (Peng et al., 1995; Hautala et al., 2003). This corresponds to data from a study with recreational runners performing a self-paced marathon road race on an almost flat profile (Gronwald et al., 2021a). DFAa1 as a dimensionless index of correlation properties of HR time series and complex regulation has

shown the ability to reflect physiological demands compared to other internal load measures. It is assumed that the kinetics of DFAa1 during exercise is based on changes in autonomic modulation due to parasympathetic withdrawal, sympathetic activation, altered non-neural factors, and the potential loss of interaction between the two branches of the ANS with increased organismic demands (Persson, 1996; White & Raven, 2014). Interestingly, and similar to the study of Gronwald et al. (2021a) DFAa1 displayed a rather large inter-individual dynamic range during the evaluated running bouts, denoting possible fluctuations in internal load situation at race pace. External load prescription assumes that physiological responses are rather static (Jamnick et al., 2020; Maunder et al., 2021) and neglect the influence of internal and external factors leading to heterogeneity in exercise tolerance and physiological responses over time (e.g., personal or environmental factors, Gronwald et al., 2020; Meyler et al., 2023).

Percentage of HR_{MAX} during the exercise bouts reached values corresponding to the transition of heavy to severe exercise intensity domain in a 3-zone-model of intensity distribution for moderate, heavy and severe exercise domain (“vigorous”, Garber et al., 2011) with significant increase but very small effect. The same applies for RF with small effect size and no change in VO_2 . These changes are in line with the expectable difference in HR and VO_2 kinetics during constant load exercise (e.g., Zuccarelli et al., 2018) leading to the assumption of a quasi steady-state condition at marathon running pace. RPE showed mean values of ~13 with a range of 11 to 15 and no significant differences in comparison of the running bouts, showing inter-individual variation across participants. Blood lactate concentration revealed values around 2 mmol/l with no alterations over time but also considerable inter-individual differences below the point of what may be considered a maximal lactate steady-state (range: 1 to 4 mmol/l; Perrey et al., 2003). A study by Santos et al. (2006) took blood lactate samples every 6 km in elite marathon runners during a 30 km race and showed values from 2.4 mmol/l at 6 km to 3.2 mmol/l at 30 km. In a marathon field study, blood lactate values of 4.0 mmol/l could be observed immediately after the race (Gronwald et al., 2021a). Overall, the calculated EF assessing potential decoupling mechanism of internal-to-external load relationship revealed values under 5% showing almost no alteration in all internal load metrics in comparison of the running intervals. Therefore, this ratio may bear great potential for assessing possible decoupling mechanisms during prolonged running exercise bouts in comparison of different exercise intensity domains (Gronwald et al., 2024).

The comparison of EDR with gas exchange derived RF revealed high correlation coefficients with a low mean difference across all paired values including the resting condition and all running bouts; with higher values for MAE analysis. However, limits of agreement were relatively wide and the absolute divergences in breaths/min could be still clinically relevant on an individual level depending on the field of application. These results were also confirmed in an analysis across the entire intensity spectrum using the same sensor technology (Rogers et al., 2022a). A possible confounding factor in the EDR detection especially during running exercise might be the fact that the assessment of spectral estimates of HRV that are typically involved in RF estimation might be hindered as an overlap with a stride frequency component in terms of cardio-locomotor-respiratory-coupling (CLRC, Niizeki et al., 1993) and corresponding aliasing and signal distortion effects are hardly avoidable (Bailon et al., 2013). As the intensity of CLRC might be individual in different marathon runners (Hottenrott et al., 2020) this might contribute to the observed individual differences in the accuracy of RF estimation approaches based on ECG and/or RR-interval data.

Further questions need to be clarified about suitability of different subsystem parameters of internal load and an “optimal” and feasible real-time monitoring approach for the control of exercise intensity (e.g., HR drift and the potential underestimation of RPE; Cartón-Llorente et al., 2022). Here, a dimensionless, global, and systemic internal load indicator like DFAa1, in addition to RPE and RF as indicators of acute performance decrement (Passfield et al., 2022), also to detect ongoing compensatory mechanism and “homeodynamic” regulation pattern could provide the potential for exercise prescription and further investigation in prolonged running exercise of different intensities (Rogers & Gronwald, 2022; Gronwald et al., 2024).

Interpreting the results of our study, a few limitations should be taken into account. Our study included a small sample size of 15 participants. The number of running intervals was limited and therefore transfer of the applied exercise prescription for typical durations of running training (e.g., >30 min) may not be appropriate, as these longer durations may show further decoupling in internal-to-external load relationships. However, data of the present report show the relationship of DFAa1 with other internal load indicators at a fixed external load of marathon running pace and its stabilization as quasi-steady-state conditions with regard to traditionally used objective and subjective time-varying and instant data for exercise prescription and real-time internal load feedback (e.g., HR, BLC, RPE).

For EDR assessment Kubios HRV Premium software estimation algorithm was used based on the combination of the cyclic cardiac beat-to-beat time domain changes in RR-intervals associated with respiratory sinus arrhythmia and the single-channel ECG-associated R wave amplitude changes seen during the respiratory cycle (Lipponen & Tarvainen, 2021). One aspect that definitely affects the results of this estimation algorithm is the design to accommodate a wide range of applications, from short resting measurements to long-term recordings and sports applications (e.g., not specifically to signal quality aspects of running exercise). Therefore, adaption of this algorithm specialized to endurance sports applications with different types of exercise would potentially enhance validity within the estimation of RF via EDR and narrow the range of upper and lower limits of agreement. In addition, approximately 25% (see Table 1) of data had to be excluded for non-linear HRV and EDR analysis due to data quality and artifact rate which can still affect the use in sport-specific field conditions. As mentioned before, the CLRC in running (Niizeki et al., 1993) and corresponding aliasing and signal distortion effects (Bailon et al., 2013) might be specific challenges that need to be refined in future advances in sensor technology and HRV signal analysis to further improve signal integrity and reliability. In that regard, EDR analysis together with the assessment of DFAa1 bears the potential of a more comprehensive internal load assessment in post-exercise and real-time analysis based on simple low-cost chest belt recordings (Gronwald et al., 2021b; Gronwald et al., 2024).

Conclusion

DFAa1 is defined as an indicator of relative internal load and proxy of physiological demands. It showed no alterations in comparison of multiple intervals of continuous running at marathon race pace in female and male long-distance runners. The comparison of EDR with gas exchange derived RF during running revealed high correlations and low mean differences, but rather large limits of agreement. This shows the necessity of further improvement of the methodology before being used in a wide range of individuals and different sports applications. In addition, further research and development of sensor technologies and analysis algorithms are needed to realize the benefits of the chest strap form factor in sports practice. The present report showed that a fixed external load based on marathon running pace implies considerable inter-individual differences in all internal load metrics. In this context, the relationship of DFAa1 to other traditionally used internal load metrics in quasi-steady-

state conditions bears the potential for further evaluation of exercise prescription in general and the enlightenment of decoupling mechanisms in exercise bouts of different duration and type.

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