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DISSERTATION

Cardiac autonomic modulation as a human physiologic response to extreme environments

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von Lea Christiane Rundfeldt
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ABSTRAKT

Hintergrund: Extreme Umwelten wie klimatische Exposition, außergewöhnliche physische Belastung, oder Schwerelosigkeit, fordern die menschliche Anpassungsfähigkeit und Resilienz heraus. Wohlbefinden und Leistungsfähigkeit können mittels kardialer autonomer Modulation, gemessen durch Herzratenvariabilität (HRV), unter Extrembedingungen wie Ultramarathon oder Bettruhe in Kopftieflage erfasst werden. Höhere vagale Aktivität ist mit besserer Ausdauer und Leistung assoziiert, und auch hochintensives Intervalltraining kann parasympathische Prädominanz und autonomes Gleichgewicht steigern. Diese Dissertation erforschte die Bedeutung von morgendlicher Kurzzeit-HRV-Messung unter Extrembedingungen. Eine höhere Vagusaktivität und erhaltenes autonomes Gleichgewicht infolge von Ausdauer- und Intervalltraining wurden erwartet. Zudem sollte die Assoziation zwischen Parasympathikus-Aktivität sowie Wohlbefinden, und Anpassungs- sowie Leistungsfähigkeit in extremen Umwelten dargestellt werden.

Methodik: Die morgendliche Kurzzeit-HRV wurde durch Herzfrequenzaufzeichnungen erfasst. HRV-Messung (liegende und stehende Position) vor dem Wettkampf wurde, in Relation mit dem Trainingsstatus, zur Leistungsvorhersage in Teilnehmern (n=25) eines 100 km langen Ultramarathons (gehend) verwendet. Veränderungen in HRV-Indizes (liegend) und psychometrischen Korrelaten wurden während eines 690 km langen subarktischen Ultramarathons (n=16) analysiert. Zudem wurden zeitlicher Verlauf und Veränderungen in HRV-Indizes (liegend) und orthostatischer Toleranz während 60-tägiger Bettruhe in Kopftieflage (n=23) in einer Kontroll-, als auch einer Intervalltrainingsgruppe, untersucht.

Ergebnisse: HRV-Ausgangswerte zwischen Finishers und Nicht-Finishers (Teilnehmer, die das Ziel nicht erreichten) beider Ultramarathons waren vergleichbar. Eine höhere Wettkampfgeschwindigkeit war im gehenden Ultramarathon mit einem größerem Abfall vagaler Aktivität während aktivem Aufstehen assoziiert. Bei besser vs. schlechter trainierten Athleten stellte sich ein höherer Parasympathikus-Tonus im Stehen dar (HF_{nu} +11.5, p 0.01). Während einem subarktischem Ultramarathon nahm die Vagusaktivität im Liegen infolge der schweren Belastungen ab, erholte sich aber teilweise direkt nach dem Rennen. Eine niedrigere Ruhe-Herzfrequenz (repräsentativ für Vagusaktivität) war zudem mit höherer Geschwindigkeit assoziiert (r -0.6, p 0.04). Zudem spiegelten HRV-Messungen den zeitlichen Verlauf und das Ausmaß von autonomer Dysbalance und orthostatischer Intoleranz (teils persistierend) während und infolge von Bettruhe wider. Regelmäßiges Training konnte dies jedoch abmildern und die Vagusaktivität erhalten.

Fazit: HRV-Ausgangswerte konnten partiell zur Vorhersage von Ultramarathonleistung verwendet werden, wobei ein orthostatischer Test zusätzliche Information über die dynamische autonome Anpassungsfähigkeit lieferte. Kontinuierliche HRV-Messungen repräsentierten das Ausmaß und den zeitlichen Verlauf von HRV-Detrimenten und orthostatischer Intoleranz während Bettruhe, welche durch Intervalltraining abgemildert werden konnten. Die morgendliche Kurzzeit-HRV-Messung stellt somit eine einfache, schnell anwendbare nichtinvasive Methode zur Überwachung von Leistungs- und Anpassungsfähigkeit sowie Wohlbefinden in extremen Umwelten dar. Kardiale autonome Modulation, und ihre dynamische Adaptabilität, spielen zusammenfassend eine entscheidende Rolle für die menschliche Anpassungsfähigkeit und Resilienz.

ABSTRACT

Background: Extreme environments, like climatic exposition, arduous exercise, or microgravity, pose great challenges for human adaptability and resilience. Cardiac autonomic modulation in terms of Heart Rate Variability (HRV) may serve to monitor wellbeing and performance during extraordinary challenges, e.g., ultramarathon competition or head-down tilt (HDT) bed rest. Higher vagal drive relates to wellbeing and better endurance training status, and high-intensity interval training may also enhance parasympathetic predominance and preserve autonomic balance. This dissertation investigated the value of short-term morning HRV assessment under strenuous conditions. Hypothetically, greater parasympathetic drive, maintained autonomic balance and orthostatic tolerance, would be observed through both endurance exercise and interval training. Also, the association between vagal drive and wellbeing with better adaptivity and greater performance in extreme environments was aimed to be established.

Methods: Heart rate (HR) recordings (at morning) served to assess short-term HRV indices. Performance prediction by pre-race HRV in supine and standing position, related to training experience, was analyzed in competitors (n=25) of a 100 km walking ultramarathon. Additionally, changes in supine HRV and psychometric correlates were investigated throughout a 690 km subarctic ultramarathon (n=16). Finally, the time-course and extent of changes in supine HRV and orthostatic reactivity promoted through 60-day HDT were investigated (n=23) in subjects undergoing high-intensity exercise, and control subjects.

Results: Pre-race HRV was comparable between finishers and non-finishers (who withdrew before course completion) in both ultramarathons. In walking ultramarathon athletes, a higher decrease in pre-race vagal drive during position change predicted greater velocity. Standing vagal drive was higher in better vs. poorly trained competitors (HF_{nu} +11.5, p 0.01). During subarctic ultramarathon, supine vagal drive significantly decreased due to in-race challenges but partly recovered in successful athletes directly post-race. A lower baseline resting HR (representing higher parasympathetic drive) predicted a greater velocity (r -0.6, p 0.04). Furthermore, HRV assessment served to monitor the extent and time-course of cardiovascular deconditioning with impaired autonomic balance and orthostatic intolerance (partly persisting post-HDT) through HDT without countermeasures. Regular exercise mitigated detriments, thus preserving vagal drive.

Conclusion: Baseline supine HRV may partly predict ultramarathon performance, with additional information on dynamic autonomic adaptability through orthostatism. Continuous HRV measurements mirrored the extent and time-course of detriments promoted by HDT, which were mitigated through exercise. The non-invasive and easily implemented short-term morning HRV measurement sensitively depicted physiological responses to different extreme environments. It may therefore serve to monitor performance, adaptability, and wellbeing, ultimately relating to adaptive capacity and resilience.

1. BACKGROUND

Continuous reactive adaptation to current organismic demands and external stressors characterizes the human physiology (1, 2). This adaptivity can be investigated through assessment of cardiac autonomic control in terms of Heart Rate Variability (HRV), as HRV, and specifically vagal tone modulations, may mirror human adaptivity and resilience (3-5). Cardiac autonomic control consists of the balanced interplay of the parasympathetic and the sympathetic branch of the autonomic nervous system (ANS), thus adjusting the heart rate (HR) to acute requirements and conditions (6). Generally, enhanced vagal activity promotes a decreased HR, whereas greater sympathovagal balance increases the HR (6). At rest, and in healthy subjects, vagal drive predominates (6). As HRV typically exhibits sex differences, women display greater indices of parasympathetic tone and total HRV, whereas in men, a shift in sympathovagal balance towards sympathetic predominance can be observed (7). Conversely, reduced vagal drive and total HRV are related to pathological states, such as cardiovascular deconditioning, adverse outcomes and increased mortality (6).

HRV assessment as a fast, non-invasive and easily implemented tool has gained interest in both clinical applications (e.g., cardiovascular and metabolic diseases) (6) and sport sciences, exemplarily, for athletic monitoring (8). Usually, HR recordings to assess HRV in athletes are implemented in the morning upon awakening and during supine rest (9). Performing an orthostatic challenge (i.e., position change from supine to upright) during the recording may provide additional information, as a light and persistent shift towards increased sympathovagal balance is promoted through orthostatism (10). The resulting assessment may then more sensitively reflect training status and predict performance (10, 11). Also, an athlete’s current training status, and the point in the training period when he is performing, should be considered, as HRV changes depend on training periodization and a “spot evaluation” may thus have poor significance (12). The specific type of training also influences the corresponding HRV parameter changes (13), while endurance athletes particularly present higher indices of total HRV, as well as vagal drive (with lower resting HR), at rest (9, 13, 14). These adaptations have been observed to directly correlate with the amount of endurance training (e.g., greater distance, longer training time) (14-16). However, not only prolonged aerobic exercise, but also high-intensity interval training may promote increased total HRV and vagal drive (17) in healthy subjects, as well as cardiovascularly deconditioned patients. Additional benefits include enhanced aerobic capacity, improved left-ventricular and vasomotor function, as well as optimized blood pressure (BP) profile (18, 19). Thus, high-intensity short duration exercise may both enhance total HRV and vagal predominance and mitigate cardiovascular deconditioning with altered autonomic control and orthostatic intolerance.

Also, HRV assessment may serve for performance prediction (13). Previous studies observed a higher resting vagal drive at baseline to predict better endurance performance, such as faster race times, or greater distance covered (20, 21). Pre-race HRV measurements may also allow differentiation between more and less successful participants, such as finishers vs. non-finishers, who withdraw before race completion and possibly experience adverse events (21, 22). This is especially relevant in ultramarathon, which is defined by course lengths exceeding marathon distance (i.e., 42.195 km) (22). Ultramarathon competition has gained substantial interest over the past years (23). With distances up to 1000 km and durations over 20 days (24), athletes undergo extremely prolonged and strenuous exercise. Also, ultramarathons may account for extreme altitudes or elevation gains (25), and arduous weather conditions, such as great heat (26), or subarctic climates (27). In addition, competitors face impaired resting conditions, as they mostly sleep on the track or in primitive checkpoint facilities, and thus experience severe sleep deprivation (28). Therefore, ultramarathon is an excellent model to study the human adaptive potential under extreme and adverse conditions (29). This can be investigated through HRV assessment, which may mirror adaptability (3) and resilience (5), and serve to recognize higher performing athletes already pre-race (22).

Yet, what is the greater value of investigating human physiology in extreme environments? Considering aggravating environmental challenges and destruction of various living spaces (30), exploring alternative habitats in deep space may become necessary (31). This ultimately leads to the question how humans will adapt and cope in the environment of space habitats (32). Consequently, earth-based research on the human adaptive potential in extreme conditions and adverse environments is vital (33). This is exemplarily performed in arduous climatic surroundings, or during extraordinary physical challenges (e.g., ultramarathon), as well as during simulated adverse conditions, like prolonged microgravity (9). Weightlessness exposure promotes various detriments, such as musculoskeletal deconditioning (32) and cardiovascular decompensation displayed by increased resting HR, altered autonomic balance with reduced total HRV and parasympathetic predominance, as well as orthostatic intolerance (34, 35). Implementation of 6° head-down tilt (HDT) during prolonged bed rest (i.e., head-down bed rest) is considered the most significant microgravity analog (36, 37). Similar to volume centralization observable in spaceflight, comparable cardiovascular deconditioning during HDT is promoted by fluid shifts in the upper body (36, 37). Additionally, bed rest serves to investigate consequences of immobilization, which is a common feature of hospitalization especially in elder patients and holds great clinical importance (38), considering that inactivity presents one of the major death causes in western regions (39). However, previous studies have not yet determined an exact time-course of cardiovascular and cardiac autonomic deconditioning promoted by prolonged immobilization and microgravity (simulation) (40). Therefore, assessing physiological consequences of inactivity and developing effective countermeasures is crucial both for public health and rehabilitation concepts, as well as upcoming deep space missions. Exercise may counteract detrimental effects of microgravity, such as musculoskeletal and cardiovascular deconditioning (33, 41). Still, training usually needs to be combined with additional treatments, such as volume loading or whole-body vibration (32, 42), to effectively mitigate said detriments. Short-duration high-intensity training represents a promising approach, but has rarely been studied in spaceflight and earth-based analogs (43).

To summarize, HRV assessment holds clinical importance in the diagnosis and prospective observation of pathological conditions. Also, it may be used to monitor wellbeing and adaptive capacity under extreme conditions, as well as to predict adaptivity and performance. This dissertation investigated the value of short-term morning HRV assessment, a fast, easily implemented, and non-invasive diagnostic tool, in extreme environments. First, the benefit of pre-race HRV assessment to contextualize endurance training experience and for performance prediction in ultramarathon was analyzed. Also, changes in HRV indices and psychological correlates over the course of an extreme ultra-endurance competition were assessed. Said analyses were performed in both a 100 km race in eastern Germany, and a 690 km competition in the subarctic Yukon Territory. Further, changes in HRV indices and hemodynamics in subjects participating in a 60-day head down bed rest trial undergoing regular lower body interval training in simulated microgravity, compared to a control group, were investigated. It was hypothesized that greater indices of vagal drive, and maintained autonomic balance and orthostatic tolerance, would be observed in subjects with better endurance training experience, as well as through short-duration high-intensity exercise. Also, subjects who would better cope with strenuous conditions and perform greater were again expected to display enhanced vagal drive and wellbeing. Finally, short-term morning HRV assessment was speculated to depict physiological responses to extreme environments and thus mirror human adaptive ability and resilience.

2. METHODS AND MATERIALS

This joint investigation consists of three different studies. Two separate trials were performed in two respectively different ultramarathon races [Study A (44) and B (45)], and the third investigation took place in a 60-day HDT trial [Study C (46)].

2.1 Study A [Subarctic Ultramarathon: Cardio-Regulation and Mood in the *Yukon Arctic Ultra* (*YAU*)]

This study (44) was performed as part of a larger investigation regarding "*Physiological changes of participants of the Yukon Arctic Ultra – an ultramarathon in extremely cold climate*". The Montane® *Yukon Arctic Ultra* (*YAU*) annually proceeds in the beginning of February in the Canadian Yukon Territory, where a total distance of 690 km must be covered in ≤14 days. Especially the first and last segments of the race display significant elevation gains (up to 1000 m), and athletes face the extreme climate of subarctic winter (temperatures as low as -47°C and air humidity up to 100%; no significant difference in weather conditions between editions). Medical screenings are regularly performed at 10 checkpoints which are distributed along the course (mostly ~50 km apart), and participants may be excluded due to medical conditions, such as severe frostbite. Apart from the checkpoints, which consist of mere tents or indoor facilities, athletes make resting and eating arrangements outside on the track, whilst pulling their gear on a sled-like "pulka" (44).

Subjects were recruited through information material distributed by the race organizers, whereas all competitors were eligible for study inclusion. During 4–5 days before the race, volunteers met with the investigators, could ask further questions, and gave their written informed consent. The study was approved by the ethics committee of the Charité University of Medicine, Berlin (document number EA4/109/12) and all measurements and procedures complied with the Declaration of Helsinki (54th Revision 2008, Korea). As all subjects had previously participated in marathons and/or ultramarathons (maximal distance: 478 ± 219 km), they were considered experienced athletes (44). Table 1 depicts subject characteristics.

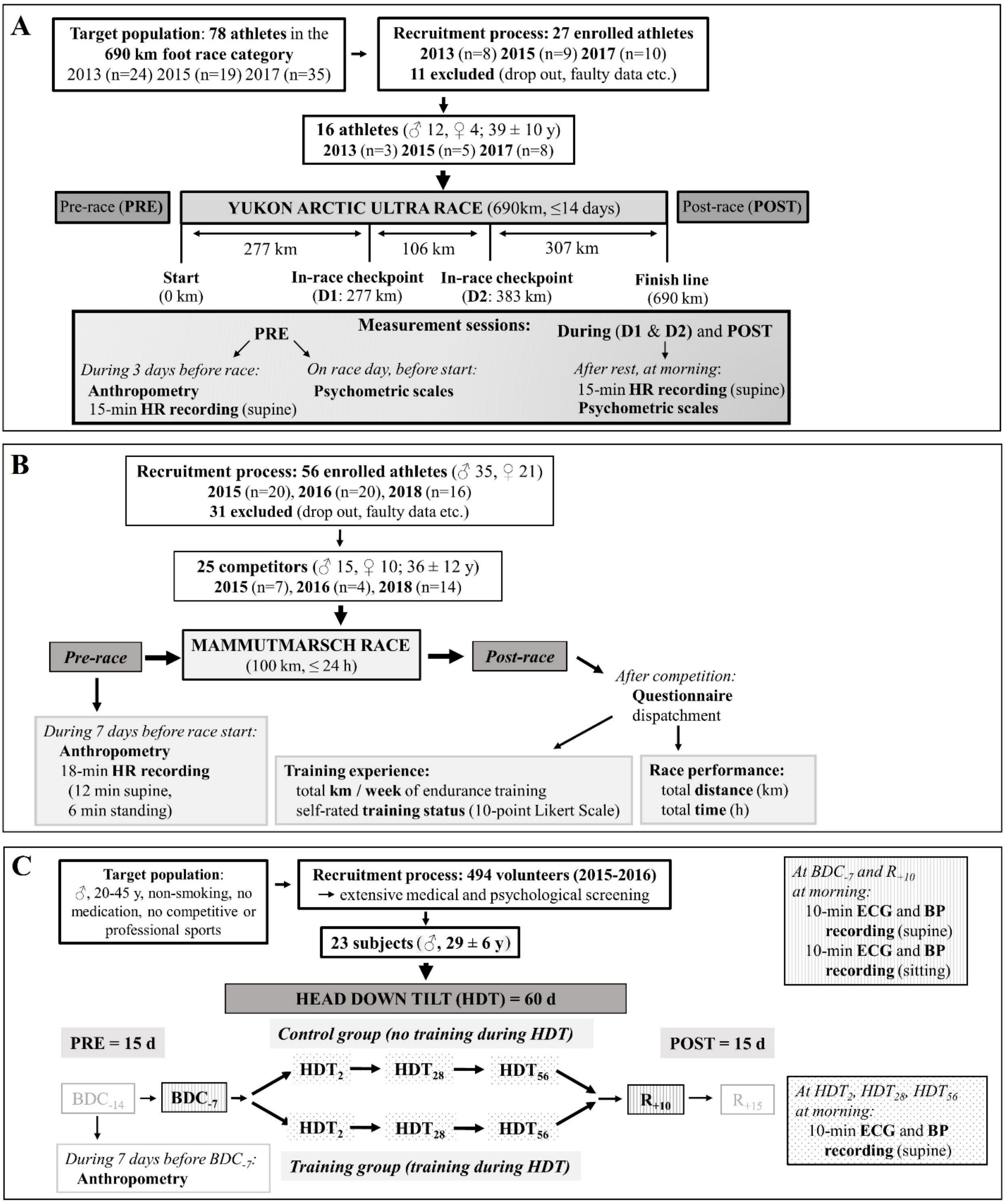

FIGURE 1. Study Protocol. *Study design and respective measurements employed in the YAU [(**A**); adapted from (44)], MM [(**B**); adapted from (45)] and RSL study [(**C**); adapted from (46)].*

Subject recruitment and experimental protocol are depicted in detail in Figure 1A. Assessments took place during the 3 race-preceding days (PRE), after 277 km at an in-race checkpoint (During 1, D1), after 383 km at an in-race checkpoint (During 2, D2), and after 690 km at the finish (post-race, POST). Checkpoints were chosen due to essential practical concerns that would allow measurements, such as indoors facilities and accessibility by car (44). At baseline (PRE), anthropometric data was collected, and 15-min beat-to-beat HR recordings were performed during supine rest (mentioned respectively as SUP) upon awakening (between 5 and 10 a.m. after ≤6–8 h sleep) using the widely applied and validated for HRV assessment HR monitor RS800CX (Polar Electro Oy, Kempele, Finland) (47). Athletes were lying on a bed or sleeping mattress, and attention was dedicated to providing a quiet and agreeable environment with reduced light and comfortable temperature. Participants were advised to breathe normally, avoid speaking and moving, and to refrain from consuming food, beverages or stimulating agents in the 2 h before the measurement (44). On the morning of the race start, psychometric scales were administered. At in-race checkpoints or the finish, after rest (≤6 – 8 h) upon awakening, HR recordings, and psychometric assessment again took place. As for psychometrics, the Borg scale and the Profile of Mood States (POMS) were assessed [amongst others, see (44)]. The Borg scale served to determine subjective "Total Quality of Recovery" (Borg TQR) and consists of a 6–20 numerical scale (6 = "very, very poor recovery"; 20 = "very, very good recovery") (48). The Profile of Mood States administered in the short form (POMS-SF) (49) is highly validated for athletic monitoring and may correlate with both HRV indices and performance (50, 51). Athletes were requested to classify the extent of "mood states" experienced in the last hours on a 1–5 numerical scale (1 = "not at all"; 5 = "extremely"). Sub-scores were then summarized to provide a total score for each main mood dimension: Vigor (the only positive dimension), Tension, Fatigue, Depression, Confusion and Anger (all negative dimensions) (44, 49).

Study	**Group**		**Age, y**	**Weight, kg**	**Height, cm**	**BMI, kg/m^2**
YAU	**ALL**	(n=16; ♂ 12 ♀ 4)	39 ± 10	75 ± 12	175 ± 8	24.3 ± 3.1
	FIN	(n=10; ♂ 7 ♀ 3)	40 ± 9	74 ± 12	174 ± 8	24.5 ± 3.5
	NON	(n=6; ♂ 5 ♀ 1)	36 ± 10	76 ± 14	177 ± 10	23.9 ± 2.4
MM	**ALL**	(n=25; ♂ 15 ♀ 10)	36 ± 12	71 ± 10	177 ± 9	22.6 ± 1.5
	FIN	(n=14; ♂ 10 ♀ 4)	35 ± 12	71 ± 11	177 ± 10	22.6 ± 1.5
	NON	(n=11; ♂ 5 ♀ 6)	36 ± 13	70 ± 10	176 ± 8	22.6 ± 1.5
RSL	**ALL**	(n=23; ♂ 23)	29 ± 6	77 ± 7	181 ± 6	23.5 ± 1.9
	TRAIN	(n=12; ♂ 12)	30 ± 7	78 ± 7	181 ± 7	23.6 ± 1.8
	CTRL	(n=11; ♂ 11)	28 ± 6	76 ± 8	181 ± 5	23.4 ± 2.0

TABLE 1. Subject characteristics in *YAU, MM* and *RSL* studies. *Data reported as mean ± SD for all subject samples (ALL) and subgroups (FIN, NON; TRAIN, CTRL; see section 2.5 for details on subgroup assignment). No significant differences between subgroups.*

2.2 Study B [Supine and Standing HRV in Training: The *Mammutmarsch* (*MM*)]

This study (45) was conducted as part of a larger investigation called "*Baseline characteristics, performance predictors and physiological changes in a 100 km walking ultramarathon*". The *Mammutmarsch* (*MM*) annually proceeds in May near Berlin (Germany), while the 100 km course leads over paved streets and forest trails (elevation gains <100 m) and must be completed by walking only in ≤24 h, resulting in a very prolonged, but low-intensity exercise. Competitors exhibit a wide range of training levels and experience, and the *MM* is considered an amateur ultramarathon. A call among competitors in the years 2015, 2016 and 2018 was distributed through information material sent out by the race organizers, as well as social networks.

Interested athletes contacted the study team and were personally invited to the laboratory. All potential subjects received information material about the study design and were able to ask questions before giving their written and informed consent to participate (see Table 1 for subject characteristics). The study complied with the Declaration of Helsinki (54th Revision 2008, Korea) and was approved by the ethics committee of the Charité University of Medicine, Berlin (document number EA1/163/14) (45).

Study	Group	Distance, km	Time, h	Velocity, km/h
YAU	**ALL**	352 ± 107	217 ± 79	2.8 ± 0.7
	FIN	690 ± 0 *	268 ± 30 *	2.6 ± 0.3
	NON	367 ± 105	131 ± 57	3.1 ± 1.0
MM	**ALL**	85 ± 18	19 ± 6	4.9 ± 1.2
	FIN	100 ± 0 *	23 ± 2 *	4.4 ± 0.4 §
	NON	67 ± 9	13 ± 4	5.4 ± 1.7

TABLE 2. Performance data. *Data reported as mean ± SD for all competitors (ALL), as well as athlete subgroups FIN and NON (see section 2.5 for details on subgroup assignment). *: significant difference between subgroups (p <0.05). §: trend to significant difference between subgroups (p <0.1>0.05).*

Subject recruitment and experimental protocol are reported in detail in Figure 1B. During the 7 days preceding the race (between 8 and 12 a.m.), pre-race data collection took place in a laboratory at the Charité Berlin. All subjects were instructed to avoid exhausting training and not consume coffee, alcohol, or other stimulants, during the previous 12 h. First, anthropometric data were assessed, then beat-to-beat HR was recorded for 12 min in supine (SUP), and, upon verbal instruction, for 6 min in standing (mentioned as STD) position in a quiet and comfortable room at constant ambient temperature. HR recordings were performed with a validated HR monitor (Polar RS800CX) and during the measurement, participants were advised to breathe normally and avoid speaking and moving (45). After the competition, a questionnaire was sent to all athletes, to assess their individual performance in the *Mammutmarsch*, as well as training experience. Subjects provided their total accomplished distance and time, as well as specific reasons for withdrawal before race completion (if applicable). Also, they stated details about their training experience and fitness level in a standardized form, as they were required to declare the total distance of endurance training (walking or running) per week, mentioned here as km/week, in the 6 months preceding the race. Then, training experience was characterized based on the resulting median of the weekly training km (24.5 km/week). Thus, athletes were either categorized as highly (HT: ≥24.5 km/week; n=12) or poorly trained (PT: <24.5 km/week; n=12). Also, subjects rated their subjective endurance training status at the time of the *MM* on a 10-point Likert scale (0 = “extremely poor”; 10 = “excellent”) (45).

2.3 Study C [High-Intensity Exercise Mitigates Cardiovascular Deconditioning (*RSL*)]

This study (46) was performed as part of the European Space Agency (ESA) sponsored study “***R**eactive jumps in a **S**ledge jump system as a countermeasure during **L**ong-term bed rest – RSL Study*” at the DLR :envihab [German Aerospace Agency (DLR), Cologne, Germany] between 2015 and 2016. The study was conducted in accordance with the Declaration of Helsinki (54th Revision 2008, Korea) and approved by the ethics committee of the Ärztekammer Nordrhein in Duesseldorf (Germany). Potential subjects received an information session, followed by extensive psychological and medical screenings (52). After giving written informed consent to partake in the investigation, 24 men enrolled in the study. Subjects (n=23; see section 2.5 and Table 1 for subject characteristics) were randomly allocated to a training (TRAIN; n=12, who performed regular short-duration high-intensity exercise involving the lower body during HDT), and a control group (CTRL; n=11, no countermeasures) (46). The training was performed in a sledge jump system (Novotec Medical GmbH, Pforzheim, Germany): subjects were lying in supine position on a lightweight sledge sliding on rails and fixed to their body with shoulder straps. As cylinders pull the sledge towards foot plates, subjects performed countermovement jumps. The training protocol started at the first day of HDT and took place 5–6 times/week for ~20 min, while the average load accounted for ≥80% of body weight (46). Further details about the sledge system and training protocol are reported by Kramer et al. (52).

Figure 1C depicts subject recruitment details and experimental protocol. Baseline anthropometric data was collected during 15 days before the start (BDC_{-15}) of the 60-day bed rest (which was followed by 15 days of recovery). Hemodynamic and autonomic cardiovascular data were collected on the 2nd (HDT_2), 28th (HDT_{28}) and 56th day (HDT_{56}) of HDT, to evaluate short-, mid- and long-term effects. Also, all data were collected at baseline 7 days before the start of HDT (BDC_{-7}) and 10 days after the end of HDT (R_{+10}) in both supine (SUP) and sitting (mentioned as SIT) position, to assess the response to orthostatic stress. Blood pressure (BP) was assessed via continuous finger plethysmography and beat-to-beat HR was recorded with one-lead ECG (both sampled at 200 Hz). All measurements took place between 9 and 12 a.m. (>18 h after previous exercise) and lasted for ≥10 min in each position. Subjects were instructed not to consume caffeine during the preceding 4 h, and were advised not to move, talk, or fall asleep during the recordings (46).

2.4 HRV Assessment

All ECG and BP series (in the *RSL* study) were inspected by an experienced operator using a distinctive software (Kubios HRV ver. 2.1, Kuopio, Finland). Premature beats or artifacts were removed with the filter adjusted at the "low" level and threshold set at <0.3 % of beats recognized as artifacts (53). Only files of sufficient length were considered: ≥15 min in the *YAU*, ≥18 min in the *MM*, and ≥20 min (for BDC_{-7} and R_{+10}) or ≥10 min (for HDT_2, HDT_{28}, HDT_{56}) in the *RSL* study. To standardize the analysis, the last 10 min (*YAU*), or the last 10- and 5-min (*MM*), and the last 10-min segments (*RSL*) for each body position were chosen for HRV analysis and converted in the normal-to-normal (NN) interval series. Then, HRV could be assessed as validated indices of cardiac autonomic modulation based on time-, frequency-domain and complexity (54). Regarding time-domain, NN50 statistics (reflecting the rate of vagal "outflow bursts") (55) were calculated: the hourly number of increases (NN50+) or decreases (NN50–) between consecutive NN intervals >50 ms (55), and the percentage of such differences regarding the total number of NN intervals (pNN50+ and pNN50–) (56). In the frequency domain, high frequency (HF) band, which derives from vagal drive and is synchronous with respiratory sinus arrhythmia, was assessed (reported as normalized units, i.e., HF_{nu}, in the *MM* study) (54). Also, low frequency (LF) band (depending on both vagal and sympathovagal activity) was computed (54). Thus, the LF/HF ratio, reflecting sympathovagal balance, was assessed (54). Regarding non-linear and complexity analysis, HR sample entropy (SampEn) was analyzed. It determines the irregularity of NN interval series and indicates greater vagal drive or sympathovagal deactivation (57). Furthermore, both the short-term (α1) and long-term (α2) self-similarity coefficient of NN intervals were analyzed by detrended fluctuation analysis (DFA) (58). Both parameters, here mentioned as DFA_1 and DFA_2, derive from vagal activity, as increased sympathovagal balance or decreased vagal drive promote higher DFA_1 (59). The DFA_2 may be associated with higher alertness and influenced by sleep stages (60). In the *RSL* study, breathing rate was assessed as the central frequency of the power spectrum of the ECG-derived respiration signal (61). Except for manually calculated NN50 statistics, all HRV indices were assessed with the free available Kubios HRV software, which is widely used in sports sciences (53).

2.5 Statistics

All data are reported as arithmetic means ± standard deviations (m ± SD) in the *YAU* and *MM* study or marginal means ± standard error (m ± SE) in the *RSL* study, if not otherwise stated. A log-transformation was applied to frequency domain indices in order to attain normal distribution (62) in the *YAU* and *RSL* study. The significance level was set at $p < 0.05$.

2.5.1 Specific Statistics in the YAU and MM Study

From 27 enrolled competitors in the *YAU*, 16 were included in the statistical analysis (Fig. 1A). Due to the extreme race conditions, several participants withdrew before course completion. Thus, the entire sample (ALL) was divided into finishers (FIN: athletes who reached the 690 km finish, complete measurements available until POST) and non-finishers (NON: athletes who withdrew before course completion; all measurements available until D1). According to the distribution, changes in HRV parameters and psychometric measurements over the entire racecourse in FIN were tested with one-way repeated measures analysis of variance (One-Way RM ANOVA) and post-hoc Student's-Newman-Keuls-Test, or Friedman ANOVA on ranks with post-hoc Tukey's Test. As all competitors reached the first checkpoint, two-way repeated measures ANOVA (Two-Way RM ANOVA) served to directly compare both groups regarding PRE and D1 (44). Data from 25 participants in the *MM* were eligible for the analysis (Fig. 1B). The entire sample (ALL, n=25) was divided into subgroups: athletes who reached the 100 km finish were categorized as "finishers" (FIN), and subjects who prematurely withdrew were classified as "non-finishers" (NON). Also, athletes were assigned into dichotomous variables (HT: highly trained, and PT: poorly trained, see section 2.2) regarding self-reported training volume by using standard median split technique. The difference in HRV indices due to position change during HR recording (SUP vs. STD) was computed by subtracting the value measured in SUP from the value recorded in STD and then referred to as "delta" (represented by "Δ" before each HRV index label). Between-subgroup differences at pre-race (e.g., FIN vs. NON) were analyzed with unpaired Student's t-Test or a Mann-Whitney rank sum test, when normality check failed (45).

In both the *YAU* and *MM* studies, normal distribution was tested with Shapiro-Wilk-Test and variance with the Equal-Variance-Test. Correlations between HRV indices and i) psychometrics or ii) training experience, as well as correlations between performance and i) HRV indices or ii) training experience were assessed with Pearson Product-Moment-Correlation or, if normality was not passed, Spearman's Rho. All statistical analyses were carried out with SigmaPlot ver. 12.3 (Systat Software, San José, CA, USA) (44, 45).

2.5.2 Specific Statistics in the RSL Study

From 24 enrolled subjects, 23 (ALL) were included in the analysis (one subject discontinued the trial before the start of HDT due to unrelated medical reasons), and all measurements could be performed (see Fig. 1C). To assess the time-course of HRV parameter and hemodynamic changes during and after HDT compared to baseline, data of HDT_2, HDT_{28}, HDT_{56}, and R_{+10} were expressed as percentage changes from BDC_{-7} by dividing the values measured at each time point during HDT by the corresponding value measured in BDC_{-7} supine (= 100 % reference). Due to the properties of the logarithm (applied to HF and LF), normalized variables were expressed as the difference between the log-transformed value at each time point and the log-transformed value at baseline (= reference zero level). The time-course of HRV parameter changes in supine position within and between subjects was assessed with two-factorial linear mixed models. "Subjects" were entered as random factors and bed rest "time" (HDT_2, HDT_{28}, HDT_{56}, and R_{+10}) and intervention "group" (CTRL and TRAIN, see section 2.3) were included as fixed factors. Significant effects of "time" and "group" or their interaction were followed up using contrasts (BDC_{-7} = reference for "time"). When only the factor "time" was significant, contrasts were performed irrespective of the intervention (i.e., CTRL and TRAIN were pooled). Regarding the orthostatic challenge (i.e., the position change from SUP to SIT) implemented before and after HDT, variables were expressed as delta (Δ) scores. The Δ-score represents the difference between the value measured before HDT (BDC_{-7}) and the respective value measured after bed rest (R_{+10}) per subgroup (CTRL and TRAIN) and position (SUP and SIT). Thus, Δ-scores represent changes from baseline values (i.e., BDC_{-7}) and express the effects of HDT (respectively for group and position). Within-participant and between-participant differences in Δ-scores were assessed using mixed models. "Position" and "group" were included as fixed and "subjects" as random factors. When the factor "position", or "group", or their interaction was significant, contrasts were performed to follow up on single comparisons. To evaluate a significant effect of HDT in a given group and posture, contrasts were used to determine whether each Δ-score differed significantly from zero (= non-zero contrasts). Normality and homogeneity were tested by visual inspection of plots of residuals against fitted values. A sequentially rejective correction procedure was applied to correct for multiple comparisons. All statistical analyses were performed with the software package R (The R Foundation for Statistical Computing, Vienna, Austria) (46).

3. RESULTS

3.1 Study A (*YAU*)

Of the entire sample (ALL, n=16), 10 athletes completed the 690 km course (FIN). The remaining 6 competitors withdrew prematurely (NON), e.g., due to fatigue or musculoskeletal complaints (see Table 2).

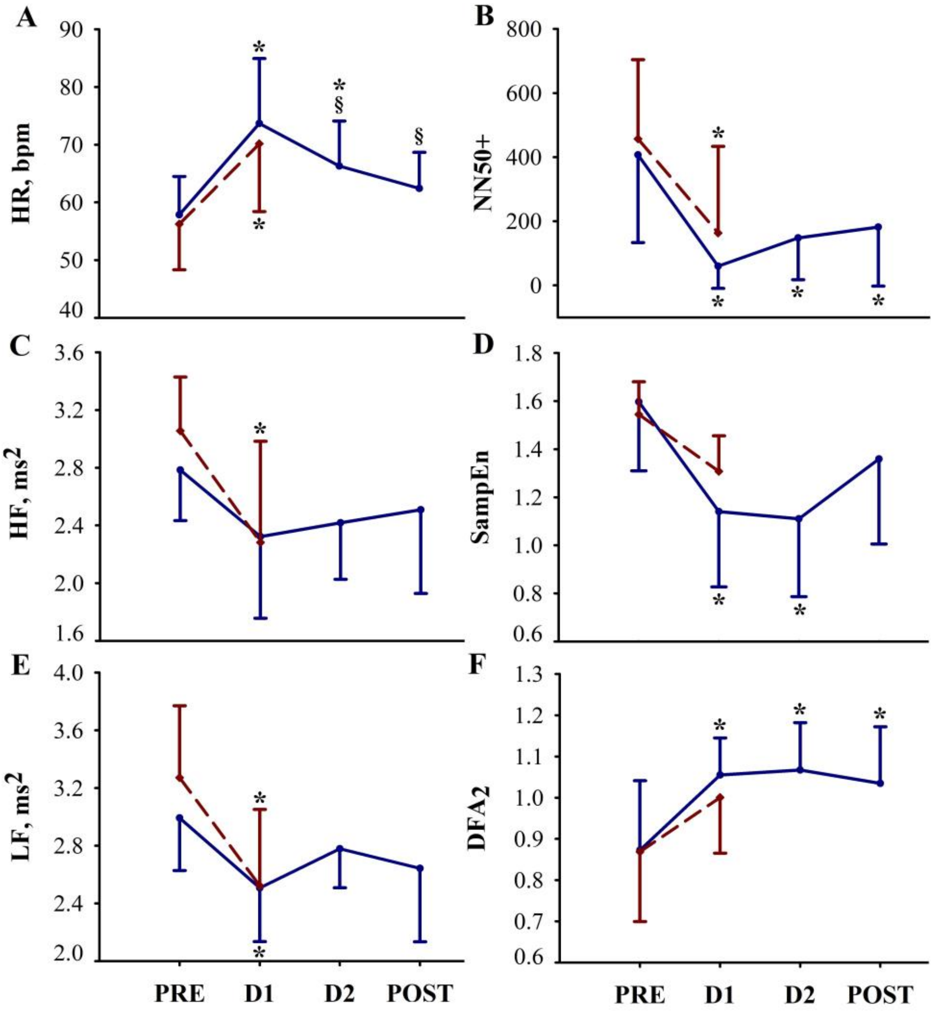

FIGURE 2. Changes in HRV over the race in FIN and NON (*YAU*), *adapted from (44). Vagal modulation (**A-D**) and sympathovagal balance (**E-F**) indices (reported as mean ± SD) in FIN (blue line) and in NON (red dash) across time-points (see 2.5.1 for subgroup details). *: significant difference vs. PRE within subgroup (p <0.05). §: significant difference vs. D1 within subgroup (p <0.05). No significant differences between subgroups.*

Due to no significant differences in baseline HRV between male and female, data were pooled regardless of gender for further analyses. Similarly, there were no differences in baseline HRV between FIN and NON (Figure 2). Mean HR significantly increased in both groups at D1. In FIN, it then gradually decreased again and was no longer significantly different at POST vs. PRE (Fig. 2A). At D1, a significant decrease in vagal drive indices was observed in both groups (which was only partially restored at POST in FIN, Fig. 2B–D). As indicated by significant decreases in HF (Fig. 2C), as well as increased DFA_1, only in NON, this decrease at D1 was partly greater than in FIN. Conversely, only FIN exhibited significantly higher DFA_2 over the entire race with respect to PRE (Fig. 2F). Further regarding sympathovagal balance, LF decreased at D1 in FIN and NON (Fig. 2E), leading to no significant changes in LF/HF. As for correlation analysis, in FIN at PRE, a lower HR was associated with both greater velocity (r -0.6, p 0.04), and higher pNN50+ (r -0.7, p 0.03). At D1, the negative correlation between HR and NN50- (r -0.8, p 0.02) persisted in FIN.

Regarding psychometric correlates, there was a significant decrease in positive mood in both groups at D1, as POMS Vigor decreased, while POMS Fatigue augmented. In FIN, this was mainly restored at POST. As for correlations between HRV and psychometrics, at PRE, NON displayed a significant positive correlation between NN50+ and Borg TQR (r 0.9, p 0.01), while a higher pNN50+ would also predict lower POMS Fatigue (r -0.8, p 0.04). At D1, Borg TQR correlated positively with DFA_2 (r 0.8, p 0.03) in FIN.

3.2 Study B (*MM*)

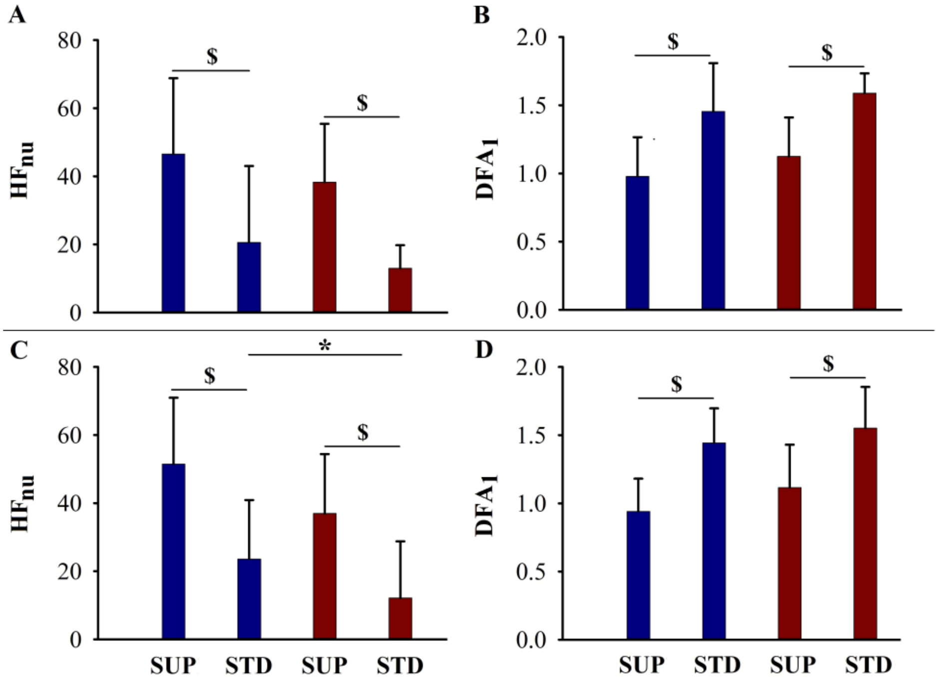

FIGURE 3. Pre-race HRV in FIN vs. NON, and HT vs. PT (*MM*), adapted from (45). *HRV indices (data reported as mean ± SD) assessed pre-race in (**A**, **B**) FIN (blue bar) vs. NON (red bar), and (**C, D**) HT (blue bar) vs. PT (red bar) in supine (SUP) and standing (STD) position (see 2.5.1 for subgroup details). $: significant difference for SUP vs. STD within subgroup (p <0.05). *: significant between-subgroup difference in the same position (p <0.05).*

From a total of 25 athletes (ALL), 14 reached the 100 km finish (FIN) and 11 withdrew before course completion (NON), e.g., due to exhaustion or musculoskeletal complaints. FIN displayed a lower overall velocity than NON (-1 km/h, p 0.09). Table 2 further depicts performance data.

Standing position promoted a reduced vagal drive vs. supine in all athletes (Figure 3). Due to no significant gender differences in pre-race HRV, data were pooled irrespective of sex for further analyses. Similarly, there were no significant differences in pre-race HRV in FIN vs. NON (Fig. 3A, B). Regarding correlations between HRV and performance in FIN, velocity was negatively correlated with Δ-HF_{nu} and was positively associated with Δ-DFA_1 (Figure 4), a greater vagal drive decrease due to position change therefore predicting a higher velocity. No correlations between pre-race HRV and performance were found in NON.

Training experience data was obtained from 24 subjects, as one athlete was not available to follow-up. All competitors amounted to a mean 30 ± 23 km walked or run per week, while subjective endurance training status was rated at averagely 7.5 ± 1.4 / 10. In ALL, a greater km/week related to higher STD-HF_{nu} (r 0.5, p 0.01). No correlations between training experience and performance could be depicted in ALL.

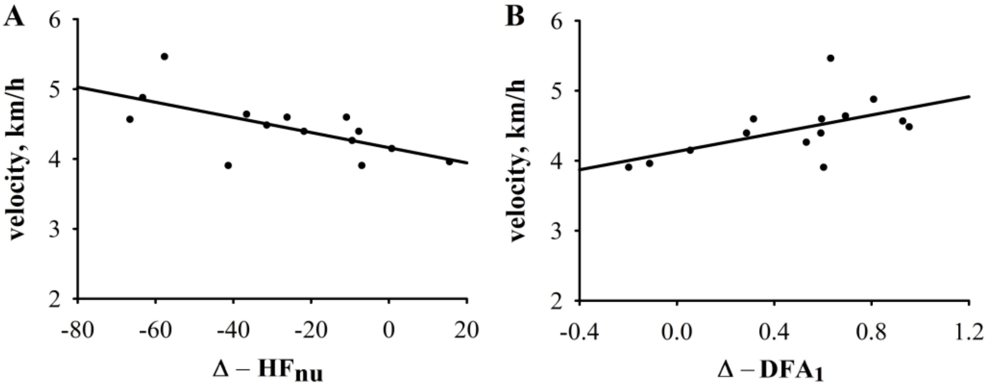

FIGURE 4. Correlations between pre-race HRV and velocity in FIN (*MM*), adapted from (45). *(**A**) Correlation between Δ-HF$_{nu}$ and velocity (r -0.7, p 0.01). (**B**) Correlation between Δ-DFA1 and velocity (r 0.6, p 0.04).*

As for subgroup assignment by training experience, STD-HF$_{nu}$ was greater in highly trained (HT) than in poorly trained (PT) subjects (+11.5, p 0.01; Fig. 3C). Also, a higher STD-DFA$_1$ predicted a greater velocity in HT (r 0.6, p 0.04). No correlations between pre-race HRV and performance could be observed in PT.

3.3 Study C (*RSL*)

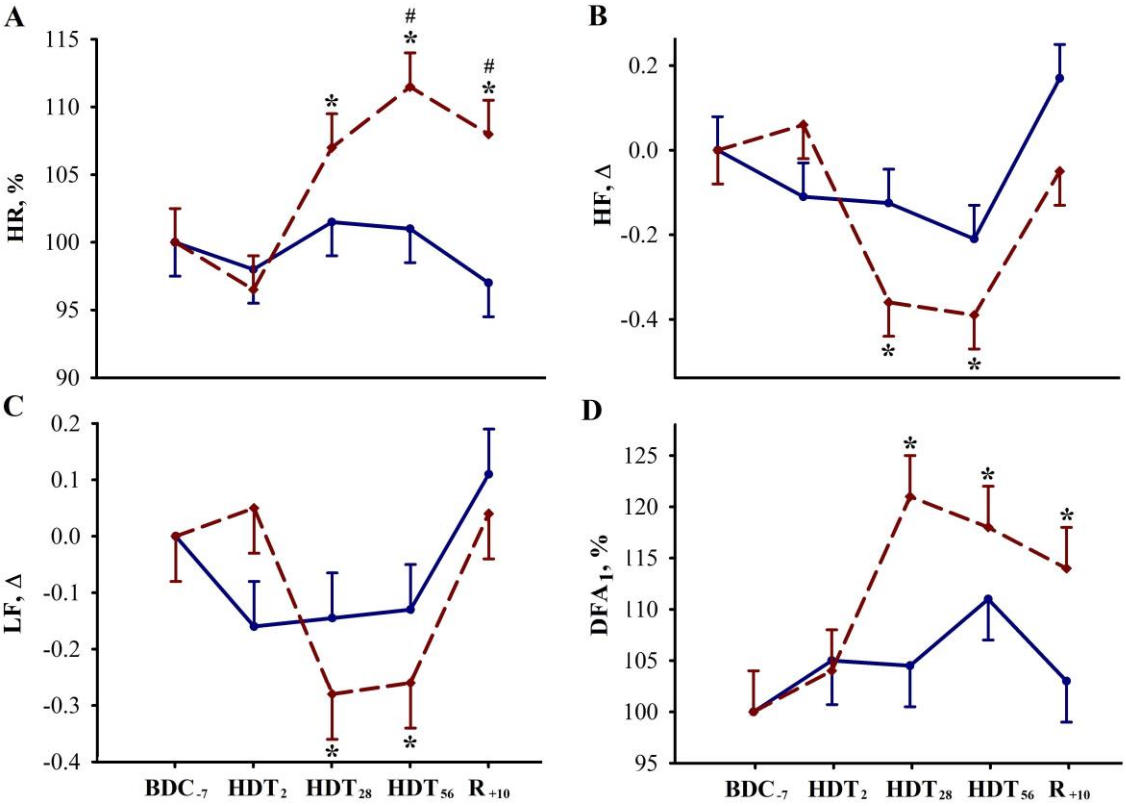

FIGURE 5. Changes in HRV through HDT (*RSL*), adapted from (46). *Time-course of HR(V) indices for TRAIN (blue line) and CTRL (red dash) in supine position across time-points (see 2.3 for subgroup details). Data presented as mean ± SE with respect to baseline supine (HF, LF; SUP-BDC$_{-7}$ = 0) or percent changes from baseline supine (HR, DFA$_1$; SUP-BDC$_{-7}$ = 100%). *: significant difference vs. BDC$_{-7}$ within subgroup ($p < 0.05$). #: significant between-subgroup difference ($p < 0.05$).*

Continuous measurements of hemodynamic and cardiac autonomic modulation variables served to assess the extent and time-course of alterations promoted by HDT (Figure 5). Resting HR significantly increased only in CTRL from HDT_{28} on and was higher than in TRAIN at HDT_{56} and R_{+10} (no changes in TRAIN; Fig. 5A). Also, in CTRL, systolic blood pressure (SBP) significantly decreased at R_{+10} vs. BDC_{-7}. HRV indices did not significantly change in TRAIN. In CTRL, both LF and HF significantly decreased at HDT_{28} and HDT_{56} but recovered to be no longer different vs. BDC_{-7} at R_{+10} (Fig. 5B, C). Conversely, DFA_1 increased from HDT_{28} on and remained increased until R_{+10} in CTRL (Fig. 5D). No changes or between-group differences were observed for LF/HF.

Both before and after HDT, in both groups, orthostatic challenge promoted an increase in HR, LF/HF and DFA_1, while HF decreased. However, HDT had a differential effect on the amplitude of the respective changes, i.e., Δ-scores (Figure 6). HR Δ-scores were not significantly different from zero in TRAIN. In CTRL, SUP-HR Δ-scores were significantly higher than the SIT Δ-score, as well as SUP-HR Δ-scores in TRAIN (Fig. 6A). SUP-SBP Δ-scores only in CTRL were negative and significantly different vs. BCD_{-7}, and also different from the Δ-score in SIT (which did not change). As for cardiac autonomic control, at R_{+10}, CTRL exhibited a positive and significantly different Δ-score for SIT-HF (i.e., HF was higher vs. BDC_{-7}; Fig. 6B). Supine and sitting HF Δ-scores in CTRL were also different vs. TRAIN, who exhibited a positive and significantly different Δ-score for SUP-HF. In CTRL, SIT-LF/HF and SIT-DFA_1 Δ-scores were negative and different vs. BDC_{-7} (Fig. 6C, D). LF/HF and DFA_1 Δ-scores did not change in TRAIN.

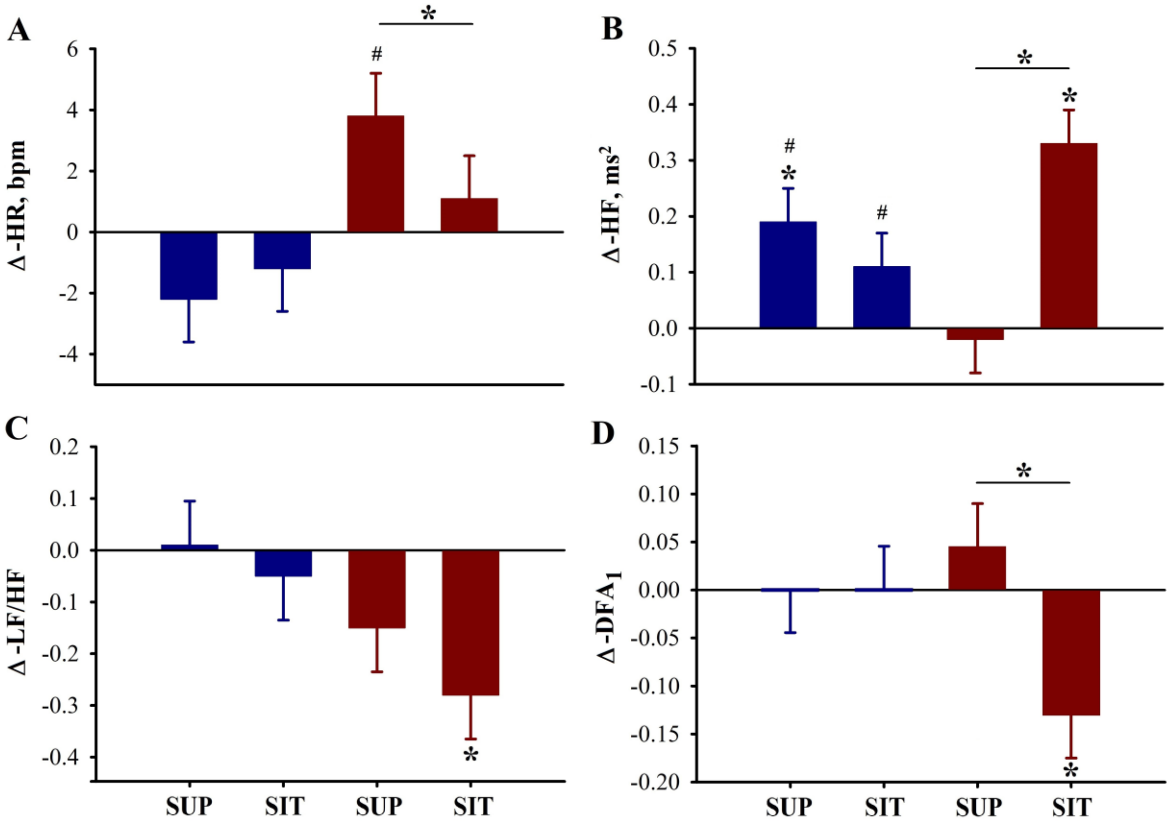

FIGURE 6. Changes in orthostatic reactivity after HDT (*RSL*), adapted from (46). *Δ-scores of HR(V) indices, i.e., the difference (Δ) between the value measured at* BDC_{-7} *and the corresponding value measured at* R_{+10} *for supine (SUP) and sitting (SIT) position in TRAIN (blue bar) and CTRL (red bar), reported as mean ± SE (see 2.3 for subgroup details). *: Δ-score significantly different from zero (p <0.05). *: significant difference for SUP vs. SIT Δ-score within subgroup (p <0.05). #: significant between-subgroup difference in the same position (p <0.05).*

4. DISCUSSION

The overarching aim of this investigation was to evaluate cardiac autonomic modulation, in terms of HRV, as a human physiologic response to and in extreme environments. The resulting dissertation is based on three different studies investigating short-term morning HRV under exceptional conditions, assessing i) the effect of respective environments on cardiac autonomic control, ii) associated influential factors, and iii) the value of (baseline) HRV assessment to predict human performance in and adaptability to extreme challenges. All three trials occupy particular value, as they were conducted in unique and not yet comparably investigated environments. This section will discuss the most important findings and contextualize them beyond the singular study. Overall, this dissertation shows that short-term HRV monitoring was effective and sensitive to detect physiological responses to different challenges that may be assimilated as an extreme environment.

According to Millet, ultramarathon is an excellent model to study human adaptive potential, reflected by cardiac autonomic modulation, in extreme and adverse conditions (29). HRV may predict ultra-endurance performance (13), as greater race velocity or total distance have been associated with higher resting vagal drive at baseline (20, 21). Thus, short-term morning HRV measurements in ultra-endurance athletes may both mirror human adaptability and serve to recognize potentially more and less successful competitors (e.g., finishers and non-finishers) already pre-race (21, 22). This dissertation investigated cardiac autonomic modulation in two extraordinary ultramarathons. To our knowledge, the *YAU* study was the first to assess performance predictors at baseline, as well as respective changes during the race, in HRV parameters and psychometric correlates in competitors of a subarctic ultramarathon (44). The *MM* study analyzed the relationship between pre-race HRV, and both performance and training experience, in the particular setting of a 100 km walking ultramarathon (which represents a greatly prolonged but lower-intensity exercise) (45). Notably, to our knowledge, only one other study had investigated cardiac autonomic modulation in a 24-hour walking race to this date (63). It was hypothesized that more successful athletes, i.e., finishers, could already at baseline be recognized through higher indices of vagal drive and total HRV, compared to non-finishers. However, in both *YAU* and *MM* studies, no differences in pre-race HRV indices between FIN and NON subgroups were respectively found (44, 45). Still, as there is little research investigating baseline differences and race success predictors by specifically comparing finishers and non-finishers (21, 22, 64), limited background exists to compare with the lack of pre-race HRV differences in FIN vs. NON (which may also have been influenced by the unique characteristics of both *YAU* and *MM* competitions).

Associations between pre-race HRV and performance were investigated in both *YAU* and *MM* competitors. In FIN (*YAU*) athletes, negative correlations between supine resting HR (which again was correlated with vagal drive indices) and mean velocity at PRE and D1 were found, a lower HR therefore predicting a greater velocity (44). As the negative correlation between HR and NN50 statistics at PRE and D1 indicated the lower HR to be promoted by greater parasympathetic tone, a higher vagal drive during supine rest was concluded to predict greater velocity and performance, in line with previous investigations (20, 21). The importance of vagal drive preservation for performance and coping will be further underlined by findings of

HRV changes throughout the *YAU* race (44). In *MM* competitors, an orthostatic challenge (supine to standing) in contrast to an exclusively supine recording was implemented (45). Limited research on the association between pre-race HRV and its change during orthostatic challenge, and ultra-endurance competition performance, has been conducted so far (20, 65). Specifically, orthostatic provocation promotes a light and persistent shift towards increased sympathovagal balance and decreased parasympathetic drive (10), as reflected by higher DFA_1 (66) and decreased HF (10). Thus, the dynamic changes in HRV indices through orthostatic challenge may more sensitively reflect the link between HRV and training status (10, 11), such as exercise volume (67), as well as ultra-endurance performance (20). Indeed, in FIN (*MM*), only Δ-values depicted the association between HRV and performance, as a greater decrease in vagal tone (HF_{nu}), or higher sympathovagal activation (DFA_1) upon position change predicted a higher velocity (45). These correlations are unlikely random, as they were observed in two completely different parameters: HF_{nu} as a spectral index (54), and DFA_1 as a recursive index of signal geometric complexity (62). Yet, an exact physiological explanation is challenging and may additionally require training experience assessment. As training periodization (such as training status and the current time-point at which an athlete performs, e.g., training vs. competition) significantly influences HRV changes (12), HRV measurements in extreme athletic environments may be contextualized through training history. In the *MM* study, a standardized questionnaire served to determine self-rated endurance training status and training volume (mean km walked or run per week), thus allowing categorization as highly (HT) or poorly trained (PT) (45). In line with previous findings on increased parasympathetic tone in direct correlation with longer training distances and duration (14-16), the HT group accounted for greater resting vagal drive than competitors with lower training volume (PT; see Fig. 3C). Similarly, correlation analysis for the entire sample ALL (*MM*) showed higher km/week and greater endurance capacity to relate to higher resting vagal tone (45), as supported by recent findings (8, 15). Yet, considering training periodization, converse to here assessed isolated pre-race resting HRV, vagal drive may decrease shortly before an upcoming competition, while increasing and then predominating sympathovagal balance could facilitate cardiac performance (e.g., with greater inotropic function) (68). Thus, the ability to supersede parasympathetic predominance promoted by long-term aerobic training may improve race performance (68), including a lower total time and higher velocity. This autonomic adaptability to external provocation exhibited by FIN (*MM*) might be crucial for successful ultra-endurance competition (20), allowing the ANS to instantly adapt the HR to the different level of effort and supply the required energy (68). However, in NON (*MM*), no correlations between HRV and performance were found. Although resting HRV was comparable in both FIN and NON (*MM*) competitors (Fig. 3), hypothetically, NON (*MM*) athletes may not have been able to sufficiently override vagal predominance (aiming to facilitate prolonged cardiac performance and race success) (45). Also, orthostatic stimulation may more specifically mirror exercise-induced HRV changes (i.e., parasympathetic responsiveness) and thus demonstrate associations between pre-race HRV and aerobic training response (11). Indeed, greater sympathovagal balance in standing position predicted higher velocity in the better trained HT subgroup (45).

Finally, changes in HRV parameters, and psychometric correlates were assessed over the course of the *YAU* competition, which exposed athletes to a three-folded stress stimulus (44): i) strenuous ultra-endurance exercise, ii) the extreme environment of subarctic winter and iii) impaired rest and sleep deprivation (28). The greatest impact on cardiac autonomic control and mood was observed during the first race segment PRE–D1, possibly related to the stress of entering the race (69), the great distance of 277 km (and significant elevation gains) and arduous weather conditions in early February (which tend to alleviate over the race duration). A decrease in vagal drive and total HRV usually occurs during acute exercise and is observable directly afterwards (70, 71). Similarly, *YAU* athletes at D1 exhibited an increased resting HR and reduced vagal drive indices, such as NN50 statistics and SampEn (Fig. 2A–D) (44). Lower SampEn values may indicate impaired reactivity to environmental stimuli (72) and enhanced sympathovagal modulation was suggested by differences in the distribution of HR accelerations (pNN50-) and decelerations (pNN50+) (56). Furthermore, the observed correlations between NN50 statistics and HR suggested the decrease in vagal drive to have mediated the respective changes in resting HR at PRE and D1 (44). Considering significant decreases in HF (Fig. 2C), and increased DFA_1 (representing vagal withdrawal) (59) at D1 only in NON (*YAU*), the decrease in parasympathetic tone may have been greater than in FIN (*YAU*). This could display the inability of NON (*YAU*) competitors to adapt to the arduous race conditions already at the beginning, concurrent with reduced HRV (44). However, in FIN (*YAU*), vagal tone indices recovered already during the race (possibly due to the very long duration, as well as successful adaptation to adverse conditions) (44). Comparably, previous studies found restoration of impaired vagal drive after ultra-endurance races, depending on exercise intensity and training status (20, 64, 73). After D1, the increased HR (mirroring impaired recovery and physiological strain), decreased again, and was no longer different at POST vs. PRE (Fig. 2A). Concurrently, vagal drive indices partly recovered post-race (Fig. 2 B–D). This restoration of autonomic balance towards the finish is considerable, especially regarding the distance of 307 km and greatest elevation gains between D2 and POST (44). As, after D1, correlations between resting HR and vagal drive indices were no longer detectable, other factors may have contributed to the restoration of HR(V) – such as psychometric correlates. The POMS questionnaire sensitively depicted decreased positive mood after the first race segment (PRE–D1), but in FIN (*YAU*), this was partly restored until POST (44). Previous studies support the hypothesis that the recovered wellbeing may have contributed to vagal tone restoration (51, 74). Indeed, a higher vagal drive (NN50+) at baseline predicted a better recovery (Borg TQR) in NON. Additionally, only FIN (*YAU*) displayed enhanced DFA_2 over the entire race compared to PRE (Fig. 2F), and Borg TQR was positively correlated with DFA_2 at D1 (44). Higher need of alertness during the subarctic ultramarathon may hence have mediated the increase in DFA_2 (59), and then contributed to greater recovery in optimally coping FIN (*YAU*). All this highlights the significant interplay of cardiac autonomic balance and psychometric correlates for adaptation and athletic achievement in extreme environments (44).

In contrast to the great challenge of arduous exercise during ultramarathon competition, bed rest trials as a microgravity analog simulation present an equally grave strain on human physiologic adaptability and resilience (46). Exemplarily, cardiovascular deconditioning is one of the most problematic features observed after spaceflight and microgravity analog trials (32, 75). The *RSL* study investigated the value of short-term morning HRV measurements to monitor the exact time-course and extent of changes in cardiac autonomic control parameters and hemodynamics induced by the “extreme environment" of a 60-day HDT bed-rest challenge in a countermeasure (TRAIN) and control (CTRL) group, with, to our knowledge, the highest number of subjects in such a specific study so far (46). In subjects not undergoing countermeasures (CTRL), short-term morning HRV assessment reliably depicted detriments in cardiovascular control and autonomic balance reflected by i) a continuous increase in resting HR, ii) significant alterations in HRV from mid-term HDT on (HDT_{28}), and iii) persisting detriments after a 10-day recovery, suggesting prevailing cardiovascular deconditioning. However, these alteration were effectively mitigated by a short-duration high-intensity lower body training (possibly through activation of central cardiac modulation mechanisms), which may thus serve to counteract adverse effects of prolonged inactivity or microgravity exposure (46).

Generally, the extreme load of prolonged HDT manifests in a significant fluid shift to the upper body, mediating a decreased vagal drive in HRV and increased vasomotor sympathetic tone, as well as decreased baroreflex sensitivity (37), whereas findings on sympathovagal balance alterations are partly contradictive (34). Similar to previous findings (40), HR significantly increased in CTRL from HDT_{28} on, remaining elevated until the recovery period, and was significantly higher than in TRAIN at HDT_{56} and R_{+10}. Also, CTRL exhibited a marked decrease in SBP at R_{+10} (whereas no hypotensive effects were detected in TRAIN), which suggested long-term impairment of BP regulation and possible orthostatic hypotension through 60-day HDT without countermeasures (46). Regarding HRV, HF and LF were reduced at HDT_{28} and HDT_{56} (but this was restored through a 10-day recovery). Additionally, both LF/HF (although only trending for significance), a marker of sympathovagal balance, and DFA_1 (an increase indicating greater sympathetic activation), had increased at HDT_{28} and HDT_{56} (46). Thus, the observed HRV alterations in the CTRL group were concluded to be promoted through reduced vagal drive (confirmed by respiratory rate assessment) and increased sympathovagal balance (46). Conversely, in TRAIN, resting HR, as well as vagal drive and sympathovagal balance indices, did not significantly change. This is in line with recent studies showing beneficial adaptations in cardiac autonomic modulation through anaerobic short-term exercise (17) even for cardiovascularly compromised patients (18, 19). In our study, the stable resting HR in TRAIN during HDT may represent a training-induced vagal tone enhancement, which would be supported by the significantly different positive Δ-score in SUP-HF (Fig. 6B) (46). Previous countermeasure trials underline the importance of exercise to mitigate cardiovascular deconditioning (33, 41), but training alone may be insufficient, as earlier studies showed only the combination of aerobic and/or resistive exercise with additional countermeasures (e.g., plasma volume restoration, whole body vibration) to alleviate detrimental effects of HDT (75, 76). Another trial demonstrated high-intensity resistive exercise only when coupled with aerobic exercise during lower-body negative pressure application to preserve orthostatic tolerance during bed rest (77). Considerably, the here implemented isolated exercise protocol effectively mitigated cardiovascular autonomic deconditioning (mainly affecting fast vagal modulation) (46), in support of previous observations (17).

As orthostatic intolerance is one of the most common and problematic features of cardiovascular deconditioning in microgravity (78), a supine-sit challenge at baseline and during recovery was additionally implemented to evaluate the response to orthostatic stimulation after HDT. In all subjects, the position change at both timepoints promoted an expectable increase in LF/HF and DFA_1, whereas HF decreased (10, 11, 46). However, as the amplitude of changes differed, HDT impacted HRV indices more in one or the other position. Exemplarily, in CTRL, SUP-HR Δ-scores were significantly higher than in the sitting position, and in TRAIN (where Δ-scores in both positions were closer to zero) (46). The significant negative Δ-score in SUP-SBP for CTRL indicates a possible hypotensive effect (underlined by the decrease in supine SBP at R_{+10} only in CTRL), and the significant difference in SBP Δ-scores between SUP and SIT suggests posture-dependent long-term hemodynamic alterations through HDT (46). Impaired sympathovagal activation upon orthostatic challenge, and consequently possible orthostatic intolerance (35, 40) in CTRL at R_{+10} was further indicated by a significant positive Δ-score for SIT-HF and reduced Δ-scores for DFA_1 and LF/HF in sitting position vs. BDC_{-7}. TRAIN exhibited less altered orthostatic reactivity, especially without changed sympathovagal balance or hypotension. Also, HF Δ-scores were positive in both positions for TRAIN. Thus, the implemented high-intensity exercise may have promoted preservation and/or faster recovery of physiological autonomic reactivity to orthostatism during the challenge of prolonged HDT bed rest (46).

Several possible limitations deserve attention. Both *YAU* and *MM* subject samples consisted of athletes from three different editions (44, 45). Yet, as ultra-endurance races still account for less competitors than other, more popular sports events (79), it is difficult to recruit larger samples in singular editions. Regarding the *YAU*, a sample of n=16 was deemed appropriate, as from a total of 78 athletes over the three editions, 27 primarily enrolled (Fig. 1A). Also, for the *MM* study, a sample of n=25 was regarded to be sufficient, as research on HRV in ultra-endurance athletes usually accounts for 10-30 subjects (20, 63, 73). Female proportions of the entire sample accounted for 25% (*YAU*) and 40% (*MM*) (44, 45). Greater female samples are generally required in ultra-endurance research. Notably, no sex differences in pre-race HRV could be detected in both *YAU* and *MM* athletes (44, 45). Still, HRV may also be influenced by the menstrual cycle (80), body composition (81) and physical activity (82, 83), and most evidence on sex differences was obtained in standard populations (7), from which female ultramarathoners may differ. In the *YAU*, there were significant differences in the distances between measurement points, which were chosen due to practical concerns, also aiming to standardize measurement conditions (see section 2.1) (44). In the *RSL* study, only men were recruited (46), as is common in HDT trials (37). Future trials should investigate the value of short-duration high-intensity exercise to counteract cardiovascular deconditioning in microgravity analog studies with greater sample sizes and female subjects. Also, diversity in study design and training concepts complicate a direct comparison of the sledge jump protocol with previous trials (46). Finally, HDT may demonstrate similar findings as actual microgravity, but still represents an earth-based analog with specific limitations (36).

4.1 Conclusion

This dissertation assessed short-term morning HRV as a human physiologic response to and in extreme environments. First, the value of baseline HRV measurement for performance prediction, as well as its association with psychological correlates and training experience, was examined in competitors of two unique ultramarathon races. A higher vagal tone during supine rest, as well as greater sympathovagal activation reactive to orthostatic stimulation at baseline may predict better adaptability and performance in extreme environments, such as ultramarathon. Incorporating an orthostatic challenge may provide additional information on the dynamic adaptability of the ANS to physiologic challenges, compared to HRV assessment during exclusively supine rest. The extent of early vagal withdrawal, as well as the timing and potential of its recovery during ultra-endurance performance may be crucial for successful adaptation to extreme challenges. Also, self-reported endurance training experience may reliably relate to vagal drive and thus serve to contextualize HRV measurements. Finally, assessment of short-term morning HRV and psychological profile may serve to monitor and partly predict performance in such extreme ultra-endurance competitions. Practical applications include training, underlining the importance of greater vagal drive (and its restoration after successful coping with physiological challenges), as well as mood states and motivation, especially in prolonged ultra-endurance exercise and adverse environments. Furthermore, HRV assessment served to monitor the exact time-course and extent of hemodynamic and cardiac autonomic alterations and detriments promoted through a 60-day HDT trial. Cardiovascular and autonomic deconditioning may occur with differential temporal dynamics during mid- and long-term HDT, and partially persist after a recovery period (with differential orthostatic reactivity), in subjects not undergoing countermeasures. Conversely, regular short-duration high-intensity exercise involving the lower body successfully mitigated some autonomic cardiovascular detriments promoted by HDT and accelerated their respective recovery. Thus, the implemented training protocol may represent an effective countermeasure against cardiovascular deconditioning during prolonged inactivity and simulated microgravity. Further studies should investigate specific application, training protocols and benefits of high-intensity training also regarding clinical applications, such as prolonged bed rest during hospitalization, and cardiovascular pathologies.

To conclude, short-term morning HRV monitoring effectively and sensitively depicted physiological responses to different extreme challenges and conditions. This investigation underlines the crucial role of cardiac autonomic modulation and its dynamic ability to adapt to internal and external stressors and conditions for human physiologic adaptation and resilience in extreme environments.

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STATUTORY DECLARATION

I, Lea Christiane Rundfeldt, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic „Cardiac autonomic modulation as a human physiologic response to extreme environments“ (title in German: „Kardiale autonome Modulation als physiologische Reaktion des Menschen in extremen Umwelten“), independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.

Date Signature

DECLARATION OF CONTRIBUTION

Lea Christiane Rundfeldt contributed the following to the below listed publications:

Publication 1:

Lea C. Rundfeldt and Martina A. Maggioni, Robert H. Coker, Hanns-Christian Gunga, Alain Riveros-Rivera, Adriane Schalt and Mathias Steinach: „**Cardiac Autonomic Modulations and Psychological Correlates in the Yukon Arctic Ultra: The Longest and the Coldest Ultramarathon**", Frontiers in Physiology, 2018

Contribution:

This publication was generated in a shared first authorship by Lea Christiane Rundfeldt and Martina Anna Maggioni. The two first authors therefore declare their contribution individually.

L. Rundfeldt assisted M. Maggioni with designing the data analysis concept and publication outline and both first authors carried out the literature research. L. Rundfeldt performed raw data analyses, such as screening of available HR recordings and computation of HRV indices with Kubios HRV software, analysis of psychometric scores, as well as all statistical analyses, under fundamental mentorship of M. Maggioni. L. Rundfeldt and M. Maggioni wrote the manuscript together. All tables (Table 1–3) and figures (Figure 1–9) in the publication were primarily drafted by L. Rundfeldt. As corresponding author, L. Rundfeldt handled all communication with the publishing journal and performed the submission and review process, under the guidance of M. Maggioni. L. Rundfeldt and M. Maggioni handled the substantial review procedure with equal contribution, in agreement with all coauthors. The corresponding tables (Table 1 and 2) and figures (Figure 1 and 2) in this dissertation were generated by L. Rundfeldt in conformity with the original tables and figures incorporated in the publication.

M. Maggioni primarily designed the study data analysis concept and publication outline with assistance of L. Rundfeldt and both first authors carried out the literature research. M. Maggioni fundamentally mentored L. Rundfeldt regarding HRV data pre-processing and analysis, as well as statistical analyses. Together, M. Maggioni and L. Rundfeldt wrote the manuscript, and M. Maggioni shared substantial expertise in respect to the writing process. Also, M. Maggioni edited the tables and figures that L. Rundfeldt drafted. M. Maggioni furthermore contributed expert knowledge to the manuscript submission process and communication with the publishing journal conducted by L. Rundfeldt. The substantial review procedure was handled by L. Rundfeldt and M. Maggioni with equal contribution, in agreement with all coauthors.

Publication 2:

Martina A. Maggioni, Paolo Castiglioni, Giampiero Merati, Katharina Brauns, Hanns-Christian Gunga, Stefan Mendt, Oliver S. Opatz, **Lea C. Rundfeldt**, Mathias Steinach, Anika Werner and Alexander C. Stahn: „**High-Intensity Exercise Mitigates Cardiovascular Deconditioning During Long-Duration Bed Rest**", Frontiers in Physiology, 2018

Contribution:

L. Rundfeldt assisted with interpretation of the results, provided critical feedback during the manuscript writing process, and reviewed the final manuscript together with all the coauthors before submission. The corresponding table (Table 1) and figures (Figure 1, 5 and 6) in this dissertation were generated by L. Rundfeldt in conformity with the original figures incorporated in the publication.

Publication 3:

Martina A. Maggioni and **Lea C. Rundfeldt**, Hanns-Christian Gunga, Marc Joerres, Giampiero Merati and Mathias Steinach: "**The Advantage of Supine and Standing Heart Rate Variability Analysis to Assess Training Status and Performance in a Walking Ultramarathon**", Frontiers in Physiology, 2020

Contribution:

This publication was generated in a shared first authorship by Lea Christiane Rundfeldt and Martina Anna Maggioni. The two first authors therefore declare their contribution individually.

L. Rundfeldt was responsible for subject recruitment and communication in the 2019 edition and primarily carried out the literature research. Both L. Rundfeldt and M. Maggioni designed the data analysis concept and publication outline and together performed all the measurements in the 2019 edition. L. Rundfeldt performed raw data analyses, such as screening of available HR recordings and computation of HRV indices with Kubios HRV software, analysis of training experience questionnaires, as well as all statistical analyses, with support by M. Maggioni. Also, L. Rundfeldt principally wrote the manuscript. All the tables (Table 1 and 2, Supplementary Table S1–S5) and figures (Figure 1–5, Supplementary Figure S1) included in the publication were primarily designed by L. Rundfeldt. Finally, L. Rundfeldt handled the review process with support of M. Maggioni. The corresponding tables (Table 1 and 2) and figures (Figure 1, 3 and 4) in this dissertation were generated by L. Rundfeldt in conformity with the original tables and figures incorporated in the publication.

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L. Rundfeldt was revised by M. Maggioni, who also contributed expert knowledge and critical feedback. Additionally, M. Maggioni edited the tables and figures designed by L. Rundfeldt. As corresponding author, M. Maggioni was responsible for all communications with the publishing journal and the reviewers and assisted L. Rundfeldt in the review process.

Signature of L. C. Rundfeldt (doctoral candidate)

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ORIGINAL RESEARCH
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Cardiac Autonomic Modulations and Psychological Correlates in the Yukon Arctic Ultra: The Longest and the Coldest Ultramarathon

Lea C. Rundfeldt [1*†], Martina A. Maggioni [1,2†], Robert H. Coker [3], Hanns-Christian Gunga [1], Alain Riveros-Rivera [1,4], Adriane Schalt [1] and Mathias Steinach [1]

[1] Charité - Universitätsmedizin Berlin, Institute of Physiology, Center for Space Medicine and Extreme Environments Berlin, Berlin, Germany, [2] Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy, [3] Institute of Arctic Biology, University of Alaska-Fairbanks, Fairbanks, AK, United States, [4] Department of Physiological Sciences, Pontificia Universidad Javeriana, Bogotá, Colombia

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Universidade Católica de Brasília,
Brazil
Beat Knechtle,
University of Zurich, Switzerland
Alessandro Tonacci,
Istituto di Fisiologia Clinica (CNR), Italy

***Correspondence:**
Lea C. Rundfeldt
lea-christiane.rundfeldt@charite.de

† These authors have contributed equally to this work.

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Studies on human physical performance in extreme environments have effectively approached the investigation of adaptation mechanisms and their physiological limits. As scientific interest in the interplay between physiological and psychological aspects of performance is growing, we aimed to investigate cardiac autonomic control, by means of heart rate variability, and psychological correlates, in competitors of a subarctic ultramarathon, taking place over a 690 km course (temperatures between +5 and −47°C). At baseline (PRE), after 277 km (D1), 383 km (D2), and post-race (POST, 690 km), heart rate (HR) recordings (supine, 15 min), psychometric measurements (Profile of Mood States/POMS, Borg fatigue, and Karolinska Sleepiness Scale scores both upon arrival and departure) were obtained in 16 competitors (12 men, 4 women, 38.6 ± 9.5 years). As not all participants reached the finish line, comparison of finishers (FIN, $n = 10$) and non-finishers (NON, $n = 6$), allowed differential assessment of performance. Resting HR increased overall significantly at D1 (FIN +15.9; NON +14.0 bpm), due to a significant decrease in parasympathetic drive. This decrease was in FIN only partially recovered toward POST. In FIN only, baseline HR was negatively correlated with mean velocity [r −0.63 (P.04)] and parasympathetic drive [pNN50+: r −0.67 (P.03)], a lower HR and a higher vagal tone predicting a better performance. Moreover, in FIN, a persistent increase of the long-term self-similarity coefficient, assessed by detrended fluctuation analysis (DFAα2), was retrieved, possibly due to higher alertness. As for psychometrics, at D1, POMS Vigor decreased (FIN: −7.0; NON: −3.8), while Fatigue augmented (FIN: +6.9; NON: +5.0). Sleepiness increased only in NON, while Borg scales did not exhibit changes. Baseline comparison of mood states with normative data for athletes displayed significantly higher positive mood in our athletes. Results show that: the race conditions induced early decreases in parasympathetic drive; the extent of vagal withdrawal, associated to the timing of its recovery, is crucial for success; pre-competition lower resting HR predicts a better performance; psychological profile is reliably depicted by POMS, but not by Borg fatigue scales. Therefore, assessment of heart rate variability and psychological profile may monitor and partly predict performance in long-duration ultramarathon in extreme cold environment.

Keywords: cold, exercise performance, extreme environments, fatigue, heart rate variability, mood, subarctic ultramarathon, ultra-endurance

INTRODUCTION

Human physiology is characterized by continuous reactive adaptation to internal and external conditions and stressors (Ramirez et al., 1999; Hawley et al., 2014). Subjects exposed to extreme conditions and environments display astounding adaptive potential, which ultimately ensures optimal adjustment to current organismic demands and external stress (Kälin et al., 2012; Gunga, 2014). Assessment of autonomic cardiac modulation by means of heart rate variability (HRV) has shown to be a reliable tool to evaluate not only physiological changes (Taralov et al., 2015; Kobayashi et al., 2016), but also psychological aspects of human reactive adaptation to different stressors (Souza et al., 2013). Therefore, HRV assessment may describe human resilience, as it represents a bridge between physiology and psychology, and, by integrating these two aspects, it mirrors human adaptive ability (Thayer et al., 2009; Spangler and Friedman, 2015). Particularly in endurance athletes, training effects, performance level and physical wellbeing may be contextualized through HRV assessment (Atlaoui et al., 2007; Plews et al., 2013a; Buchheit, 2014; Bellenger et al., 2016a). Successful adaptation to increased training load, resulting in improved performance, is associated with increased HRV, as well as enhanced parasympathetic predominance at rest (Plews et al., 2013b; Stanley et al., 2015; Lucini et al., 2017). Assessment of autonomic cardiac modulation conducted directly post-exercise or after competitions, demonstrated a decrease in HRV and a parasympathetic withdrawal (Bricout et al., 2010; Buchheit et al., 2010; Bellenger et al., 2016a), which, however, was effectively recovered depending on the intensity of the preceding exercise (Martinmäki and Rusko, 2008; Manzi et al., 2009; Stanley et al., 2015), and on the individual's training status (Bricout et al., 2010; Buchheit et al., 2010; Bellenger et al., 2016a). This has been vastly evidenced in endurance exercisers (Buchheit et al., 2010; Plews et al., 2012; Da Silva et al., 2014; Kiviniemi et al., 2014), and investigations of cardiac autonomic function in response to extreme endurance exercise, such as ultramarathon, display similar findings (Gratze et al., 2005; Scott et al., 2009; Foulds et al., 2014), even though specific studies related to cardiac autonomic modulation during ultramarathon, are still scarce in comparison. On the other hand, in ultra-endurance athletes, physical exertion has been commonly associated with mental fatigue and increased mood disturbance (Anglem et al., 2008; Siegl et al., 2017), especially in participants who experience adverse incidents, then perform poorly or are forced to prematurely withdraw (Parry et al., 2011; Joslin et al., 2014).

Ultramarathon is mostly defined by course lengths exceeding marathon distance and is characterized by the combination of extremely challenging highly intensive exercise (e.g., track lengths >300 km or great elevation gains), often under strenuous environmental conditions, with concurrently impaired possibilities to recover. The Yukon Arctic Ultra (YAU) is considered to be one of the world's toughest ultramarathons (Coker et al., 2017), as it combines the great course distance of 690 km with extreme environmental conditions typical of a subarctic winter. Except for several in-race checkpoints, there are no indoors sleeping vacancies, so that competitors have to camp on the race-course and experience complete environmental exposition. Therefore, YAU competitors are challenged by a *three-folded stress stimulus* of (i) long-term strenuous exercise, (ii) extreme cold exposure, and (iii) impaired resting conditions, due to in-race camping. So far, among studies on ultra-endurance exercise, research objectives mostly diverge from evaluation of autonomic cardiac function in ultramarathon runners (Degache et al., 2014; Hurdiel et al., 2015; Mrakic-Sposta et al., 2015; Wüthrich et al., 2015; Tonacci et al., 2017), which, to our knowledge, was only implemented in three previous studies (Gratze et al., 2005; Scott et al., 2009; Foulds et al., 2014). However, these investigations differed regarding (i) the race length (e.g., 160 km ultramarathon, or Ironman competition with a total distance of 226.35 km), (ii) study protocol (i.e., pre- vs. post-race comparison only), (iii) environmental conditions (mild climate, summer), and (iv) terrain characteristics (e.g., mountain, large altitude variation, etc.). Therefore, this is the first study to assess cardiac autonomic modulation in competitors of an extremely long (i.e., 690 km) ultramarathon on a mostly flat course, in subarctic climate, which may provide essential insights into the adaptive capacity, as, aside from exercise, HRV is associated with numerous external and internal factors (Rajendra Acharya et al., 2006; Shaffer et al., 2014).

With outdoor temperatures ranging from +5 to −47°C and the air humidity accounting for up to 100%, YAU competitors face extreme subarctic weather conditions. Comparable scientific knowledge is, however, insufficient. Autonomic balance has been observed to shift toward greater parasympathetic predominance during Antarctic stays (Farrace et al., 2003; Harinath et al., 2005), but these results were obtained in expeditioners confined to indoors housing. Moreover, a significant interplay between autonomic cardiac regulation and psychological wellbeing has been observed (Sakuragi et al., 2002; Karavidas et al., 2007; Sgoifo et al., 2015), so that psychometric assessment may also serve to contextualize findings about HRV (Bellenger et al., 2016b; Flatt et al., 2017b). Increased performance and greater parasympathetic drive in cardiac autonomic regulation are associated with increased psychological wellbeing (Cervantes Blásquez et al., 2009; Bisschoff et al., 2016). Conversely, fatigued states and increased mood disturbance have been related to decreased indexes of total HRV, as well as parasympathetic tone (Nuissier et al., 2007; Leti and Bricout, 2013; Schmitt et al., 2013; Flatt et al., 2017a). In this context, impaired resting conditions present another vital influence on cardiac autonomic regulation. Assessment under concurrent sleep-deprivation, which itself is again related to impaired both cognitive and physical performance (Marcora et al., 2009; Fullagar et al., 2015), shows decreased HRV indexes in the parasympathetic domain (Dettoni et al., 2012; Glos et al., 2014; Tobaldini et al., 2014).

To our knowledge, exposition to such a particular combination of stress-stimuli as presented by competition in the YAU has never been investigated regarding cardiac autonomic function and psychological profile. Therefore, we assessed autonomic cardiac regulation in terms of HR/HRV, as well as psychometric measurements including mood states, indicators of sleepiness, exertion and recovery, to investigate adaptation to extreme conditions and performance, by analyzing cardiac autonomic control and its interplay with mood and fatigue. We hypothesized that higher performing competitors, compared to less successful athletes, would exhibit differential profiles of autonomic cardiac regulation associated with optimal psychometric profile, overall characterized by higher adaptability and greater resilience to the extreme challenges of the three-folded stress stimulus.

MATERIALS AND METHODS

Subjects and Study Implementation

This study is part of a larger investigation regarding "*Physiological changes of participants of the Yukon Arctic Ultra - an ultramarathon in extremely cold climate*," where it is planned to assess a variety of different physiological parameters and their interplay.

From a total number of 78 athletes partaking in the 690 km foot-race category of the YAU during the years 2013, 2015, and 2017, 27 (20 men, 7 women) volunteers enrolled in the study (8 in 2013, 9 in 2015 and 10 in 2017). Due to issues related to data collection, from the 27 participants, only 16 (ALL: 12 men, 4 women) were included in the data analysis (see section Statistics). The majority ($n = 15$) were of Caucasian descent and one was of Asian origin. Their anthropometric data are presented in **Table 1**.

TABLE 1 | Subject demographics.

Group	Gender	*n*	Age, years mean (S.D.)	Weight, kg mean (S.D.)	Height, cm mean (S.D.)	BMI, kg/m^2 mean (S.D.)
FIN	Men	7	42 (10)	80 (9)	176 (6)	25.7 (3.0)
	Women	3	38 (10)	61 (2)	168 (10)	21.7 (3.4)
	All	10	40 (9)	74 (12)	174 (8)	24.5 (3.5)
NON	Men	5	33 (7)	79 (12)	179 (10)	24.7 (1.7)
	Women	1	51 (0)	58 (0)	170 (0)	20.1 (0)
	All	6	36 (10)	76 (14)	177 (10)	23.9 (2.4)
ALL	Men	12	38 (10)	80 (10)	177 (7)	25.2 (2.5)
	Women	4	41 (11)	60 (2)	169 (9)	21.3 (2.9)
	All	16	39 (10)	75 (12)	175 (8)	24.3 (3.1)

Subject demographics at baseline for all participants (ALL) and in subgroups (FIN and NON). No significant differences between groups.

The recruitment for this study was conducted with the support of the event organizers. A call for participants, with a brief description of the study and planned measurements, was transmitted to the athletes who had enrolled in the 690 km foot-race category. The organizers were encouraged to predominantly contact experienced athletes, who had a long history of completed endurance events and/or who had completed the YAU before. Athletes who were interested in the study contacted the principal investigator via e-mail and received further detailed information. The potential study participants had several weeks to ask questions via e-mail and to decide whether to partake in the study or not. There were no further inclusion or exclusion criteria: all athletes enrolled in the 690 km foot-race category were eligible to enter the study. All athletes were required to present to the event organizers a health certificate issued by their home physician, in order to be enrolled in the race. During a meeting in Whitehorse, Yukon Territory, Canada, 4–5 days before the race start, the potential study participants met with the investigators in person, had the chance to ask further questions and to finally give their informed written consent to partake in the study. The study was approved by the Charité Ethics Board (review number EA4/109/12), and all measurements and procedures complied with the Declaration of Helsinki (54th Revision 2008, Korea)[1] regarding the treatment of human subjects.

All study participants included in the final analysis had completed either one marathon (9.6 ± 24.4) or ultramarathon (14.4 ± 24) prior to their study-participation. The mean longest ultramarathon distance completed by the athletes before their YAU participation was 380 ± 220 km. In addition, seven of the study participants had previously participated in the YAU foot-race in various distance categories, with a mean longest completed distance of 478 ± 219 km. Thus, the study participants were experienced endurance athletes, which is also reflected by their self-reported sedentary HR of 52.6 ± 7.3 bpm. From one participant, this background data was not made available.

The Yukon Arctic Ultra: The Longest, the Coldest Ultramarathon

The Montane® YAU ultra-endurance race takes part in the beginning of February, covering a 690 km distance between Whitehorse and Dawson City in the Canadian Yukon Territory. Besides the foot-race, the YAU also allows the competition in cross-country-skiing and mountain-biking. The first and last sections of the trail account for elevations between 500 and 700 m, however, especially in the last 200 km, the terrain along the Yukon river partly exhibits great elevation gains (up to 1,000 m). The YAU is not an orientation race, as the trail is marked and prepared with snow-mobiles. Via GPS devices, athletes can be tracked on the course and have the possibility to call for assistance in case of an emergency. To

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[1] https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/

further increase their safety, the time until the race has to be completed is limited to 14 days and additionally, medical screenings are administered at the 10 checkpoints which are located (mostly about 50 km apart) along the route. Despite these partly indoor vacancies (otherwise, tents were provided), during the race, competitors face complete exposition to the subarctic environment, with outdoor temperatures in February ranging between +5°C (highest temperature measured in February 2013 in Whitehorse) and −47°C (lowest temperature measured in February 2015 in Dawson City). Additionally, the extremely high air humidity (up to 100% as measured in February 2013 in Whitehorse) contributes to the possible onset of frostbite, which, along with other (medical) concerns, may lead to immediate disqualification. Importantly, the weather conditions between editions were not significantly different (**Figure 1**), detailed information on weather conditions can be assessed in respective weather archives[2].

Notably, participants walked between 12 and 15 h per day whilst pulling their gear on a sled-like pulk (accounting for 30–40 kg; additionally, participants were allowed up to three drop bags) and, apart from the checkpoints, had to eat, rest and make toiletry arrangements in the outdoor conditions of the Yukon Territory.

More detailed information about the Montane® YAU is provided on the official website of the event[3].

Experimental Protocol and Measurements

Experimental protocol details are depicted in **Figure 2**. At two out of ten in-race checkpoints, we respectively implemented two in-race assessments, so that, in summary, measurements were performed: (1) at baseline during the 3 days preceding the race in Whitehorse (PRE), (2) at the Carmacks in-race checkpoint at 277 km (During 1, D1), (3) at the Pelly Crossing in-race checkpoint at 383 km (During, D2) and (4) immediately after completion of the race in Dawson City at 690 km (POST).

The in-race checkpoints had to be selected for measurement implementation due to essential practical concerns. They had to be indoors facilities buildings with sufficient space, comfortable ambient temperature and low noise in order to perform measurements under controlled conditions, as well available electricity and that it was accessible by car for the investigators. Exemplarily, several of the race checkpoints were mere tents that did not meet these criteria and therefore, the study checkpoints were chosen as they were. Thus, the distance between the race start (i.e., PRE) and the first in-race assessment (D1) accounted for over a third (277 km) of the entire race-course and additionally, in this period, athletes would face the most strenuous weather conditions (which tend to ameliorate toward the second half of the month; see **Figure 1**). As the second assessment was performed at 383 km (D2), the distance between D1 and D2 (as well as the time to cover it, which accounted for only 30 h in some subjects) was the shortest between the measurements and, in fact, more than 50% less than the other two distances. Conversely, the distance between D2 and POST was again very high (307 km) and additionally, the terrain in the last third of the course accounted for the greatest elevation gains (see section The Yukon Arctic Ultra: The Longest, the Coldest Ultramarathon).

Baseline Assessment

During the three race-preceding days (PRE), baseline anthropometric data (age, weight and height) were obtained. Weight was measured using a calibrated scale (Seca® GmbH, Hamburg, Germany) and height was taken from the participants' interview.

Fifteen minutes baseline recordings of beat-to-beat HR to assess HRV were collected with a HR monitor (RS800CX Polar Electro Oy, Kempele, Finland), which is widely used and validated for HRV assessment (Wallén et al., 2012). HR recordings were performed in supine position upon awakening (between 5 and 10 a.m.) directly after participants had slept 6–8 h the previous night. The athletes had not consumed food, beverages or stimulants (e.g., coffee) in the 2 h before the recording and were instructed to breathe normally, avoid speaking and moving during the data collection. Additionally, it was ensured by the investigators that subjects would not fall asleep. With the limitations of this specific in-field study, special attention was devoted to performing data collection sessions in a quiet and comfortable setting, with participants lying in a bed or on a sleeping sleeping mattress, ambient temperatures between 17 and 23°C, and reduced light.

On the morning of the race start before departure, additionally, psychometric assessment was performed (see **Figure 2** and section Psychometric Assessment).

In-Race and POST Assessment

Upon arrival at the in-race checkpoints, as well as at the race finish, psychometric scales were administered according to physiological needs and conditions of arriving competitors. Participants then had a few hours of rest (ranging from 4 up to 6–8 h) and upon awakening (at morning, between 5 and 10 a.m.), HR data was collected, as at baseline, to assess HRV. Afterwards, before departure, psychometric assessment again took place (see **Figure 2** and section Psychometric Assessment).

Indoors ambient conditions between the different measurement facilities were comparable, with special attention dedicated to a quiet and comfortably warm setting with reduced light exposure.

Moreover, throughout the entire race, participants were continuously (day and night) monitored by means of a heart rate monitor (RS800CX Polar Electro Oy, Kempele, Finland - sample rate 15 s).

Data Analysis

Performance Assessment and Heart Rate Continuous Recordings

The official time at the end of the race for each participant who reached the finish line was collected, together with the times and the respectively completed distance for each participant who had to withdraw. Subsequently, the mean running velocity of the race

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[2] http://climate.weather.gc.ca/historical_data/search_historic_data_e.html.

[3] http://arcticultra.de/en.

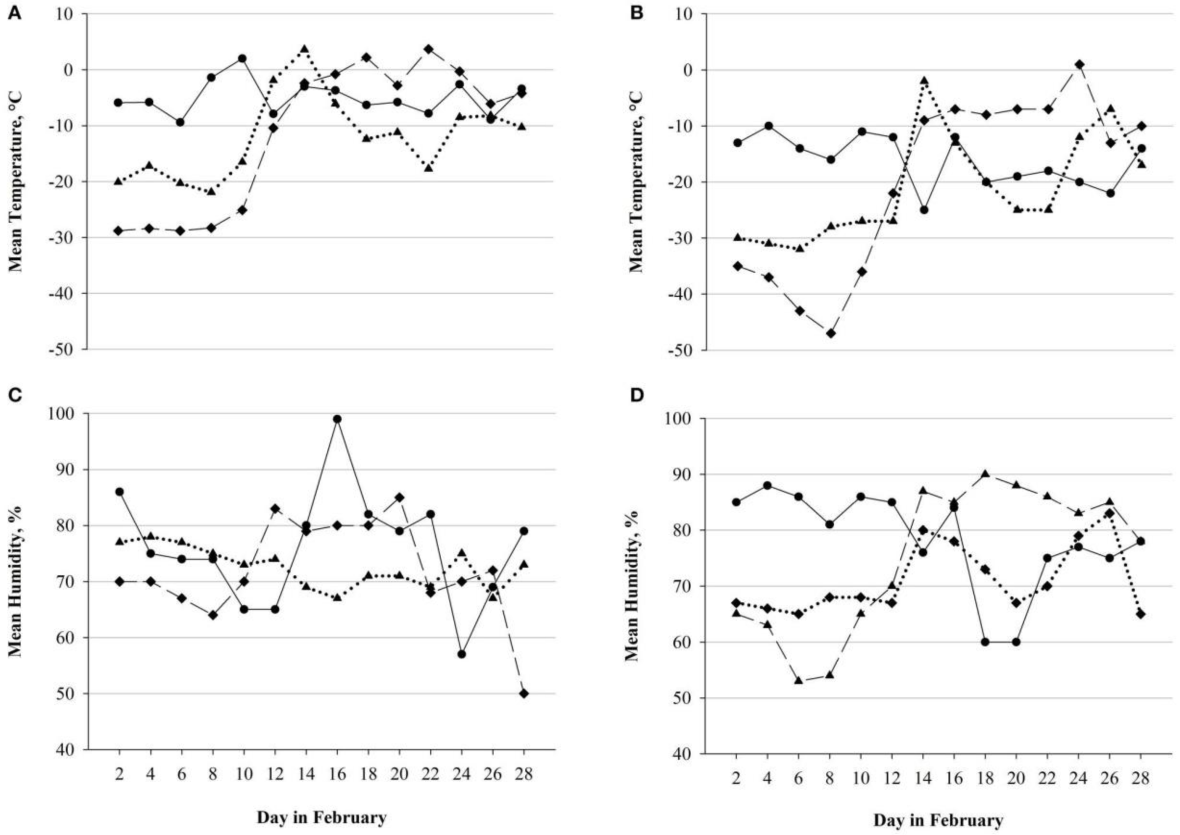

FIGURE 1 | Weather conditions across editions. Mean temperature and mean humidity as measured in Whitehorse and Dawson City in February 2013 (solid line), February 2015 (long dashed line), and February 2017 (dotted line). **(A)** Mean temperature in Whitehorse. **(B)** Mean temperature in Dawson City. **(C)** Mean humidity in Whitehorse. **(D)** Mean humidity in Dawson City. No significant differences between editions or locations[4].

was calculated from the total recorded time and the total distance covered [$time\ (h)/space\ (km) = velocity\ (km/h)$]. By collection of in- and out-going times at in-race measurement points for each participant, both split times and velocities could be computed, allowing detailed assessment of performance. Additionally, continuous HR measurements served to determine exercise intensity, as well as resting quality (in respect to HR expressed as a percentage of calculated maximal HR). The continuous HR recordings, collected during the race, were screened for quality (no more than 3% signal lost/disturbed). The average, maximum and minimum HR were determined per each selected race period, and the values were normalized with respect to the individual age-related maximal HR (HR_{max}) (Tanaka et al., 2001). This provided further information about exercise intensity and quality of rest. Specifically, data were divided into four time-segments, according to the selected period of recording: (i) HR recorded in the first 36 h following the race start (D1a), (ii) during the 24 h before arriving at D1 (D1b), (iii) during the 24 h before arriving at D2 (D2a), and (iv) HR recorded during the last 24 h before finishing the race (D2b). Collected HR data were then expressed as a percentage of the HR_{max}, for average exercise intensity (ExHR) and average resting HR (RestHR). This approach was selected to allow comparison with parameters assessed at checkpoints (i.e., psychometric and HRV analysis) and, by classifying data, served to better interpret findings.

Heart Rate Variability Assessment

An expert operator visually inspected the R–R interval series, and with the support of a dedicated software (Kubios HRV ver. 2.1, Kuopio, Finland), premature beats or artifacts were removed. The filter threshold was set at the "low" level (Tarvainen et al., 2014) and only files 15-min long and including less than 0.3% of beats recognized as artifacts were considered; then the last 10 min were selected for HRV assessment, to better standardize the analysis. After providing the normal-to-normal (NN) interval series, HRV was assessed as validated indices of autonomic cardiac modulation, based on time-domain, frequency-domain,

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[4] Data taken from https://www.timeanddate.com/weather/canada/whitehorse/historic, https://www.timeanddate.com/weather/canada/dawson-city/historic and http://climate.weather.gc.ca/historical_data/search_historic_data_e.html (last accessed December 13, 2017).

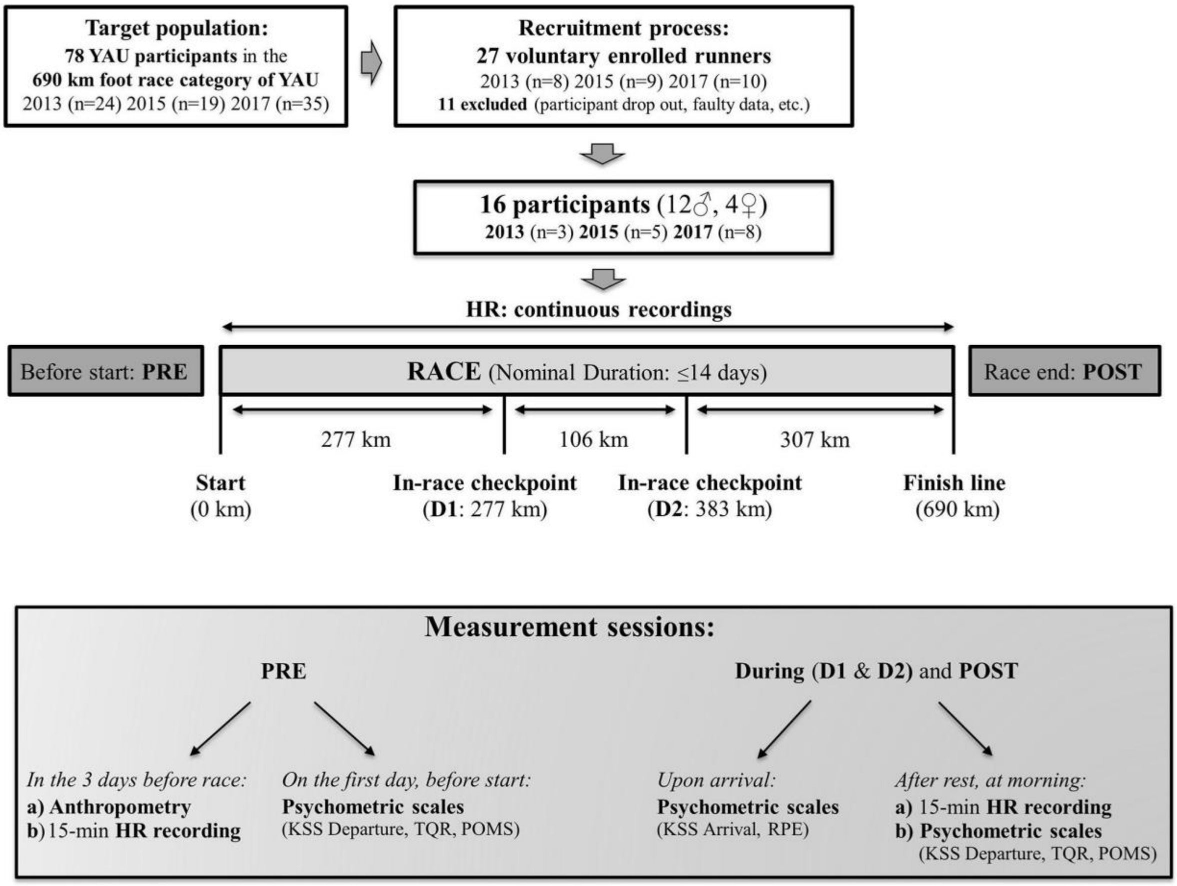

FIGURE 2 | Experimental protocol. Schematic representation of the study protocol and experimental procedure employed in all three YAU races of 2013, 2015, and 2017, except for psychometric assessment (included only in the 2015 and 2017 races).

and complexity (European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Specifically, as for time-domain analysis, the root mean square of the successive RR differences (RMSSD), an indirect index of vagal activity, was calculated. Furthermore, NN50 statistics were computed, specifically, the hourly number of increases (NN50+) or decreases (NN50−) between consecutive NN intervals larger than 50 ms (Ewing et al., 1984), as well as the percentage of such differences with respect to the total number of NN intervals (pNN50+ and pNN50−) (Bigger et al., 1988; Merati et al., 2015). The NN50 statistics may reflect the rate of "vagal bursts," as bursts of vagal outflow are producing NN intervals greater than 50 ms (Ewing et al., 1984). In the frequency domain, the total spectral power density (TP) was assessed together with its components: (i) high frequency (HF) band (0.15–0.40 Hz), which depends mainly on parasympathetic activity and is synchronous with the respiratory sinus arrhythmia; (ii) low frequency (LF) band (0.04–0.15 Hz), which depends on both parasympathetic and sympathetic activity; and (iii) LF/HF ratio, which is currently considered a marker of sympathovagal balance (Ewing et al., 1984). In the non-linear domain, as for complexity analysis, the following indices were assessed: (i) the HR sample entropy (SampEn), which measures the level of irregularity of the NN interval series and mirrors vagal activations or sympathetic deactivations (Porta et al., 2008); (ii) the short-term self-similarity coefficient ($\alpha 1$) and long-term self-similarity coefficient ($\alpha 2$) of NN intervals, as assessed by detrended fluctuation analysis (DFA), mentioned here respectively as DFA$\alpha 1$ and DFA$\alpha 2$ (Peng et al., 1995). Both indices may be affected by parasympathetic tone, whereas, for example, higher DFA$\alpha 1$ is associated with sympathovagal balance increase or vagal tone decrease (Penttilä et al., 2003). The significance of DFA$\alpha 2$ has not yet been completely elucidated, as there is indeed only scarce evidence within the literature. However, it seems to be associated with alertness (Ivanov et al., 1999) and may be influenced by sleep stages, being higher in awake states and REM sleep with respect to light and deep sleep (Schumann et al., 2010).

All HRV indices, except for NN50 statistics (manually calculated), were assessed by means of the Kubios HRV software, ver. 2.1 (Kuopio, Finland), a free available software to assess HRV, widely used in the scientific literature, especially in the field of sport sciences (Tarvainen et al., 2014).

Psychometric Assessment

Karolinska Sleepiness Scale

The Karolinska Sleepiness Scale (KSS) (Kaida et al., 2006), which has been highly validated to sensitively depict objective sleepiness (Kaida et al., 2006; Sallinen et al., 2008), was administered both after rest (before departure in the morning: KSS Departure) and upon arrival (KSS Arrival) (see **Figure 2**). The athletes were asked to rate their subjective sleepiness on a numerical scale ranging from 1 to 10. Specifically, 1–6 are assigned to an "active state" of alertness (1 corresponding to "extremely alert" and 6 to "some signs of sleepiness") and 7–10 to a "sleepy state" (7 corresponding to "sleepy, but no difficulty remaining awake" and 10 to "falling asleep all the time").

Borg Scales

After rest (before in-race departure and at the finish), subjects were administered the Borg Total Quality of Recovery (TQR) questionnaire (Senttä and Hassmén, 1998). The Borg TQR has been demonstrated to sensitively represent the individual recovery status (Freitas et al., 2014), whereas the use of recovery and wellness indicators has exhibited important validity in the monitoring of athletes (Buchheit, 2015; Bisschoff et al., 2016). It consists of a 6–20 numerical scale, with 6 being equivalent to "very, very poor recovery" and 20 to "very, very good recovery," so that the obtained score allows determination of the athlete's subjectively evaluated quality of recovery.

Upon arrival at checkpoints or the finish (**Figure 2**), participants were administered the Borg Rating of Perceived Exertion (RPE) scale (Borg, 1982; Scherr et al., 2013), which is commonly used in athletes to monitor exertion and also the current subjective workload, additionally, in association with cardiac autonomic regulation (Parry et al., 2011; Thorpe et al., 2016; Siegl et al., 2017) and performance (Suzuki et al., 2006). It again consists of a 6–20 numerical scale, 6 being "very, very light" and 20 "very, very hard," the individual score indicating the athlete's degree of subjectively perceived exertion.

Profile of Mood States

At morning, after rest, mood states in the YAU participants were investigated through the Profile of Mood States questionnaire in the short-form (POMS-SF, here referred to as POMS) (Curran et al., 1995). This extensively validated tool is commonly used in athletic monitoring (Hedelin et al., 2000; Leti and Bricout, 2013; Bisschoff et al., 2016) and has been variously observed to be associated with both HRV and performance (Hedelin et al., 2000; Leti and Bricout, 2013; Comotto et al., 2015; Bisschoff et al., 2016). The POMS required participants to state the extent of emotions currently experienced during the last hours (respectively operationalized as "not at all" providing a subscore of 1; up to "extremely," providing a subscore of 5). Analysis of individual subscores in emotional subcategories subsequently provided an individual raw score representing the 6 main mood states Depression, Vigor, Fatigue, Tension, Confusion and Anger, as well as a total sum score of mood disturbance (POMS Total).

The psychometric assessment of mood states in our subjects was further analyzed by comparison with normative data for an athletic sample (Terry and Lane, 2000). This data had been obtained in mixed general athletic samples as well as, amongst others, subgroups of athletes at different competition levels and situations (pre- or post-competition, etc.) (Terry and Lane, 2000). In accordance with Terry, raw scores were transformed to a normalized T-Score (using the individual raw score, group mean and group standard deviation) through the formula: $T\text{-}Score = 50 + [10 + (raw\ score - group\ mean)]/SD$. This transformation converted raw scores to normalized scores on a standard scale with a mean of 50 and a standard deviation of 10, so that individual results could be compared with normative sample data. POMS normative scores of athletes from various sport disciplines, plotted against college student norms originally obtained by McNair in 1971 (McNair et al., 1971), show a distinctive pattern of mood states in athletes compared to sedentary populations, which is referred to as the *Iceberg Profile* (**Figure 9A**). Specifically, athletes have been found to account for significantly higher Vigor, whereas all other (negative) dimension scores remain below mean values for non-athletes, i.e., "under the surface." This distinctive profile has been proposed to indicate greater mental health and reduced mood disturbances in athletic subjects compared to sedentary populations. By plotting individually obtained values against normative data, this specific pattern of higher positive mood and mental health in our participants compared to normative data of sedentary subjects could therefore be assessed.

Moreover, by analyzing result scores of the administered psychometric scales, the so-called *psychological wellbeing* (Scully et al., 1998; Johnston et al., 2015; Saw et al., 2016) was evaluated. A higher psychological wellbeing would correspond to an overall low score both for POMS Total (i.e., higher Vigor, lower Fatigue, Tension, Confusion, Anger and Depression scores) and for fatigue scales (i.e., Borg RPE and KSS), and inversely higher scores for Borg TQR.

Statistics

Data are reported as means ± standard deviations (m ± SD), if not otherwise stated. From 27 enrolled competitors, we included 16 in the statistical analysis (due to early dropouts before D1, as well as related to HRV-data availability and quality). As a result of the extreme conditions of the competition, several participants withdrew before course completion (see section Performance). Therefore, after the race, the entire sample of all participants (ALL) was divided into two subgroups: finishers (FIN: measurements throughout the race until POST) and non-finishers (NON: measurements until D1) (see **Table 1**). Normal distribution was tested with

Shapiro-Wilk and variance with the Equal-Variance-Test. A log-transformation was applied to frequency domain indices to attain normal distribution (Castiglioni et al., 2011). According to the distribution, the variance of HRV parameters, exercise intensity and rest quality and psychometric measurements over the entire race-course in FIN was tested with one-way repeated measures analysis of variance (One-Way RM ANOVA) and *post-hoc* Student's-Newman-Keuls-Test, or Friedman ANOVA on ranks with *post-hoc* assessment through Tukey's Test (i.e., RMSSD, pNN50+, POMS Depression, Borg TQR). In addition, differences in weather conditions between editions were assessed with One-Way RM ANOVA. As all participants reached the first checkpoint, we could implement a direct comparison between the two groups regarding PRE and D1 by applying two-way repeated measures ANOVA (Two-Way RM ANOVA), after normality was passed. Psychometric assessment of mood states was further analyzed through comparison with normative data for athletic samples. In accordance with Terry (Terry and Lane, 2000), raw values of mood states were conversed to standardized T-Scores. Hence, T-Scores could be plotted against the athletic sample mean in order to assess expression of the *Iceberg Profile*, which represents specific mood profiles in athletic subjects. Unpaired Student's *t*-Test was used on raw values, as well as computed T-Scores, to allow comparison of significant differences between YAU participants and normative data for mixed athletic samples. In order to further analyze mood states in YAU competitors, comparison of baseline values (as individual raw scores) with normative data obtained in athletes directly pre-competition, as well as with normative data obtained in athletes post-competition, was performed by application of unpaired Student's *t*-Test. Correlations between HRV indices and psychometric measurements, as well as correlations between HRV indices or psychometric measurements and performance were assessed with Pearson Product-Moment-Correlation or, if normality was not passed, Spearman Correlation. All statistical analyses were performed using SigmaPlot 12.3 (Systat Software, San José, CA, USA). The significance level was set at $p < 0.05$.

RESULTS

Performance

Of the 16 participants included in the statistical analysis (ALL), 10 successfully completed the course (FIN). Due to general fatigue, cardiovascular distress or gastrointestinal problems, as well as injuries (e.g., sprained ankle), 6 withdrew from the competition at earlier points (NON). Baseline anthropometric characteristics of the two subgroups, based on the completion of the race, are presented in **Table 1**. Details of performance are depicted in **Table 2**. The official times recorded among FIN ranged between 225 and 312 h. Specifically, analysis of split times displayed that FIN accounted for 82 ± 15 h (velocity 3.6 ± 0.6 km/h) to reach D1, whereas for NON it took 91 ± 17 h (moving at a velocity of 3.2 ± 0.8 km/h). For FIN, 41 ± 6 h were required to reach D2 (velocity 2.7 ± 0.4 km/h) and 125 ± 21 h to reach the finish line (at a velocity of 2.5 ± 0.4 km/h), so that the overall total finish time (i.e., excluding resting time at checkpoints) was 248 ± 36 h (velocity 2.8 ± 0.3 km/h). A positive correlation between the split times PRE-D1 and D2-POST with respect to the finish time was retrieved (r 0.83, p 0.01 and r 0.93, p 0.001, respectively). This shows that the participants, who were the fastest both in the first and in the last part of the race, also accounted for the best overall time at the end of the race. Such correlations were not found for the split time between D1 and D2.

TABLE 2 | Performance data.

Group	Gender	Distance, km mean (S.D.)	Finish Time, h mean (S.D.)	Velocity, km/h mean (S.D.)
FIN	Men	690.0 (0.0)	254.6 (21.8)	2.7 (0.2)
	Women	690.0 (0.0)	300.2 (18.8)	2.3 (0.2)
	All	690.0 (0.0)	268.3 (29.7)	2.6 (0.3)
NON	Men	384.3 (107.4)	139.3 (60.0)	3.1 (1.2)
	Women	278.4 (0.0)	92.0 (0.0)	3.0 (0.0)
	All	366.7 (105.4)	131.4 (57.0)	3.1 (1.0)
ALL	Men	350.3 (106.4)	206.6 (71.4)	2.9 (0.8)
	Women	358.3 (124.3)	248.1 (105.2)	2.5 (0.4)
	All	352.3 (106.7)	217.0 (79.3)	2.8 (0.7)

Performance data of subgroups (FIN and NON), as well as of all participants (ALL). The overall completed distance and total time to cover it are reported, as well as the overall mean velocity (including resting times). No significant differences between groups.

Heart Rate Continuous Recordings

A total of 185 recordings fulfilled the criteria for analytic inclusion. They were obtained in 15 participants, as one competitor of the 2017 race belatedly volunteered to participate in the study and could not be equipped with the measuring device anymore. As reported above, recorded HR was used as a marker of exercise intensity, by normalizing absolute values with respect to the calculated HR_{max}. A similar approach was used to define the quality of rest during the actual race, i.e., the time spent at checkpoints was excluded. Results of ExHR (mean HR during exercise as percentage of HR_{max}) and RestHR (mean HR during rest in between checkpoints, as a percentage of HR_{max}), classified according to the 4 segments mentioned above, i.e., D1a (36 h after race start), D1b (24 h before arriving at D1), D2a (24 h before reaching D2), D2b (24 h before arrival at the finishing line), are displayed in **Table 3**.

Heart Rate Variability

For all 16 participants, a total of 53 R-R interval recordings were available and exhibited sufficient quality for analysis and assessment of HRV. 16 recordings corresponded to PRE, 13 to D1, and 10 to both D2 and POST. Morning HR pre-, post- and in-race (at checkpoints) is depicted in **Figure 3** and HRV results are depicted in **Figures 4**, **5**. **Figure 4** shows the significant decrease of parasympathetic tone in both groups at D1 compared to PRE, and in the following in-race time-points as for FIN only. **Figure 5** depicts sympathovagal balance indices, where a significant decrease at D1 in both groups of log LF lead to no variations of log LF/HF, whereas in FIN a significant increase of DFAα2 across all time-points was retrieved, and in NON

TABLE 3 | Average exercise intensity and rest quality.

Group	*n*	HR rec. time-point	ExHR, % mean (S.D.)	RestHR, % mean (S.D.)
FIN	9	D1a	70.9 (5.3)	47.4 (8.3)
	8	D1b	62.0 (3.9)*	38.4 (4.4)*
	8	D2a	62.1 (2.6)*	40.0 (3.6)*
	7	D2b	59.1 (4.4)*	37.0 (4.7)*
NON	6	D1a	66.9 (5.6)	44.6 (4.9)
	6	D1b	59.1 (5.8)*	40.0 (3.6)
ALL	14	D1a	69.5 (5.6)	46.4 (7.2)
	14	D1b	60.7 (4.8)	39.1 (4.0)
	10	D2a	60.9 (3.5)	39.6 (3.7)
	8	D2b	57.0 (7.1)	36.2 (4.9)

*Average HR during exercise (ExHR) and at rest (RestHR) across time-points. Data are presented as a percentage of the maximal HR (HRmax) for mean HR during exercise (ExHR) and mean HR during rest periods (RestHR). D1a: HR recorded in the first 36 h; D1b: HR recorded during the 24 h before arriving at D1; D2a: HR recorded during the 24 h before arriving at D2; D2b: HR recorded during the last 24 h before reaching the finish line. Data for the whole sample (ALL) are also reported. *$p < 0.05$ vs. PRE within subgroup (One-Way RM ANOVA).*

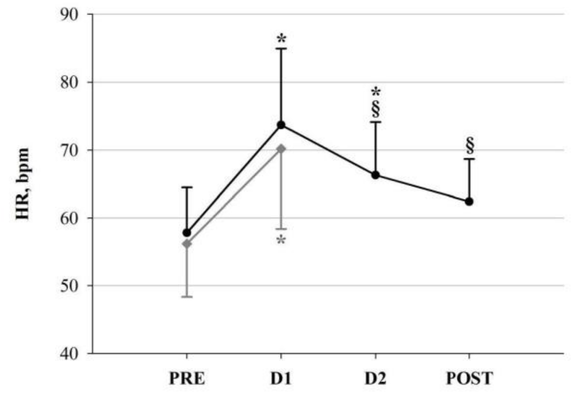

FIGURE 3 | Heart Rate (HR) in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup (One-Way RM ANOVA). $^{\S}p < 0.05$ vs. D1 (One-Way RM ANOVA).

only a significant increase at D1 as for DFAα1. Between PRE and D1, an overall decrease in TP could be observed in both groups (FIN: −1,964.7 ms^2 and NON: −3,699.6 ms^2), but the decrease was only significant (*p* 0.02) in NON. In fact, in NON the decrease in parasympathetic drive was to some extent greater when compared to FIN, as indicated by DFAα1, which was significantly higher at D1 only in NON (**Figure 5**), and by the difference between PRE and D1 in values of RMSSD: −34.1 ms in NON (*p* 0.01) and −18.1 ms in FIN (ns) and log HF: −0.8 ms^2 (*p* 0.04) in NON and −0.5 ms^2 (ns) in FIN.

Only in FIN, a significant negative correlation between HR and mean running velocity, as well as between HR and pNN50+, was detected at PRE (**Figure 6**). The negative correlation between HR and vagal tone indices was observed also at D1, as for pNN50- (*r* −0.82 *p* 0.02), and NN50- (*r* −0.82, *p* 0.02) with respect to HR. This could not be detected in NON.

Psychometric Scales

Psychometric measurements were performed in competitors of the 2015 and 2017 races, so that a total of 45 assessments (13 in PRE, 13 in D1, 10 in D2 and 9 in POST) were included in the statistical analysis. Results of the POMS questionnaire revealed significant decreases in POMS Vigor and Tension, associated with an increase in Fatigue and POMS Total scores (**Figure 7**). No changes in POMS Depression, Confusion and Anger scores were observed, nor significant differences between FIN and NON at D1. As for fatigue scales, i.e., Borg RPE, Borg TQR and KSS, results are depicted in **Figure 8**. In NON, values of KSS Departure scores were significantly higher at D1 than at PRE. However, as for the other psychometric scores, no significant between-group differences could be detected.

Nevertheless, at PRE, positive correlations between several indices of vagal tone (RMSSD: *r* 0.86, *p* 0.03; NN50+: *r* 0.90, *p* 0.01; NN50–: *r* 0.89, *p* 0.02; pNN50+: *r* 0.87, *p* 0.03; pNN50–: *r* 0.87, *p* 0.03; log HF: *r* 0.87, *p* 0.03) and Borg TQR were detected in NON only, so the higher the vagal tone, the higher the TQR score. Moreover, at PRE, again in NON only, a negative correlation between POMS Fatigue and pNN50+ (*r* −0.82, *p* 0.04) was observed, which indicates that the lower the vagal tone, the higher POMS Fatigue. On the other hand, in FIN at D1, a negative correlation was observed between HR and KSS Departure (*r* −0.85, *p* 0.02), indicating that the lower the HR, the higher the KSS Departure score; this was associated with a positive correlation between both pNN50– (*r* 0.84, *p* 0.02) and NN50– (*r* 0.84, *p* 0.02) and KSS Departure, which confirmed that a lower HR and a higher vagal drive were coupled with higher sleepiness; additionally in FIN at D1, there was a positive correlation between Borg TQR and DFAα2 (*r* 0.81, *p* 0.03): the higher the TQR score, so the quality of recovery after rest, the higher the DFAα2.

POMS T-Scores of YAU participants were plotted against normative data to provide the above-mentioned *Iceberg Profile* (Terry and Lane, 2000), which depicts POMS Vigor to be significantly higher and all other (negative) dimension scores to be significantly lower compared to mean values for a sedentary population. Analysis of variance between YAU participants and normative athletic data revealed distinctive differences, which are depicted in detail in **Figure 9B**. In comparison to the athletic sample, raw and T-Scores at baseline were significantly lower in ALL regarding POMS Depression, Anger, Fatigue and Confusion, but also Vigor. This was similarly observed when plotting YAU scores against normative data for athletes before and after competition. Compared to pre-competition normative data, YAU subjects at PRE displayed significantly lower Depression, Anger, Confusion and Fatigue, whereas Tension and Vigor were not different (**Figure 9C**). At POST, there were no significant differences between normative data for athletes in post-competition situations and the YAU subjects, except for significantly lower Vigor in YAU (**Figure 9D**).

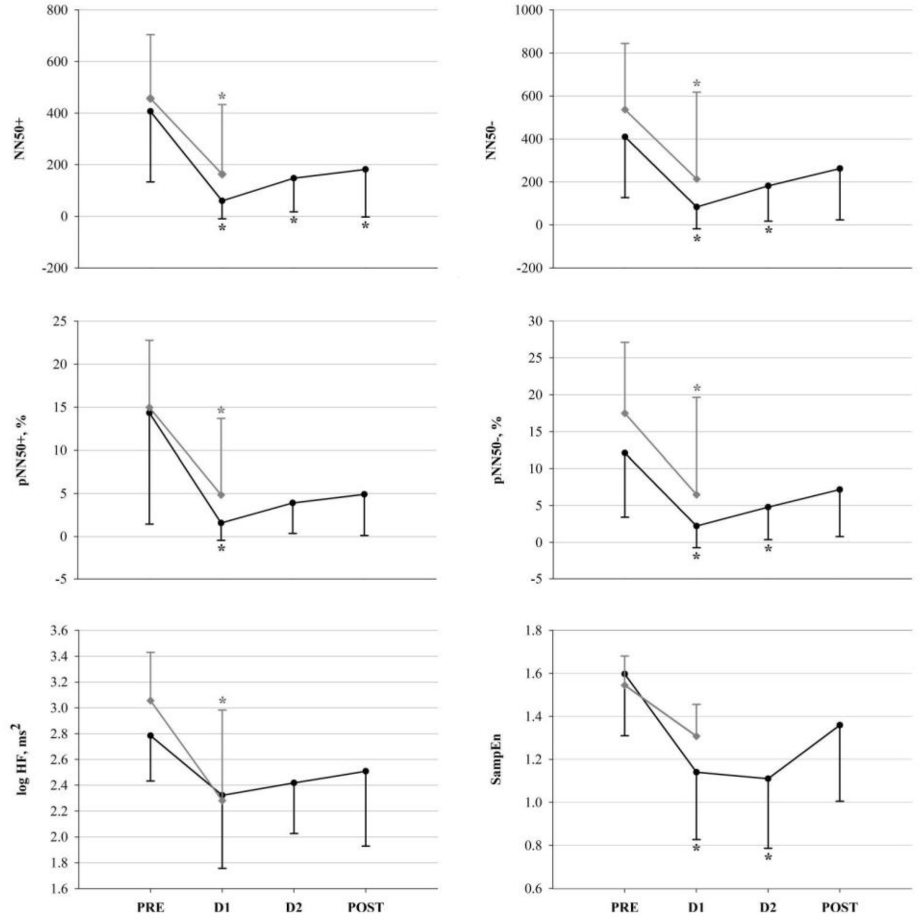

FIGURE 4 | Vagal modulation indices in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup (all variables: One-Way RM ANOVA; pNN50+: Friedman ANOVA).

DISCUSSION

To our knowledge, this is the first study investigating cardiac autonomic modulation and psychological correlates during ultramarathon in a subarctic environment. This setting provided the unique combination of three extreme environments: (i) ultra-endurance exercise performance (Perini and Veicsteinas, 2003; Scott et al., 2009), (ii) arduous environmental circumstances (Maughan et al., 2007), such as severe continuous cold exposition, and (iii) sleep deprivation/disturbances, induced by the condition of outdoor living during the race (Stein and Pu, 2012). The interplay of each single component of this *three-folded stress stimulus*, amplifies and affects several physiological and psychological aspects, which may be reflected in physical performance outcomes. Regarding race results, 10 of 16 subjects successfully completed the 690 km ultramarathon. Taking into account characteristics and conditions of the competition (see section The Yukon Arctic Ultra: The Longest, the Coldest Ultramarathon), the distribution of measurements sessions (see section Experimental Protocol and Measurements and **Figure 2**) holds great importance to interpret observations. In fact, the greatest effect on autonomic cardiac modulation, mood and fatigue was observed in the race segment between PRE and D1 (i.e., more than one third of the entire race); this may have been related to: (i) the initial stress of entering the race (i.e., performance demands coupled with environmental conditions), (ii) the different characteristics of the three parts of the race (see section Experimental Protocol and Measurements), and (iii) the running strategy of successful participants. Indeed, by analyzing the split times between measurement sessions, a positive correlation between the first and the final split time was found. This indicates that the fastest competitors in PRE-D1 (and D2-POST), were also the fastest finishers. The challenge of this first part of the race would be significantly underlined by this observation, and it provided ulterior evidence of the central role of the ability to cope with in-race demands at

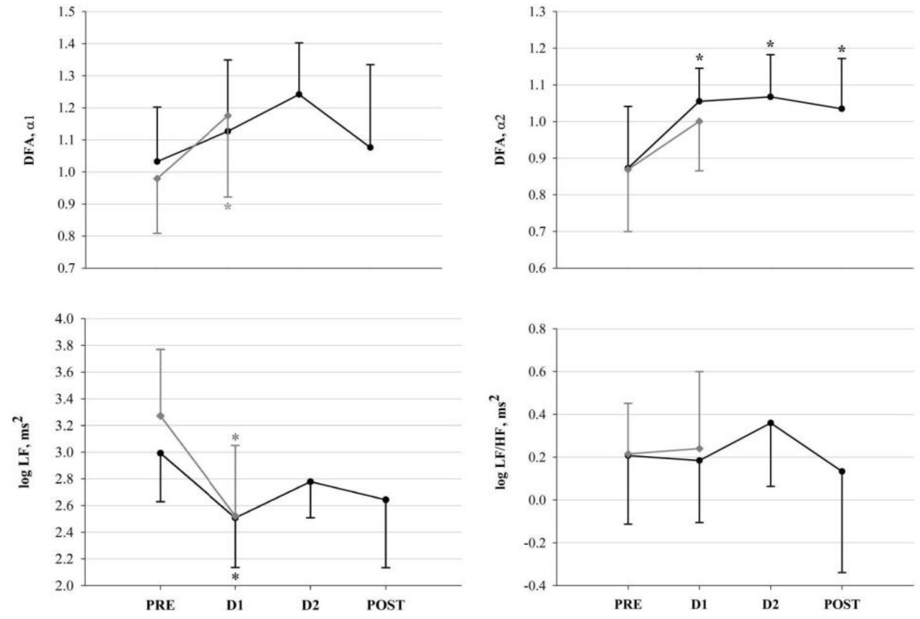

FIGURE 5 | Sympathovagal balance indices in FIN (black line) and in NON (gray line) across time-points. $^*p < 0.05$ vs. PRE within subgroup (One-Way RM ANOVA).

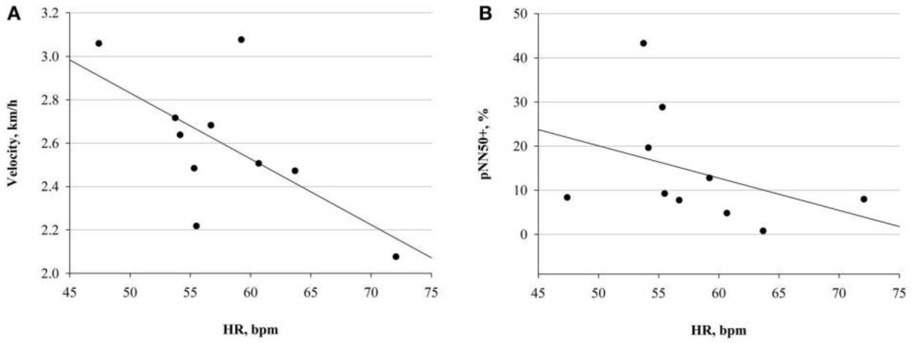

FIGURE 6 | Correlations at PRE in FIN group. **(A)** Correlation between HR and mean velocity ($r = -0.63$; $p = 0.04$). **(B)** Correlation between HR and pNN50+ ($r = -0.67$; $p = 0.03$).

very early time-points (i.e., directly after entering the race) for optimal adaptation. Therefore, during the first kilometers, successful competitors could already be recognized. This is in line with previous investigations on early recognition of successful competitors by their initial pacing strategies (Renfree et al., 2016; Bossi et al., 2017). Specifically, Lambert et al. observed more successful competitors in a 100 km ultramarathon to display higher velocities than lower performing athletes in the early race stages (Lambert et al., 2004). Moreover, the continuous significant decrease in ExHR in FIN, associated to a concomitant decrease of RestHR ($p < 0.05$ across all time-points vs. PRE), reflected decrements in performance, while the need of rest increased. On the other hand, the continuous decrease in RestHR may also indicate higher quality of recovery (Waldeck and Lambert, 2003; Silvani and Dampney, 2013), demonstrating that successful competitors had higher recovery potential, as they attained a higher quality of rest. Indeed, by comparing FIN and NON, we found a significant decrease of RestHR between PRE and D1 in FIN only.

In line with Millet (Millet and Millet, 2012), our observations show that ultramarathon might be an excellent model to test the adaptive potential under extreme conditions. However, in our case, we have to take into account not only potential effects of ultraendurance exercise, but the interplay of three factors (i.e., *three-folded stress stimulus*) on influencing

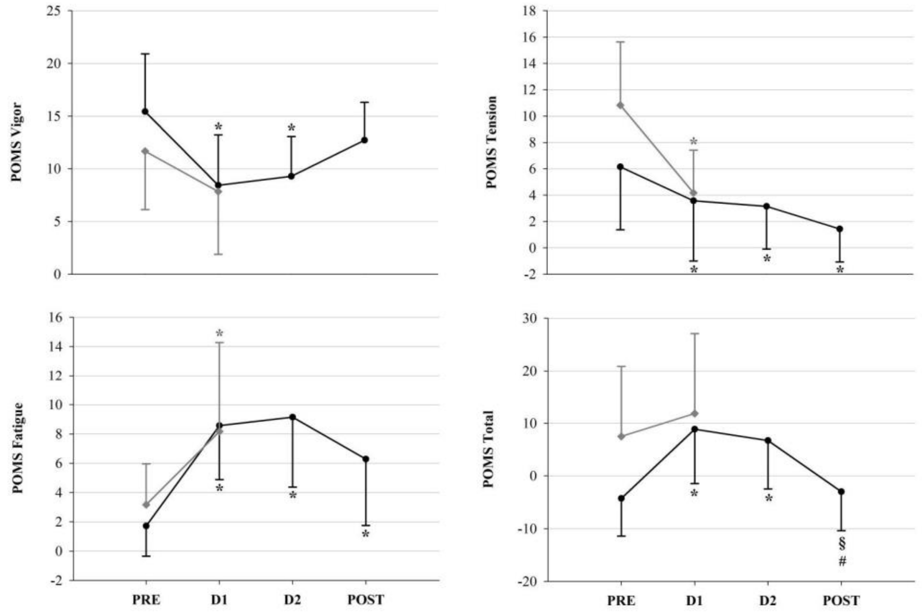

FIGURE 7 | POMS mood state scores in FIN (black line) and in NON (gray line) across time-points: $^*p < 0.05$ vs. PRE within subgroup; $^§p < 0.05$ vs. D1; $^\#p < 0.05$ vs. D2 (One-Way RM ANOVA).

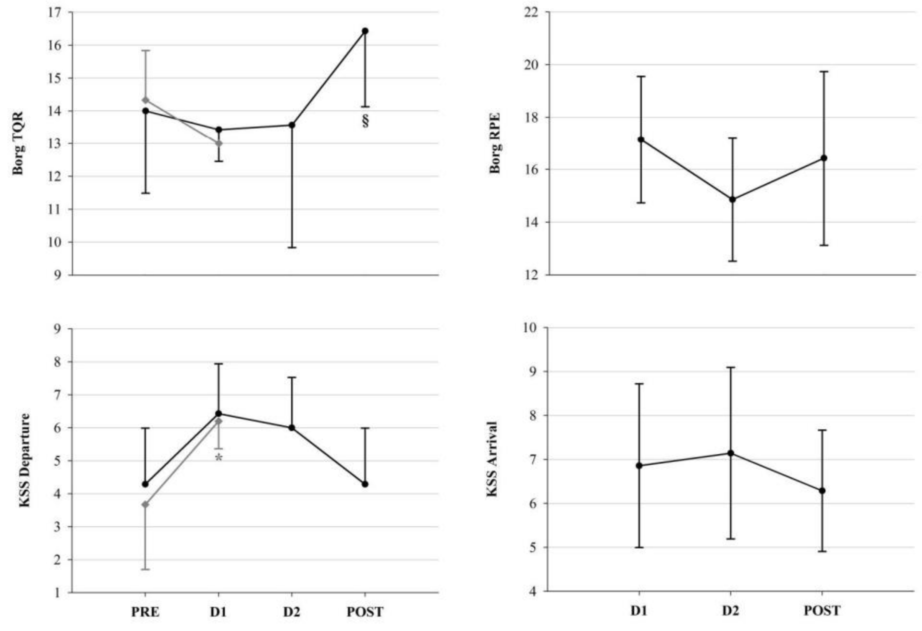

FIGURE 8 | Fatigue scale scores. Borg and KSS scores in FIN (black line) and in NON (gray line) across time-points. $^*p < 0.05$ vs. PRE within subgroup; §$p < 0.05$ vs. D1 (One-Way RM ANOVA).

autonomic cardiac modulation and psychometric aspects: strenuous exercise, living outdoors in subarctic winter and sleep deprivation/disturbances.

Autonomic Cardiac Control and Endurance

An overall reduction of vagal drive, as well as total HRV, has been widely observed during acute exercise and competition (Perini

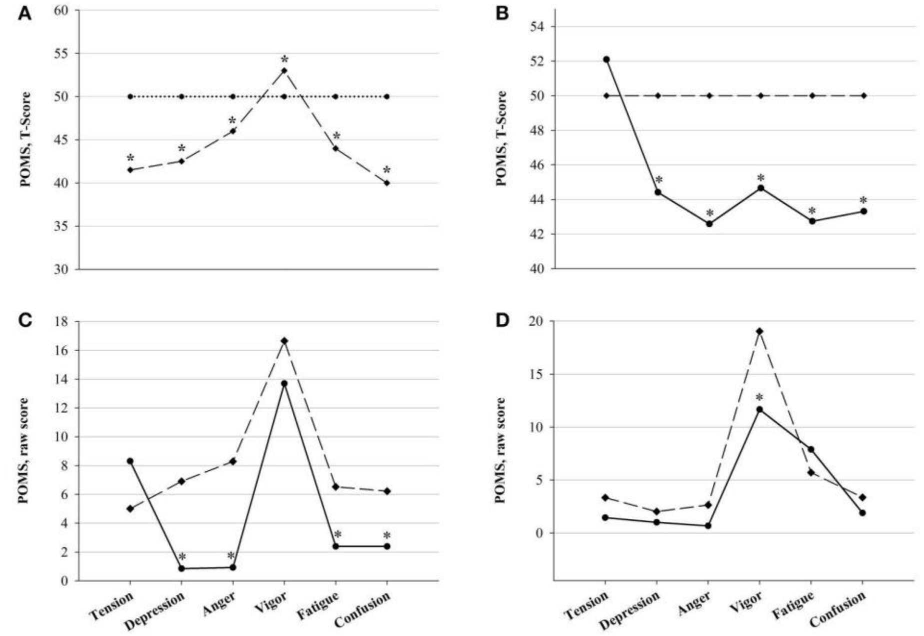

FIGURE 9 | Comparison of POMS results with normative data (Unpaired Student's *t*-Test). **(A)** *Iceberg profile* of mood states in an athletic sample (dashed line) plotted against sedentary subject norms (dotted line), **(B)** Mood states in all YAU subjects (ALL, solid line) plotted against normative data for mixed athletic samples (dashed line), **(C)** Mood states in ALL at PRE (solid line) plotted against normative data for pre-competition assessment (dashed line), **(D)** Mood states in ALL at POST (solid line) plotted against normative data for post-competition assessment (dashed line): $^{*}p < 0.05$ between groups.

and Veicsteinas, 2003; Buchheit et al., 2010). After cessation of exercise, parasympathetic predominance is gradually restored, depending on the preceding intensity, the training status and the quality of recovery, as reported also in previous studies on ultramarathon (Gratze et al., 2005; Scott et al., 2009; Foulds et al., 2014), and this is in line with our results. However, due to the very long distance of YAU we did observe already a vagal tone recovery before the end of the race, in successful participants (**Figure 4**). Considering that our HR recordings were collected after several hours of rest, early at morning during the race, the significant increase in HR (**Figure 3**) clearly describes the inability for participants to recover completely, as HR remained significantly higher at both D1 and D2. Nevertheless, at D2, HR decreased again with respect to D1 (−7.8 bpm, p 0.03), remaining, however, significantly above baseline values. At POST, HR was significantly lower than at D1 (−11.3 bpm, p 0.004) and not different from PRE. This would demonstrate that successful competitors (i.e., FIN) were able to positively adapt by recovering toward baseline conditions. This specific trend of HR can also be compared with observations in functional overreaching training interventions, where, initially, the increased training stimulus promotes a decrease in vagal drive (i.e., increase in HR), but, due to optimal adaptation, this is subsequently recovered (Buchheit, 2015; Bellenger et al., 2017). Indeed, in our participants, the increased HR between PRE and D1 was modulated via a significant attenuation in vagal tone in both groups (**Figure 4**).

This, associated with a significant concomitant decrease of log LF, lead to an overall reduced HRV (see result section for TP). Additionally, evidence of reduced parasympathetic drive at D1 was further underlined by significant decreases in SampEn in FIN (**Figure 4**). In line with previously investigated implications of reduced SampEn values, this suggest lower responsiveness to environmental stimuli under attenuated entropy (Sassi et al., 2015). Interestingly, our findings indicate that in unsuccessful participants (i.e., NON), this parasympathetic drive decrease was to some extent greater than in FIN. This was shown firstly by the significantly higher DFAα1 at D1 in NON only (**Figure 5**), which has been associated to vagal tone decrease (Penttilä et al., 2003), and secondly by the difference in values of RMSSD and log HF for NON vs. FIN between PRE and D1 (see results section). Taking into account that in athletic subjects, stress has been associated to lower HRV and depressed parasympathetic drive (Nuissier et al., 2007; Cervantes Blásquez et al., 2009), this suggests that successful participants were able to efficiently relax and therefore fall asleep. The stronger decrease of vagal tone in NON, indeed, may indicate that these participants, ultimately unable to complete the race, were characterized by an impaired ability to cope with the in-race demands, already at early points of the competition, which may be reflected by lower quality of recovery (i.e., sleep quality impairment), whereas FIN displayed higher recovery potential. In turn, this supports the hypothesis that in such extreme conditions, vagal tone modulations may mirror the individual's ability to adapt, showing in resilient individuals earlier and efficient increase of parasympathetic tone, after the large initial decrement. Between PRE and D1, we also observed the typical reduction of overall HRV, which normally occurs during exercise, as well as a significant reduction of log LF in both groups, thus leading to non-significant changes in log LF/HF ratio (**Figure 5**). Only in FIN, DFAα2 was significantly higher at D1 compared to baseline and remained higher at both D2 and POST. As the exact implications of this non-linear HRV index have not been elucidated, this is an interesting finding. Previous investigations have reported that DFAα2 would decrease after the application of clonidine, an imidazoline-derived centrally-acting α2-adrenergic agonist and hypothalamic inductor of hypotension, which affects the overall sympathetic activity by resetting it to a lower setpoint (Castiglioni et al., 2011). Conversely, increased DFAα2 values have been reported in subjects who were awake compared to when asleep, but have also been linked to sleep stages, being higher in awake states and REM sleep than during light and deep sleep (Schumann et al., 2010). These findings allow us to hypothesize a link between increased DFAα2 and hyperarousal or enhanced alertness and vigilance, which in this case would be driven by the sympathetic branch of the vegetative nervous system. As we reported a significant increase in DFAα2 during the race, which persisted up until POST, this interpretation concurs very well with our observations (**Figure 5**). Higher values of DFAα2 could have been induced by an increased need for vigilance (i.e., sleeping outdoors during the subarctic winter in the Yukon Territory), leading to sleep impairment and/or deprivation, and to a general acute stress response promoting hyperarousal. Furthermore, the negative correlation between HR and velocity, paired with the negative correlation between pNN50+ and HR at PRE only in FIN (**Figure 6**), indicated that a lower HR in association with a higher vagal tone would predict a better performance. In FIN, the persistency of the above-mentioned negative correlation of vagal indices with HR at D1 (see Results section), demonstrated how, in our study, the observed increase in HR was specifically driven by a decrease of parasympathetic tone. This mechanism was mirrored mainly by time domain indices of vagal drive, i.e., NN50 and pNN50 statistics, which are linked to mean HR. Indeed, while pNN50+ quantifies the rate of HR decelerations (increase in successive R–R intervals), NN50– quantifies the rate of HR accelerations (decrease in successive R–R intervals) (Merati et al., 2015). At PRE, our data showed a negative correlation between HR and pNN50+ (i.e., rate of successive HR decelerations) and at D1, HR correlated negatively with both pNN50– and NN50– (i.e., rate of successive HR accelerations). Differences in the distribution of HR decelerations and accelerations have been associated with the enhanced presence of sympathetic modulations, whereas the HR decelerations have been identified as a better marker of vagal activity (Merati et al., 2015). This further demonstrates that at the beginning of the race, between PRE and D1, the reduction of vagal tone determined the increase in HR. Indeed, at D2 and POST, no correlation between vagal indices and HR could be detected. During this second in-between measurement section, HR decreased with respect to D1. Concomitantly, a slight increase of vagal tone was observed (**Figures 3**, **4**). After D1, the correlation between HR and vagal tone disappeared, indicating that the increase of parasympathetic tone was not able to elicit *per se* the decrease in HR. Instead, it would be suggested that the observed HR reduction also occurred due to other concurrent factors as for example psychological states. In fact, at D2, a significant reduction of POMS Total scores (indicating increased negative mood states or disturbance) with respect to D1 was found (**Figure 7**). This was associated with a decrease of Borg RPE at D2 (although not significant), suggesting that psychological factors were involved in recovering overall wellbeing, and thus were associated with reducing the HR (see section Psychological Wellbeing).

Psychological Wellbeing

We observed an overall decrease of psychological wellbeing across the whole ultramarathon (**Figures 7**, **8**). Interestingly, the POMS Tension item exhibited significantly higher values already at PRE with respect to D1 in both groups, in particular in NON (+6.7 vs. +2.6 in NON vs. FIN). This may reflect pre-competition anxiety. In fact, during the subsequent race, POMS Tension decreased significantly across all time-points. The concurrent increase in Vigor from D2 onwards (as POST values were no longer significantly lower in respect to PRE), may be related to the recovering process of positive mood, but also to the fact that participants were succeeding in the race and the finishing line was getting closer. Between PRE and D1, a significant reduction of positive mood items (lower POMS Vigor and higher Fatigue as well as POMS Total scores) had been observed. Therefore, we can infer that psychometric measurements sensitively reflected the impact of this extremely demanding competition on different subgroups, more strongly affecting those subjects that were unable to cope with the in-race demands. Nevertheless, after D1, it was possible to recognize a particular pattern in FIN, who recovered their wellbeing and positive mood. Indeed, not only POMS Tension scores continuously decreased, but also Vigor again attained values comparable to baseline at POST. Enhanced positive mood or motivation may have furthermore contributed to the observed recovery of vagal tone. In fact, previous investigations have demonstrated associations between enhanced parasympathetic drive in the frequency domain and POMS Vigor, as well as energy index (i.e., the POMS Vigor/Fatigue ratio) (Bisschoff et al., 2016), and the Vigor subscale has been proposed as a marker of the overall autonomic nervous system modulatory activity (Nuissier et al., 2007).

As mentioned above, this finding could be related to the fact that completion of the race was approaching. On the other hand, we found significantly lower POMS Total scores (indicating reduced mood disturbance) paired with higher Borg TQR values at D2 compared to D1 (even if not reaching statistical significance). This reflects a trend of increase in psychological wellbeing. As at POST, POMS Total was similar to PRE values, but significantly higher than at D1 and D2, successful recovery of mood disturbance in FIN is accentuated.

Moreover, during the first part of the race, as mentioned above, the increased HR depicted the inability of participants to recover completely. However, this event was not reported by data of the Borg RPE scale (**Figure 8**), which, although in-race values had decreased, did not exhibit any significant changes across the race. In this sense, it is likely that in the case of the YAU competitors, the Borg RPE failed to detect the perceived exertion.

Results of Borg TQR in NON showed a significant correlation between vagal indices and TQR scores at PRE, which may suggest that the higher the parasympathetic tone, the higher the perceived quality of recovery, underlining previous findings about the effect of parasympathetic tone on perceived fatigue in athletes (Bisschoff et al., 2016). However, this correlation was not found at D1. Instead, only in NON, KSS Departure scores were higher at PRE compared to D1. As the KSS has been extensively validated to depict objective sleepiness (Kaida et al., 2006), this subjective measurement indicates greater sleepiness, probably due to impaired rest and insufficient recovery in NON compared to FIN.

At PRE, no correlation between psychometric scales and HRV indices was found in FIN. Nonetheless, at D1, KSS Departure correlated negatively with the HR and positively with vagal indices in FIN, i.e., the lower the HR and the higher the vagal tone, the higher the subjective sleepiness upon departure. On the other hand, the concomitant positive correlation between Borg TQR and DFAα2 could suggest that subjects with higher recovery and better sleep quality, were also in a state of enhanced vigilance and alertness, ready to continue on the trail. Nevertheless, we must admit that as we recorded HR early in the morning, just after awakening, and DFAα2 has been reported to be higher in awake states and REM sleep than in light and deep sleep (Schumann et al., 2010), our observations could also be influenced by the circumstances of the measurement sessions, immediately after waking up. The high adaptive potential in our FIN subjects promoting recovery of initially increased mood disturbance, exertion and sleepiness, paired with a concurrently re-increasing subjective recovery status, presents several implications. Possibly, lower sleepiness and therefore higher alertness would yield essential importance for coping with the environmental challenges of the YAU competition. Moreover, sleepiness and fatigue have been associated with impaired cognitive, as well as physical, function and performance (Fullagar et al., 2015). Therefore, the ability to recover from attenuated psychometric wellbeing in our high-achieving FIN once more underlines the importance of adaptability.

Finally, comparison of mood states with normative data for athletic samples (Terry and Lane, 2000) generally displayed lower mood disturbance in our competitors (**Figure 9**). At baseline, POMS Depression, Fatigue, Confusion and Anger were lower, with Vigor conversely being higher compared to normative scores. Further comparison with normative data for pre- and post-competitive assessment again confirmed the great mental health in our participants, who had significantly higher positive mood than compared to pre-competitive normative values. During the first stages of the race, mood disturbance significantly increased under the exhausting demands, but recovered. Therefore, at POST, mood states in YAU participants (except for Vigor scores) did not significantly differ from normative data in post-competition assessment. To conclude, the high adaptive capacity in our subjects, who attained recuperation of gravely impacted mood states after enduring the extreme in-race conditions and stress stimuli, is again underlined.

Practical applications of these findings are related to training methods, highlighting the importance of high and/or fast increasing vagal tone, and of mood states: the "mind," i.e., mood state and motivation, plays a crucial role, especially with respect to such a long-lasting and highly demanding competition. In fact, successful competitors were able to perform greatly also in the second part of the race, where the decrease of HR was not coupled directly with higher vagal drive (as instead was in the first part of the race for FIN only), and the intervention of psychological aspects could be hypothesized (see above). All in all, assessment of HRV and psychological profile may contribute to monitor and partly predict performance in such extreme environments.

LIMITATIONS

Given that this is an in-field study in extreme environments, a number of possible limitations must be addressed.

First of all, the sample size of 16 may appear small, however, considering that a total number of only 78 athletes competed in the three investigated editions and 27 of them enrolled in our study, we regard this number to be quite considerable and sufficient under these specific conditions.

Moreover, there is a substantial difference in the distance between the in-race checkpoints (i.e., D1, D2) selected to perform measurement sessions and a study protocol over three equispaced checkpoints may have been favorable. However, the choice of measurement implementation was due to essential practical concerns, as previously mentioned (see section Experimental Protocol and Measurements). These concerns also held essential importance for standardizing as much as possible measurement conditions, (i.e., indoors facility, comfortable setting regarding space, temperature, noise, and light exposure), especially regarding HR beat-to-beat recordings.

Furthermore, we aimed to allow comparison of additional data from HR continuous measurements with HRV and psychometric parameters obtained at measurement points. Therefore, continuous HR recording data were clustered and were split up in the above-mentioned four sections (see section Performance Assessment and Heart Rate Continuous Recordings).

CONCLUSION

The main findings of this study are: (i) the extent of the early vagal withdrawal, associated to the timing and potential of its recovery, is crucial for success in this specific competition, (ii) a pre-competition lower resting HR, coupled with a higher vagal tone, would predict a better performance, as already reported in the literature for endurance sports (Gratze et al., 2005; Buchheit, 2014), and (iii) psychological profile and wellbeing is reliably depicted by mood state assessment with the POMS questionnaire, but not by Borg fatigue scales, and again associated with autonomic cardiac modulation. Successful ultramarathoners were coping better already in early stages of the competition, which allowed recovery of cardiac autonomic balance and positive mood, thus associated with higher athletic achievement. Therefore, assessment of HRV and psychological profile may contribute to monitor and partly predict performance in such extreme long-duration competitions in extremely cold environments.

AUTHOR CONTRIBUTIONS

LR and MM contributed equally to the study by writing the manuscript and analyzing the data. MS designed, planned and implemented the study, secured funding sources, and performed measurements and data collection. AS assisted with the measurements and data collection. AR-R, LR, and MM performed statistical analyses. RC and H-CG contributed to the study design, provided expertise and feedback. LR formatted, and, with assistance of MM and MS, revised the manuscript.

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High-Intensity Exercise Mitigates Cardiovascular Deconditioning During Long-Duration Bed Rest

Martina A. Maggioni[1,2*†], **Paolo Castiglioni**[3†‡], **Giampiero Merati**[2,3], **Katharina Brauns**[1], **Hanns-Christian Gunga**[1], **Stefan Mendt**[1‡], **Oliver S. Opatz**[1], **Lea C. Rundfeldt**[1], **Mathias Steinach**[1], **Anika Werner**[1,4‡] **and Alexander C. Stahn**[1,5]

[1] Charité—Universitätsmedizin Berlin, Institute of Physiology, Center for Space Medicine and Extreme Environments Berlin, Berlin, Germany, [2] Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy, [3] IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy, [4] Université de Normandie, INSERM U 1075 COMETE, Caen, France, [5] Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, United States

Edited by:
Andrew Blaber,
Simon Fraser University, Canada

Reviewed by:
Piero Ruggeri,
Università di Genova, Italy
Joyce McClendon Evans,
University of Kentucky, United States

***Correspondence:**
Martina A. Maggioni
martina.maggioni@charite.de
orcid.org/0000-0002-6319-8566

[†] These authors have contributed equally to this work

[‡] Paolo Castiglioni,
orcid.org/0000-0002-8775-2605
Stefan Mendt,
orcid.org/0000-0001-8227-9655
Anika Werner,
orcid.org/0000-0002-9822-0348

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Head-down-tilt bed rest (HDT) mimics the changes in hemodynamics and autonomic cardiovascular control induced by weightlessness. However, the time course and reciprocal interplay of these adaptations, and the effective exercise protocol as a countermeasure need further clarification. The overarching aim of this work (as part of a European Space Agency sponsored long-term bed rest study) was therefore to evaluate the time course of cardiovascular hemodynamics and autonomic control during prolonged HDT and to assess whether high-intensity, short-duration exercise could mitigate these effects. A total of $n = 23$ healthy, young, male participants were randomly allocated to two groups: training (TRAIN, $n = 12$) and non-training (CTRL, $n = 11$) before undergoing a 60-day HDT. The TRAIN group underwent a resistance training protocol using reactive jumps (5–6 times per week), whereas the CTRL group did not perform countermeasures. Finger blood pressure (BP), heart rate (HR), and stroke volume were collected beat-by-beat for 10 min in both sitting and supine positions 7 days before HDT (BDC−7) and 10 days after HDT (R+10), as well as on the 2nd (HDT2), 28th (HDT28), and 56th (HDT56) day of HDT. We investigated (1) the isolated effects of long-term HDT by comparing all the supine positions (including BDC−7 and R+10 at 0 degrees), and (2) the reactivity of the autonomic response before and after long-term HDT using a specific postural stimulus (i.e., supine vs. sitting). Two-factorial linear mixed models were used to assess the time course of HDT and the effect of the countermeasure. Starting from HDT28 onwards, HR increased ($p < 0.02$) and parasympathetic tone decreased exclusively in the CTRL group ($p < 0.0001$). Moreover, after 60-day HDT, CTRL participants showed significant impairments in increasing cardiac sympathovagal balance and controlling BP levels during postural shift (supine to sitting), whereas TRAIN participants did not. Results show that a 10-day recovery did not compensate for the cardiovascular and autonomic deconditioning following

60-day HDT. This has to be considered when designing rehabilitation programs—not only for astronauts but also in general public healthcare. High-intensity, short-duration exercise training effectively minimized these impairments and should therefore deserve consideration as a cardiovascular deconditioning countermeasure for spaceflight.

Keywords: heart rate variability, hemodynamics, countermeasure, cardiovascular deconditioning, posture, long-term bed rest

INTRODUCTION

Upcoming deep space missions such as Martian expeditions will require exposure to up to 1,000 days in microgravity (Horneck, 2006). Space agencies are thus investigating adverse health effects of long-term missions and their possible countermeasures in order to reduce detrimental consequences for astronaut health (Aubert et al., 2016; Bergouignan et al., 2016; Frippiat et al., 2016; White et al., 2016; Lang et al., 2017). Space analogs simulating prolonged gravity changes therefore play a crucial role (Ploutz-Snyder, 2016). Bed rest with −6 degrees head-down tilt (HDT) is one of the best conditions to mimic the effect of long-term weightlessness on the human body—even within the limitations of this model. HDT shifts fluids from the lower domain to the upper region of the body, similar to the fluid centralization observed in spaceflight (Pavy-Le Traon et al., 2007; Hargens and Vico, 2016; Watenpaugh, 2016). Bed rest models also allow for the investigation of some of the effects of immobilization, secondary to hospitalization, and physical inactivity. Indeed, elderly patients spend over 80% of their hospital admission confined to their bed (Vernikos and Schneider, 2010; Baczynska et al., 2016), and physical inactivity is one of the leading causes of death in Western countries (Blair, 2009). Therefore, investigating the physiological consequences of physical inactivity and designing effective countermeasures is essential for planning future long-term space missions as well as for public health and rehabilitation purposes. Weightlessness negatively affects several physiological functions. For example, it can cause deconditioning of the cardiovascular system, which may be characterized by higher resting heart rate with altered autonomic control associated with orthostatic intolerance (Blomqvist et al., 1994; Sigaudo et al., 1998; Fortrat et al., 2001; Custaud et al., 2002). Despite research spanning at least four decades on weightlessness-associated cardiovascular alterations (Pavy-Le Traon et al., 2007; Hargens and Vico, 2016), the exact time courses of changes in hemodynamic regulation and autonomic cardiovascular control induced by long-term spaceflight are not fully understood (Liu et al., 2015; Aubert et al., 2016). Moreover, several countermeasures for cardiovascular deconditioning have already been tested (e.g., volume loading, lower-body negative pressure, hypergravity; Wang et al., 2011; Stenger et al., 2012; Jeong et al., 2014; Li et al., 2017), but exercise is the most investigated countermeasure (Blaber et al., 2009; Petersen et al., 2016; Ploutz-Snyder, 2016). However, despite the consensus on physical activity as a countermeasure, the type and intensity of the exercise are undergoing further investigation. Common exercise countermeasures include aerobic (Pagani et al., 2009; Cavanagh et al., 2017; Demontis et al., 2017) and resistive exercise (Holt et al., 2016; Demontis et al., 2017), as well as in combination with whole-body vibration (Belavý et al., 2010). Recent findings show high-intensity interval training (HIIT) to be salutary in cardiovascularly compromised persons (Ramos et al., 2015; Fleg, 2016; Hussain et al., 2016), improving aerobic capacity, endothelial, and left-ventricular function, vasomotor function, and blood pressure (Hussain et al., 2016). So far, however, HIIT has rarely been implemented to counteract cardiovascular deconditioning and orthostatic intolerance in microgravity settings (Hughson et al., 1994; Greenleaf, 1997; Hastings et al., 2012; Hargens et al., 2013). This study therefore aimed to evaluate whether short-duration HIIT is an effective countermeasure against cardiovascular deconditioning and orthostatic intolerance induced by 60 days of head-down-tilt bed rest. To achieve this aim, we compared subjects doing HIIT with a control group and investigated 1) the time course of hemodynamic changes and adaptations of the cardiovascular autonomic control during 60-day HDT, and 2) the cardiovascular response to a postural test performed before and after the bed rest confinement. As for the HIIT exercise, we administered specific lower body resistance training that provides neuromuscular force solicitation: the reactive jump protocol in a sledge jump system (Kramer et al., 2010, 2017b).

METHODS

This research was performed as part of the European Space Agency (ESA) sponsored study "**R**eactive jumps in a **S**ledge jump system as countermeasure during **L**ong-term bed rest—RSL Study" at the DLR *:envihab* (German Aerospace Agency (DLR), Cologne, Germany), between 2015 and 2016. Details related to the core project design, recruitment, randomization of volunteers, and training protocol are reported elsewhere (Kramer et al., 2017a,b). The study was conducted in accordance with the Declaration of Helsinki for Medical Research Involving Human Subjects (revision October 2013) and was approved by the ethics committee of the Northern Rhine Medical Association in Düsseldorf, Germany (see Kramer et al., 2017a). After the purpose, procedures, and known risks of the tests had been explained to the participants, each participant gave written informed consent. In brief, the study consisted of 15 days of baseline data collection (BDC−15 through BDC−1), 60 days of HDT bed rest (HDT1 through HDT60), and 15 days of recovery (R+0 through R+14). During the 60 days of −6 degrees HDT, the reactive jump training was administered as a countermeasure in one randomly selected subsample (TRAIN: training), whereas the other subsample (CTRL: control) did not perform any physical training (see section subjects below). The training protocol was performed using a sledge jump system (Novotec Medical GmbH, Pforzheim, Germany) composed of a lightweight sledge sliding on rails. Cylinders pull the sledge toward the plates with force exerted on the subject by adjusting the pressure settings within the cylinders. The participant was fixed supine to the sledge with shoulder straps and with feet on force plates. Participants would then perform countermovement jumps while receiving feedback on jump height and peak force from a monitor. Participants in the TRAIN group underwent the training protocol starting from HDT1. Each training session consisted of repetitive jumps and different series of countermovement jumps with an average load equal to or above 80% of the participant's body weight. Sessions lasted about 20 min, including preparation. Training took place in the afternoon between 2 and 6 pm, 5–6 times per week, for a total of 48 sessions during the 60-day bed rest. A comprehensive description of the sledge system, the training method, and timeline is reported elsewhere (Kramer et al., 2017a,b).

Subjects

Data were collected from 23 young, healthy, male participants (baseline: age 29 ± 6 [m ± SD] years, weight 77 ± 7 kg, height 181 ± 6 cm), who were not involved in competitive or professional sport activities at the time of the study (see Kramer et al., 2017a,b for details on inclusion and exclusion criteria). Participants were randomly allocated to a training group (TRAIN, $n = 12$, age 30 ± 7 years, weight 78 ± 7 kg, height 181 ± 7 cm) or to a control group (CTRL, $n = 11$, age 28 ± 6 years, weight 76 ± 8 kg, height 181 ± 5 cm), and were matched based on anthropometry (Kramer et al., 2017a). One subject of the TRAIN group and one of the CTRL group were re-ambulated after 49 and 50 days of HDT (instead of 60 days), respectively, for medical reasons (Kramer et al., 2017a). Notably, this did not affect their completion of the recovery phase and all the scheduled measurements were collected. Accordingly, these subjects were included in the data analysis.

Data Collection

To evaluate short-, mid-, and long-term exposure to HDT, autonomic cardiovascular and hemodynamic data were collected on the 2nd (HDT2), on the 28th (HDT28), and on the 56th (HDT56) day of HDT. To evaluate the response to a postural stimulus, we also collected the identical data 7 days before the start of HDT (BDC−7) and 10 days after the end of HDT (R+10, R+0 being the first day of recovery) in both sitting and supine positions (**Figure 1**). In each session, a time series of physiological data were recorded for 10 min. During the lay-to-sit challenge (in BDC−7 and R+10) data were first recorded for 10 min in the supine position and then for an additional 10 min immediately after the change of posture to the sitting position. Recordings included blood pressure at the finger artery (BP) measured via continuous finger plethysmography and one-lead electrocardiogram (ECG). Both were sampled at 200 Hz. Beat-by-beat stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR) were obtained using impedance cardiography (TensoScreen®, Medis Germany). All measurement sessions were conducted between 9 and 12 am (before lunch), at least 18 h after the previous training session. Participants were required to avoid caffeine consumption during the 4 h leading up to the measurements, and were instructed not to move, talk, or fall asleep during the recordings.

Data Analysis

An expert operator visually inspected the ECG and BP signals, identifying and manually removing possible artifacts and premature beats. Beat-by-beat time series of normal-to-normal R-R intervals were derived from the ECG tracing for heart rate variability (HRV) analysis. Beat-by-beat values of systolic BP (SBP) and diastolic BP (DBP) were obtained from the BP signal. Beat-to-beat series of R-R intervals and DBP values were interpolated linearly at 10 Hz and resampled at 5 Hz. The Welch periodogram was estimated by splitting the resampled series in 90% overlapping Hann windows of 240 s in duration, computing the FFT spectrum in each window, and by averaging the spectra over all the windows. The final periodogram was smoothed with a broadband procedure that averages adjacent spectral lines with a moving average filter whose order increases with the frequency from 3 to 11 (Di Rienzo et al., 1996). Following the guidelines on HRV analysis (Task Force of the European Society of Cardiology the North American Society of Pacing

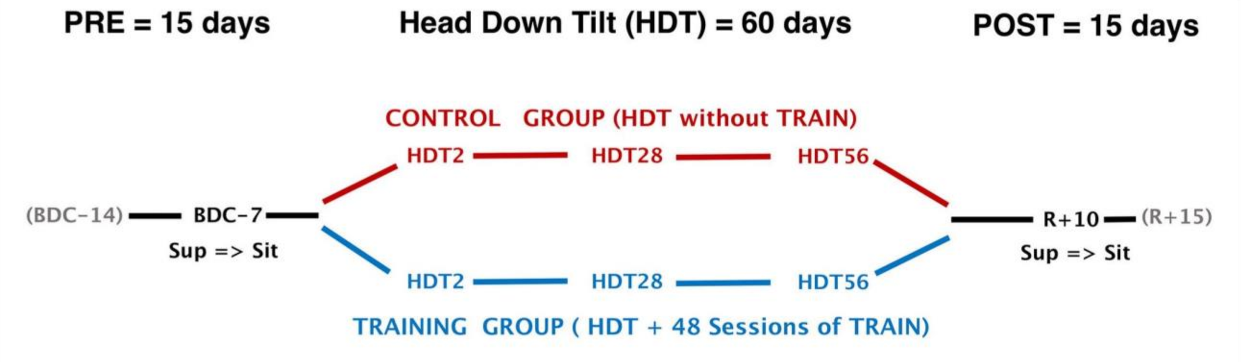

FIGURE 1 | Study design and timeline of data collection. HDT, head-down tilt; TRAIN, reactive jumps training; BDC−7, Baseline Data Collection (7 days before start of HDT); R+10, Recovery data collection (10 days after the end of HDT); Sup, measurements in supine position; Sit, measurements in sitting position.

Electrophysiology, 1996), the power spectrum of R-R intervals was integrated over a very-low-frequency (VLF, 0.005–0.04 Hz), a low-frequency (LF, 0.04–0.15 Hz), and a high-frequency band (HF, 0.15–0.40 Hz). The ratio between LF and HF powers was calculated as an index of cardiac sympathovagal balance (i.e., LF/HF). The LF power was also derived for the DBP spectrum as an index of sympathetic modulations of the vascular tone (Castiglioni et al., 2007). The short-term fractal index DFA1 was estimated by applying detrended fluctuation analysis to the beat-by-beat R-R interval series (Peng et al., 1995) and by considering block sizes not larger than 16 beats. DFA1 reflects changes in the cardiac autonomic tone, which increases when the sympathovagal balance increases or the vagal tone decreases (Tulppo et al., 2001; Castiglioni et al., 2011). The mean breathing rate was evaluated as the central frequency of the power spectrum of ECG-derived respiration (EDR) signal. The EDR signal reflects the respiratory movements of the thorax as modulations of the amplitude of the QRS complex (Schmidt et al., 2017).

Statistics

Descriptive statistics have been reported as means and standard deviations (m ± SD) unless stated otherwise. To evaluate

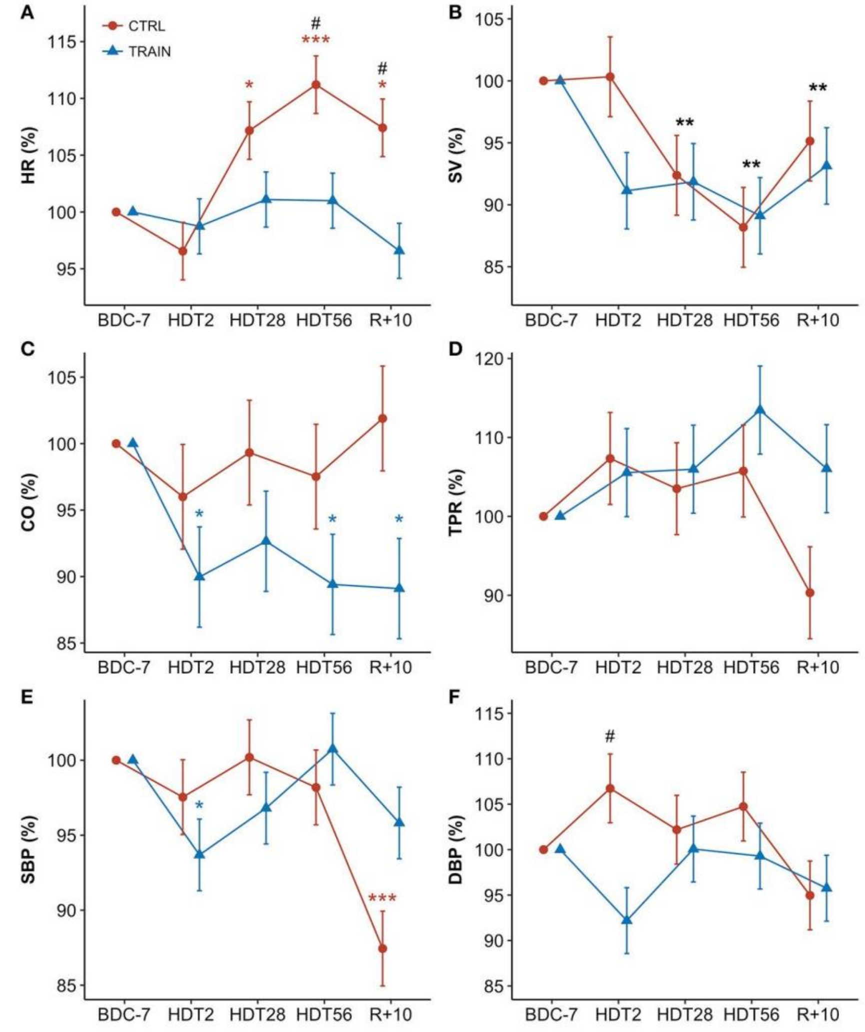

FIGURE 2 | (A–F) Time courses of hemodynamic variables for CTRL (red circles) and TRAIN (blue triangles) groups. Measurements time points in supine position: 7 days before (BDC−7) and 10 days after (R+10) HDT, and on the 2nd (HDT2), 28th (HDT28) and 56th (HDT56) day of HDT. Data are presented as marginal means ± SE of percent changes from baseline values (with BDC−7 supine =100%) at each time point. Colored asterisks indicate significance compared to BDC−7 within the single group. Black asterisks show significant difference from baseline in the whole sample of participants (when only the factor Time - and not the factor Group or their interaction - is significant). The pound sign denotes significant differences between groups at each time point. **(A)** HR, Heart Rate; **(B)** SV, Stroke Volume; **(C)** CO, Cardiac Output; **(D)** TPR, Total Peripheral Resistance; **(E)** SBP, Systolic Blood Pressure; **(F)** DBP, Diastolic Blood Pressure. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, #$p < 0.05$.

the time course of cardiovascular changes during and after bed rest compared to baseline, we expressed data of HDT2, HDT28, HDT56, and R+10 as percentage changes from BDC−7 by dividing the values recorded in each HDT time point by the corresponding value measured in BDC−7 supine (which therefore corresponds to the 100% reference). A log transformation was applied to frequency-domain indices to attain normal distribution (Castiglioni et al., 1999). Because of the properties of the logarithm, the normalized variables were expressed as the difference between the log-transformed value in each time point and the log-transformed value in the baseline, which therefore corresponds to the reference zero level. Two-factorial linear mixed models were then used to assess the time course of cardiovascular changes in supine position within and between subjects. Subjects were entered as random factors and bed rest Time (HDT2, HDT28, HDT56, and R+10) and intervention Group (CTRL and TRAIN) were included as fixed factors. Significant effects of Time and Group or their interaction were followed up using contrasts (with BDC−7 as a reference for Time). When only the factor Time was significant, contrasts were performed irrespective of the intervention (i.e., CTRL and TRAIN groups were pooled).

As for the postural test (i.e., the shift from supine to sitting) performed before and after HDT, variables were expressed as *delta scores*. The *delta score* corresponds to the difference (Δ) between the value measured before bed rest (at BDC−7) and the respective value measured after bed rest (at R+10) per group (CTRL and TRAIN) and position (supine and sitting). Therefore, *delta scores* are changes from baseline values (i.e., BDC−7) and express the effects of bed rest according to each group and each posture. Mixed models were used to assess within-participant and between-participant differences in *delta scores*. Body position and intervention group were included as fixed factors and subjects as random factors. When the factor Posture, or Group, or their interaction was significant, contrasts were performed to follow up on single comparisons and corrected for multiple comparisons by a sequentially rejective correction procedure. To test whether bed rest had a significant effect in a given group and posture, contrasts were used to determine whether each *delta score* differed significantly from zero (non-zero contrasts). Covariance matrices were determined by restricted maximum likelihood (REML) estimation. *P*-values were obtained using Satterthwaite's approximation for denominator degrees of freedom. Normality and homogeneity were checked via visual inspections of plots of residuals against fitted values. The level of significance was set at $\alpha = 0.05$ (two-sided) for all testing. All comparisons were corrected for multiple comparisons using a sequentially rejective correction procedure (Hochberg, 1988). To maximize sensitivity for detecting true differences while maintaining control over family-wise Type I errors, we followed the recommendation of choosing smaller, more focused families rather than broad ones (Westfall et al., 2011). All statistical analyses and graphical illustrations were carried out using the software package R (R Core Team, 2016) Mixed models were run using the packages lme4 and lmerTest (Bates et al., 2014; Alexandra Kuznetsova et al., 2016). Adjusted means were calculated using the lsmeans package (Lenth, 2016) and htmlTable (Max Gordon, 2017). Figures were created using ggplot2, ggpubr, and cowplot (Wickham, 2016; Alboukadel Kassambara, 2017; Claus O. Wilke, 2017).

TABLE 1 | HDT time course: significance of Time and Group factors and their interaction based on linear mixed models analysis (see text for abbreviations).

	Significance p		
	Time	**Group**	**Time × Group**
HR	<0.001	<0.05	<0.01
SV	<0.05	0.43	0.16
CO	0.67	0.055	0.65
SBP	<0.001	0.72	<0.01
DBP	0.12	0.18	0.06
TPR	0.09	0.31	0.26
RRI SPECTRAL POWERS:			
log HF	< 0.001	0.19	<0.01
log LF	< 0.001	0.74	<0.01
log VLF	< 0.001	0.75	0.20
log LF/HF	0.055	0.28	0.61
DFA1	<0.05	0.15	0.06
DBP SPECTRAL POWERS:			
log LF	0.38	0.37	0.21

RESULTS

Time Course of Hemodynamic Variables During HDT

Figure 2 shows percent changes in hemodynamic values from baseline (BDC−7 supine) measured during HDT and recovery. **Table 1** reports the results of the linear mixed models analysis. The factor Time and its interaction with Group were significant for HR. Accordingly, HR was higher than at baseline from HDT28 up to the recovery phase (R+10) in CTRL participants, while not exhibiting changes in the TRAIN group. In particular, the difference between groups in HR changes was statistically significant near the end of bed rest (HDT56) and during recovery (R+10). Time was also a significant factor for SV, which decreased during HDT, recovering only partially in R+10. The factor Group was marginally significant for CO, with values lower than baseline for only the TRAIN group. The factor Time and its interaction with Group were highly significant for SBP. **Figure 2** shows a marked fall in SBP during recovery in the CTRL group only. DBP showed a marginal significance for the interaction between Group and Time. No factors were significant for TPR (**Figure 2**) and EDR; the respiratory rate did not change significantly during and after HDT.

Time Course of Autonomic Indices During HDT

Figure 3 shows percent changes in autonomic indices from baseline. **Table 1** shows the results of the linear mixed models. Since percent changes of spectral powers were log-transformed

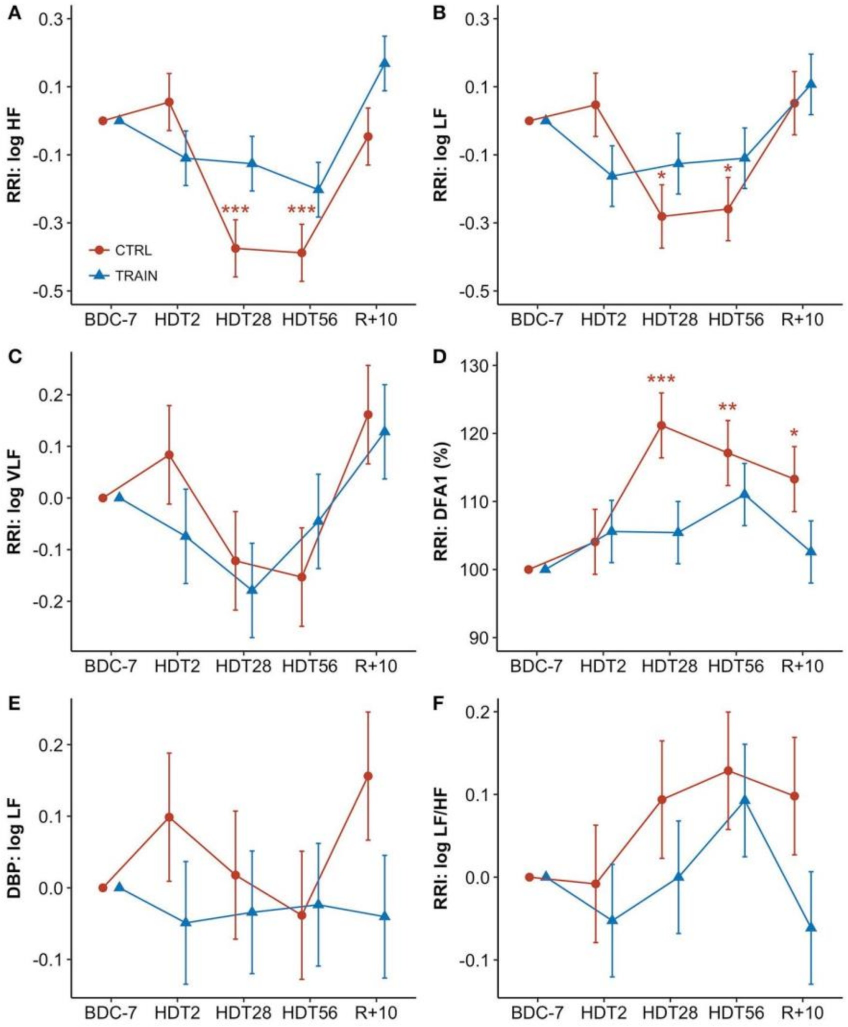

FIGURE 3 | (A–F) Time courses of autonomic variables for CTRL (red circles) and TRAIN (blue triangles) groups. Measurements time points in supine position: 7 days before (BDC−7) and 10 days after (R+10) HDT, and on the 2nd (HDT2), 28th (HDT28) and 56th (HDT56) day of HDT. Data are presented for each time point as marginal mean ± SE of differences with respect to baseline supine position (for spectral powers, BDC−7 supine = 0) or of percent changes from baseline supine position (for DFA1, BDC−7 supine = 100%). Colored asterisks indicate significance compared to BDC−7 within the single group. RRI, R-R intervals; **(A)** HF, High-Frequency power; **(B)** LF, Low-Frequency power; **(C)** VLF, Very-Low-Frequency power; **(D)** DFA1, short-term fractal index by detrended fluctuations analysis; **(E)** LF of DBP: Low-Frequency power of Diastolic Blood Pressure; **(F)**: LF/HF, Low-Frequency to High-Frequency powers ratio $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

before the statistical test, they are reported as differences vs. zero, i.e., the baseline reference (see Methods). The factor Time and the interaction between Time and Group were highly significant for the HF and LF powers of RRI (**Table 1**). Both these powers had values lower than baseline at HDT28 and HDT56 for the CTRL group only. Time was a significant factor and the interaction between Time and Group was close to the 5% significance threshold. This was also the case for DFA1, whose profile mirrored the profile of the HF power, with a significant increase at HDT28 and HDT56 for the CTRL group only. In this case, however, the increase was consistently statistically significant during recovery as well. As for the LF/HF powers ratio, Time was the only factor close to statistical significance (**Table 1**). No factors were significant for the LF power of DBP.

Hemodynamic Response to the Postural Test

Table 2 reports descriptive statistics of hemodynamic data in supine and sitting positions at BDC−7 and R+10. Not only before but also after bed rest, the shift from supine to sitting posture appears associated with an increase in HR, DBP, and TPR, and with a decrease in SV and CO in both groups. However, the bed rest had a different effect on the amplitude of the changes in the two groups, as reported in **Figure 4** (i.e., *delta scores* of

TABLE 2 | Postural test: mean (SD) in the supine (Sup) and sitting (Sit) position before (BDC−7) and after (R+10) bed rest (see text for abbreviations).

		CTRL		*TRAIN*	
	Time	**Sup**	**Sit**	**Sup**	**Sit**
HR (bpm)	BDC−7	58.5 (7.4)	69.1 (8.3)	64.2 (9.1)	70.4 (7.8)
	R+10	62.7 (7.6)	70.2 (5.9)	62.1 (10.5)	68.2 (7.8)
SV (mL)	BDC−7	106.7 (18.7)	81.5 (14.0)	101.6 (14.1)	76.8 (12.5)
	R+10	101.3 (17.9)	75.6 (11.8)	94.6 (15.9)	70.9 (9.0)
CO (L/min)	BDC−7	6.1 (1.1)	5.5 (1.0)	6.5 (1.1)	5.3 (0.8)
	R+10	6.2 (1.2)	5.2 (0.8)	5.8 (1.3)	4.7 (0.6)
SBP (mmHg)	BDC−7	129.5 (10.0)	125.4 (10.0)	129.7 (8.6)	127.6 (9.6)
	R+10	112.5 (7.8)	122.4 (7.0)	124.2 (9.3)	126.6 (8.1)
DBP (mmHg)	BDC−7	70.5 (7.5)	76.6 (7.1)	76.0 (8.6)	81.6 (9.6)
	R+10	66.5 (4.8)	77.6 (7.5)	72.7 (10.0)	81.0 (5.4)
TPR (dyn s cm^5)	BDC−7	1148 (235)	1318 (249)	1154 (246)	1395 (209)
	R+10	1026 (226)	1379 (182)	1223 (299)	1559 (188)
EDR (Hz)	BDC−7	0.27 (0.03)	0.29 (0.03)	0.27 (0.04)	0.27 (0.03)
	R+10	0.27 (0.02)	0.27 (0.03)	0.27 (0.03)	0.27 (0.02)
RRI:					
log HF (log ms^2)	BDC−7	2.85 (0.50)	2.38 (0.50)	2.58 (0.38)	2.40 (0.25)
	R+10	2.81 (0.49)	2.69 (0.45)	2.75 (0.47)	2.54 (0.34)
log LF (log ms^2)	BDC−7	3.10 (0.40)	3.02 (0.31)	3.03 (0.28)	3.09 (0.34)
	R+10	3.15 (0.29)	3.08 (0.28)	3.14 (0.21)	3.17 (0.25)
log VLF (log ms^2)	BDC−7	3.18 (0.39)	3.22 (0.37)	3.09 (0.35)	2.97 (0.21)
	R+10	3.34 (0.40)	3.30 (0.45)	3.22 (0.40)	3.15 (0.31)
log LF/HF	BDC−7	0.46 (0.07)	0.65 (0.48)	0.39 (0.05)	0.70 (0.29)
	R+10	0.34 (0.36)	0.39 (0.45)	0.38 (0.32)	0.63 (0.30)
DFA1	BDC−7	0.81 (0.15)	1.09 (0.20)	0.87 (0.16)	1.04 (0.11)
	R+10	0.90 (0.12)	0.96 (0.16)	0.89 (0.15)	1.03 (0.11)
DBP:					
log LF (log mmHg^2)	BDC−7	1.28 (0.25)	1.20 (0.33)	1.32 (0.23)	1.21 (0.14)
	R+10	1.43 (0.19)	1.26 (0.29)	1.28 (0.19)	1.26 (0.15)

hemodynamic variables) and **Table 3** (i.e., factors significance based on linear mixed models). The HR *delta score* of the CTRL group in supine position was positive and significantly higher than in sitting position (**Figure 4A**), suggesting that bed rest increased HR more in the supine than the sitting position in the CTRL group. The factor Group and its interaction with Posture were significant (**Table 3**), and bed rest did not increase HR in the TRAIN group (*delta scores* were negative). A significantly different *delta score* between groups in the supine position was also found. Bed rest decreased SV in both groups (**Figure 4B**), independent of posture (**Table 3**). CO (the product of HR and SV) reflected the combination of HR and SV *delta scores*. Both factors and their interaction were significant (**Table 3**) and *delta scores* differed between the groups in the supine position and between positions in the CTRL group (**Figure 4C**).

The factors Posture, Group, and their interaction were also significant for TPR (**Table 3**). The negative TPR *delta score* of CTRL participants in the supine position indicates that bed rest decreased supine TPR only in the CTRL group (**Figure 4D**). Posture and its interaction with Group were also significant factors for SBP (**Table 3**). Bed rest decreased SBP more in supine than in sitting position and markedly more in the CTRL group (**Figure 4E**). Effects of bed rest were less pronounced on DBP (**Figure 4F**) than on SBP.

Autonomic Response to the Postural Test

The shift from supine to sitting position was associated with an increase in the LF/HF powers ratio and DFA1, and a decrease in the HF power of RRI and the LF power of DBP—both before and after bed rest (**Table 2**). However, the prolonged bed rest period influenced these changes differently in the two groups (**Figure 5**). In fact, after bed rest, the HF power of RRI in supine position only increased in the TRAIN group (**Figure 5A**), and the DFA1 in sitting position only decreased in the CTRL group (with differences in *delta scores* between positions found only in the CTRL group, **Figure 5D**). Furthermore, bed rest decreased the sitting LF/HF index in CTRL participants only and influenced the supine LF power of DBP differently between groups (i.e., the *delta score* was positive for CTRL participants and negative for TRAIN participants, **Figures 5B** and **5C**).

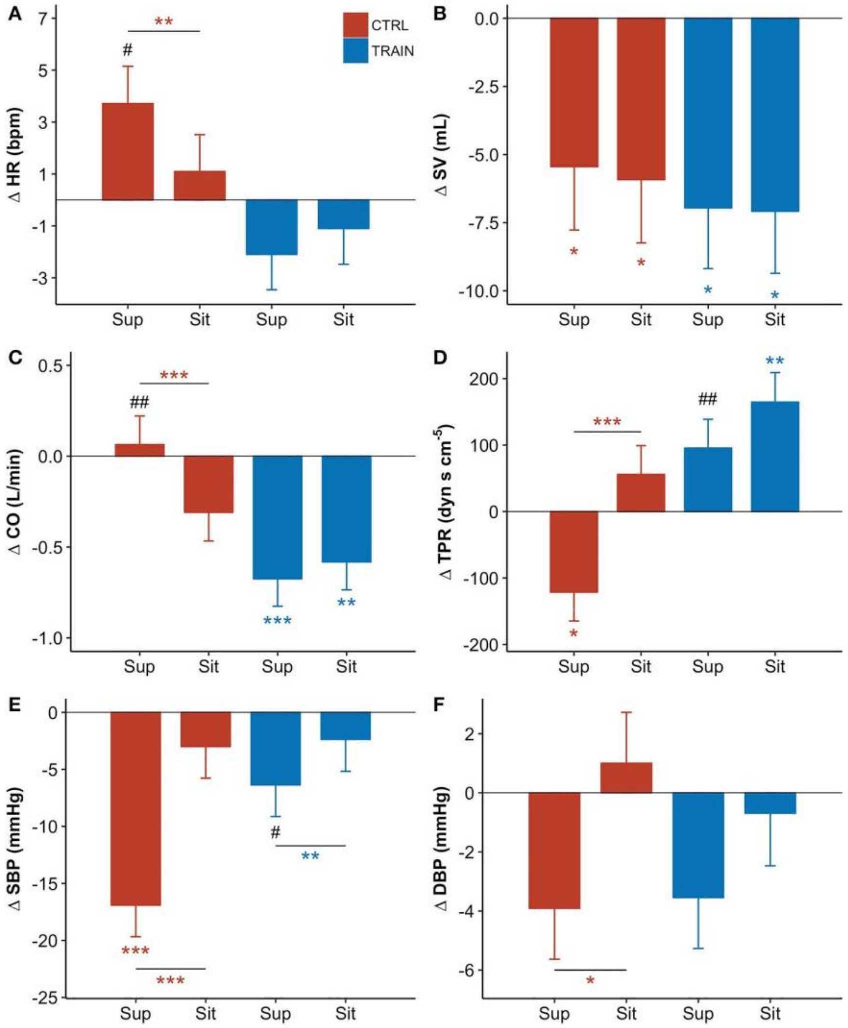

FIGURE 4 | (A–F) Delta scores of hemodynamic variables, i.e., the difference (Δ) between the value measured before bed rest (at BDC−7) and the corresponding value measured after bed rest (at R+10, see text) for the supine (Sup) and the sitting (Sit) position: adjusted means ± SE. For each panel: control group (CTRL) red bars on the left-hand side, and training group (TRAIN) blue bars on the right-hand side. Asterisks above whiskers indicate *delta scores* significantly different from zero. Horizontal lines with asterisks indicate significant differences between supine and sitting *delta scores* within the same group. Pound signs indicate significant differences between groups at the same position. **(A)** HR, Heart Rate; **(B)** SV, Stroke Volume; **(C)** CO, Cardiac Output; **(D)** TPR, Total Peripheral Resistance; **(E)** SBP, Systolic Blood Pressure; **(F)** DBP, Diastolic Blood Pressure. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$.

DISCUSSION

Our study investigated the alterations induced by 60 days of HDT in hemodynamics and autonomic modulations of heart rate. To the best of our knowledge, this study involved the highest number of participants to investigate these specific effects during long-term HDT bed rest so far. Our main findings show that: (1) HR progressively increased some days after the start of the HDT; (2) changes in SV and HR vagal modulations appeared almost synchronously; and (3) alterations in these variables and SBP were detectable several days after the end of bed rest, indicating persistent cardiovascular deconditioning. Furthermore, this high-intensity/short-duration exercise alleviated the cardiovascular deconditioning, counteracting the autonomic alterations and improving recovery. Although the adopted exercise protocol mainly involved the lower part of the body, it likely influenced central mechanisms of cardiac modulation and appears to be a promising countermeasure for long-term spaceflight missions. Different exercise-based countermeasures have been tested so far to reduce cardiovascular deconditioning. A previous study showed that low-magnitude whole body vibration with

TABLE 3 | Postural test: significance of Group and Posture factors and their interaction based on linear mixed models analysis (see text for abbreviations).

	Significance p		
	Group	**Posture**	**Group x Posture**
HR	<0.05	0.16	<0.01
SV	0.66	0.82	0.89
CO	<0.05	0.053	<0.01
SBP	0.16	<0.001	<0.001
DBP	0.76	<0.01	0.38
TPR	<0.05	<0.001	<0.05
RRI SPECTRAL POWERS:			
log HF	0.96	<0.01	<0.001
log LF	0.55	0.83	0.48
log VLF	0.99	0.26	0.23
log LF/HF	0.12	0.07	0.46
DFA1	0.48	<0.001	<0.001
DBP SPECTRAL POWERS:			
log LF	0.13	0.09	<0.001

resistive exercise prevented the increase of the autonomic index of cardiac sympathovagal balance after an HDT period lasting 60 days (as was the case in our study) without, however, improving orthostatic tolerance (Coupé et al., 2011). Other studies were different from the present in their design and duration. Nonetheless, these studies highlight the crucial role of exercise as a countermeasure against cardiovascular deconditioning. The intermittent exposure to hypergravity coupled with ergometric exercise limited the decrease in parasympathetic activity after 14 days of HDT (Iwasaki et al., 2005). Daily rowing ergometry and biweekly strength exercise training after 5 weeks of HDT prevented orthostatic intolerance only when combined with volume loading (Hastings et al., 2012). Finally, supine cycling could counteract orthostatic intolerance after 18-day bed rest only in combination with plasma volume restoration (Shibata et al., 2010). However, the vast range of different study designs and countermeasures complicate a direct comparison with the sledge jump training protocol.

Time Course of Hemodynamic Variables and Autonomic Indices During HDT

We described the time course of long-term HDT adaptations by comparing values during HDT with baseline measures in the supine position (0 degrees). Compared to sitting, the recumbent position is characterized by a fluid shift to the upper body, which increases stroke volume and stimulates baroreceptors and volume receptors, inducing cardiac vagal activation. By choosing this reference we could describe the isolated effect of the prolonged −6 degrees bed rest and this makes our results comparable with the cardiovascular changes observed during mid- and long-term spaceflight (Baevsky et al., 2007; Di Rienzo et al., 2008; Demontis et al., 2017). In the CTRL group, HR significantly increased from baseline starting from the 28th day of HDT and remained higher than baseline on the 56th day, in the recovery phase, and 10 days after the end of bed rest (R+10 supine; **Figure 2A**). A similar pattern was observed in another 60-day HDT study (Liu et al., 2015). Interestingly, this pattern was missing in the TRAIN group, where HR remained stable during and after HDT. The HR increase is a crucial feature of cardiovascular deconditioning, and the lack of this feature in the TRAIN group is a clear marker of the efficacy of the proposed training protocol as a cardiovascular countermeasure. A question arises about the type of training we adopted as short duration HIIT is not usually considered to act as a *vagal enhancer*. However, some data confirm that also this type of training may induce a parasympathetic adaptation of HR when performed in the supine position (Kiviniemi et al., 2014). An alternative hypothesis for the unchanged HR in TRAIN group after 28 days of bed rest might be constant increments in left atrial volume that could have induced a bradycardic response by stretching the sinus node. However, this unlikely occurred in our study because we found a significant reduction in SV in both groups (**Figure 2A**). SV depends on contractility, on arterial blood pressure, and on atrial pressure. Since contractility did not change (as sympathetic indices of HRV remained stable in the TRAIN group) and arterial blood pressure did not change from HTD28 onwards, we may hypothesize that atrial pressure, although not directly measured, was chronically reduced (perhaps via reduced blood volume and hence preload) in both groups of subjects. A previous echocardiographic study during bed rest confirmed that the left ventricular end-diastolic volume (a surrogate of cardiac preload) progressively decreases throughout 60 days of bed rest (Westby et al., 2016). Hence, it appears that the main reason for the unchanged HR in the trained subjects could be a training-induced enhancement of vagal tone. SV progressively decreased in both groups during HDT, reaching a minimum at the end of bed rest (i.e., about 90% of the baseline value). The SV reduction is in line with the literature on HDT, which reports a decrease in plasma and blood volume by 10 to 30% within the first 24 to 72 h of confinement (Convertino, 2007). A decrease in SV suggests a dehydration condition, which was probably due to different reasons. One factor was the increased renal sodium excretion and thus reduced water retention (Convertino, 2007). Another factor could be related to tissue compression in lying position that dehydrated areas of weight bearing because of greater interstitial flow into the microcirculation (Hargens and Vico, 2016). The reduced daily physical activity might also have been a cause of dehydration (Convertino, 2007).

The training not only failed in counteracting the decrease in SV, but it might have even accelerated it. On the 28th day of bed rest, SV was 92% of the baseline value in both groups, but on the 2nd day it was equal to 100% in CTRL participants and decreased to 91% in TRAIN participants. Moreover, on the 2nd day of bed rest SBP decreased significantly only in the TRAIN group, and DBP changes tended to be lower in the TRAIN when compared to the CTRL group (**Figures 2E** and **2F**). This contrast suggests that exercise training might quickly influence the hemodynamic balance, inducing post-exercise hypotension likely due to an early blood volume reduction. We speculate that training might accelerate the loss of plasma

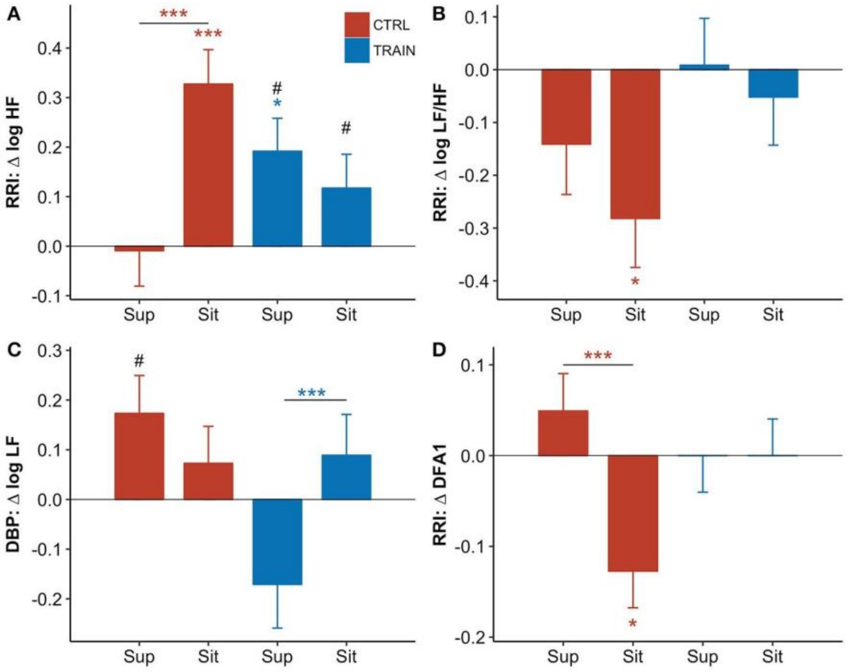

FIGURE 5 | (A–D) Delta scores of autonomic variables, i.e., the difference (Δ) between the value measured before bed rest (at BDC−7) and the corresponding value measured after bed rest (at R+10, see text) for the supine (Sup) and the sitting (Sit) position: adjusted means ± SE. For each panel: control group (CTRL) red bars on the left-hand side, and training group (TRAIN) blue bars on the right-hand side. Asterisks above whiskers indicate *delta scores* significantly different from zero. Horizontal lines with asterisks indicate significant differences between supine and sitting *delta scores* within the same group. Pound signs indicate significant differences between groups at the same position. RRI, R-R intervals; **(A)** HF, High-Frequency power; **(B)** LF/HF, Low-Frequency to High-Frequency powers ratio; **(C)** LF, Low-Frequency power; **(D)** DFA1, short-term fractal index by detrended fluctuations analysis. ***$p < 0.001$, *$p < 0.05$, #$p < 0.05$.

volume, as demonstrated by recent works showing that lower limb exercise (cycling) produced a different adaptation of the autonomic sympathetic tone in supine vs. upright position (Ried-Larsen et al., 2017). Supine position activated the stretch receptors of heart, veins, and pulmonary circulation, increasing central blood volume, and blunting the metaboreflex activation. Such a response reduced heart contractility, limiting SV, and reducing blood pressure via a non-renal mechanism. At the same time, this reaction could be responsible for a reduction in plasma renin activity and therefore for an early increase in water loss (Ried-Larsen et al., 2017). Thus, a potential additive effect on the cardiovascular system of acute blood volume changes and sympathetic response to exercise might occur in the first days of training and HDT. We can suggest that, in order to prevent such additive effect, the exercise-based countermeasures should start at full load a few days after exposure to microgravity or its analog. The only other time point at which we observed a significant difference in blood pressure between groups was 10 days after the end of HDT (R+10), when CTRL participants showed a decrease in supine SBP compared to baseline of 17 mmHg (see **Table 2**), a marked phenomenon of hypotension absent in the TRAIN group. As will be discussed later, this suggested that prolonged bed rest had long-term effects on blood pressure control mechanisms, potentially leading to orthostatic hypotension at the restoration of normal gravity conditions. The implemented training protocol showed positive effects on these cardiovascular modifications. With respect to the cardiac autonomic modulations of HR, previous studies documented a decrease in HRV total power and vagal indices in early and chronic HDT (Fortrat et al., 1998; Sigaudo et al., 1998; Pavy-Le Traon et al., 2007), as well as contrasting findings on the sympathetic cardiovascular control (Hughson et al., 1994; Sigaudo et al., 1998; Fortrat et al., 2001; Ferretti et al., 2009). In our study, the HF power decreased significantly in the CTRL group on the 28th and 56th day of bed rest (**Figure 3**). The breathing rate was stable before, during, and after HDT, always falling within the HF band (**Table 2**). In our experimental set-up, the HF power thus correctly represented the respiratory component of the parasympathetic modulations of HR. Therefore, our data indicated that HDT induced a substantial reduction in vagal modulations of HR in the respiratory band. The LF/HF powers ratio is an index of cardiac sympathovagal balance: in the CTRL group, it tended to increase on the 28th and 56th day (+9 and +13% after log transformation, **Figure 3F**). Additionally, DFA1 quantifies changes in the sympathovagal balance, but unlike the LF/HF powers ratio, it considers fractal components of the HR dynamics not related to the amplitude of the oscillations. Concurrently with the HF power reductions (**Figure 3A**), DFA1 also tended to increase in the CTRL group on the 28th and 56th day of bed rest and remained higher than baseline even 10 days (i.e., R+10) after the end of bed rest (**Figure 3D**). The LF power reflects both the vagal and sympathetic cardiac modulations; in CTRL participants it decreased in a similar fashion as the HF power, suggesting the predominance of the vagal withdrawal compared to possible sympathetic activation (**Figure 3B**). The VLF power reflects the cardiac modulations of different humoral and thermoregulatory mechanisms superimposed on the autonomic cardiac control; the time course of the VLF power during bed rest was remarkably similar in the two groups, suggesting that the effects of training on the changes in HR variability during bed rest mainly regards the fast-vagal modulations of HR (**Figure 3C**). Unlike DFA1 or the LF/HF powers ratio, the power of DBP oscillations in the LF band is a measure of sympathetic vascular control not influenced by parasympathetic modulations. This index did not show any significant effect of bed rest (**Figure 3E**). Therefore, the analysis of changes occurring during bed rest in the cardiovascular autonomic indices suggested a reduction of vagal heart rate modulations and an increase in the sympathovagal balance without evidence of an altered sympathetic tone in the CTRL group. This effect was detectable up to 10 days after the end of bed rest. The trends were different in the TRAIN group without apparent alterations during the HDT period with regard to any autonomic index. Therefore, our results suggested that during HDT the reactive jumps training reduced the cardiac autonomic deconditioning.

Hemodynamic and Autonomic Response to the Postural Test

The postural test allowed for the evaluation of the effects of the 60-day HDT on the cardiovascular system as it operates around different working points. In the supine position, the upper part of the body contains a larger volume of fluids than in sitting position. The fluid shift from sitting to supine posture is expected to increase the volume of the large vessels and the filling of the heart chambers, stimulating volume receptors which induce an autonomic response. The descriptive statistics of hemodynamic variables and autonomic indices in **Table 2** showed higher SV and vagal index, and lower HR, sympatho/vagal balance indices, and TPR in supine compared to a sitting position. Our results indicate that in the recovery phase 10 days after bed rest, the cardiovascular deconditioning affects some variables more in one position than in the other. For instance, this is the case with HR (**Figure 4A**) because the HR *delta score* of the CTRL group significantly differed from zero in only the supine position. Such a finding points out that after HDT without countermeasures, the cardiovascular deconditioning affects HR more in supine than in sitting position. HR *delta scores* of TRAIN participants were indeed closer to zero in both positions and lower than those of the CTRL group in the supine position (**Figure 4A**). This finding confirms the efficacy of the administered training protocol as a countermeasure for cardiovascular deconditioning. In contrast to HR, SV showed the same significant reduction in the recovery phase, between −5 and −7 mL, independent of the posture in both groups, suggesting that the training protocol did not affect the loss of body fluids caused by HDT. Interestingly, the postural test was able to detect a significant reduction in TPR in the CTRL group (**Figure 4D**), but in the supine position only. The difference between the two postures was also highly significant in the CTRL group. It is possible that the fall in supine TPR induced by the 60-day HDT was responsible for the significant reduction in supine SBP observed at R+10 only in the CTRL group (**Figure 2E**)—a hypotensive effect also evidenced by the negative *delta score* of supine SBP (**Figure 4E**). The significant differences between supine and sitting *delta scores* of TPR, SBP, and DBP in CTRL participants further highlighted that long-term effects of head-down-tilt bed rest depended on posture. In this regard, the crucial point is that the sledge jump training protocol showed positive effects. No significant *delta scores* were observed in the TRAIN group for TPR and BP in the supine position, indicating the ability of the administered training protocol to accelerate recovery after HDT bed rest. Interestingly, in the TRAIN group the significantly positive *delta score* of TPR in sitting position (**Figure 4D**) indicates that exercise training might even have improved the capability to increase the total peripheral resistance in sitting position. An improved endothelial function induced by training (Ashor et al., 2015) could mediate such an effect. The autonomic indices (**Figure 5**) also shows the effects of HDT related to the posture. When sitting in the recovery phase, the CTRL group had lower sympathovagal activation and vagal withdrawal (**Table 2**). This is demonstrated statistically by significant negative *delta scores* of the LF/HF powers ratio and DFA1 and by a significant positive *delta score* of the HF power in sitting position (**Figure 5**). This phenomenon, suggests an impaired autonomic response to a postural shift after prolonged bed rest, a possible marker of orthostatic intolerance. By contrast, the TRAIN group had unchanged (i.e., null) *delta scores* for the indices of sympathovagal balance in both supine and sitting positions, whereas the index of vagal modulations of HR (HF power, **Figure 5A**) increased after bed rest in a similar manner for both postures. These findings therefore suggest that the proposed training protocol allowed for a faster recovery of the physiological autonomic responses to posture changes.

CONCLUSION

Considering that a supine position on Earth mimics acute cardiovascular effects of weightlessness, which induces a robust vagal activation immediately after the fluid shift to the upper body, we described the time course of long-term changes by comparing HDT to supine baseline recordings. Our data revealed different dynamics of cardiovascular adaptations. The acute autonomic changes induced by the supine position persisted throughout short-term HDT exposure and were strongly attenuated during mid-term and long-term HDT exposure, whereas SV adaptations showed an enduring trend. The administered training protocol appeared to mitigate some of the autonomic cardiovascular adaptations occurring during mid- or long-term HDT exposure and to accelerate their recovery after bed rest. The hypotensive phenomenon observed in the TRAIN group only during short-term HDT exposure, however, suggests that administering this exercise with initially light but progressively heavier loads during the first days of bed rest is an effective countermeasure.

AUTHOR CONTRIBUTIONS

MM, PC and AS wrote the manuscript and processed the data. AS designed and directed the project. KB helped supervise the project and performed data collections with support from AW and SM. PC and MM preprocessed data for statistical analyses. AS performed statistical analyses and prepared the figures and tables. PC, MM, GM, and AS drafted the manuscript. H-CG, LR, MS, and OO provided critical feedback and contributed to the interpretation of the results. All authors discussed the results and contributed to the final manuscript.

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The Advantage of Supine and Standing Heart Rate Variability Analysis to Assess Training Status and Performance in a Walking Ultramarathon

Martina Anna Maggioni[1,2*†], Lea Christiane Rundfeldt[1†], Hanns-Christian Gunga[1], Marc Joerres[1], Giampiero Merati[2,3†] and Mathias Steinach[1†]

[1] Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Institute of Physiology, Center for Space Medicine and Extreme Environments, Berlin, Germany, [2] Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy, [3] IRCCS Fondazione Don Carlo Gnocchi, Milan, Italy

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Jinlei Nie,
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***Correspondence:**
Martina Anna Maggioni
martina.maggioni@charite.de

† These authors have contributed equally to this work

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Cardiac autonomic modulation of heart rate, assessed by heart rate variability (HRV), is commonly used to monitor training status. HRV is usually measured in athletes after awakening in the morning in the supine position. Whether recording during standing reveals additional information compared to supine remains unclear. We aimed to evaluate the association between short-duration HRV, assessed both in the supine and standing position, and a low-intensity long-duration performance (walking ultramarathon), as well as training experience. Twenty-five competitors in a 100 km walking ultramarathon underwent pre-race supine (12 min) and standing (6 min) HR recordings, whereas performance and subjective training experience were assessed post-race. There were no significant differences in both supine and standing HRV between finishers ($n = 14$) and non-finishers ($n = 11$, mean distance 67 km). In finishers, a slower race velocity was significantly correlated with a higher decrease in parasympathetic drive during position change [larger decrease in High Frequency power normalized units (HF_{nu}: $r = -0.7$, $p = 0.01$) and higher increase in the detrended fluctuation analysis alpha 1 index (DFA1: $r = 0.6$, $p = 0.04$)]. Highly trained athletes accounted for higher HF_{nu} during standing compared to poorly trained competitors (+11.5, $p = 0.01$). Similarly, greater training volume (total km/week) would predict higher HF_{nu} during standing ($r = 0.5$, $p = 0.01$). HRV assessment in both supine and standing position may provide additional information on the dynamic adaptability of cardiac autonomic modulation to physiologic challenges and therefore be more valuable for performance prediction than a simple assessment of supine HRV. Self-reported training experience may reliably associate with parasympathetic drive, therefore indirectly predicting long-term aerobic performance in ultramarathon walking races.

Keywords: heart rate, heart rate variability, autonomic modulation, ultramarathon, exercise, low intensity

INTRODUCTION

Ultramarathon, defined by track lengths exceeding marathon distance (i.e., 42.195 km), has gained considerable interest over the past decades (Hoffman and Krishnan, 2013). Ultra-endurance races may account for distances up to 1,000 km and duration up to 25 days, as well as extreme environmental conditions (e.g., subarctic or desert climates, high mountains, etc.) (Rundfeldt et al., 2018; Tiller et al., 2019). Among these competitions, 24-h-long races present a particular physiologic strain, by combining prolonged exercise and sleep deprivation with possible unexpected course conditions and weather changes (Cottin et al., 2007).

Human physiologic responses to ultra-endurance exercise include neuromuscular fatigue (Millet, 2011), increased cardiac strain (Cote et al., 2015) and persistent shifts in cardiac autonomic modulation of heart rate (Gratze et al., 2005; Rundfeldt et al., 2018), the last being assessed by means of Heart Rate Variability (HRV) (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996; Taralov et al., 2015). Prolonged low aerobic workloads are generally correlated with an increase in resting total HRV and parasympathetic drive indices, whereas high and anaerobic workloads reduce these parameters (Aubert et al., 2003; Iellamo et al., 2004).

To date, the use of HRV for assessing the state of aerobic training and performance prediction has generated conflicting results (Buchheit, 2014). Indeed, HRV may change depending on training periodization. A "spot" evaluation of the HRV therefore has poor significance, if the current training status of the athlete and the time of the training period during which he is performing are unknown (Buchheit and Gindre, 2006; Buchheit, 2014). Furthermore, HRV indices may vary with the measurement conditions, like daytime (as HRV exhibits a typical circadian profile) (Shaffer et al., 2014), the subject recumbency during recording (supine, sitting, standing) (Fürholz et al., 2013), the RR series length (from minutes to 24 h) (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996), and the temporal proximity with training or competition, including possible influences like neuro-hormonal adaptation to stress (Hynynen et al., 2011) and pre-competitive anxiety (Morales et al., 2013), as well as the effect of tapering periods (Hug et al., 2014).

Currently, HRV assessment in athletes has been mostly performed in the morning upon awakening (Aubert et al., 2003). As 5-min recordings are considered the acceptable minimum, especially for spectral measurements (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996), 5- to 10-min long beat-to-beat HR recordings to assess HRV are usually employed. Among athletes, the supine position appears to be the most sensitive tool to monitor training status and performance (Aubert et al., 2003). However, the additional informative value of HRV recording during standing, compared to measurements exclusively in supine position, remains to be elucidated.

Indeed, the standing posture may add some valuable information (Schneider et al., 2019), as it provokes a light and persistent (order of minutes) shift in sympathovagal balance (Gonçalves et al., 2015). This autonomic shift may be influenced by training-related phenomena such as an overall vagal enhancement due to aerobic training, pre-competitive anxiety, or mere stress (Hynynen et al., 2011) and fatigue carry-over from preceding exercise sessions (Schäfer et al., 2015), as well as overtraining (Hynynen et al., 2008). Thus, autonomic adaptation to orthostatic position may be associated with both aerobic training status and performance prediction, and provide additional information beyond an exclusively supine recording, also regarding external circumstances and influences (Gratze et al., 2005; Hynynen et al., 2008, 2011; Grant et al., 2009).

Therefore, this study aimed to evaluate the relationships between short-duration HRV both assessed during supine rest (12 min) and standing (6 min), with performance and training status, in a group of amateur athletes participating in a 100 km walking ultramarathon, the "*Mammutmarsch.*" We hypothesized that the better trained and then more successful competitors would account for higher resting parasympathetic predominance pre-race. Furthermore, we assumed that the cardiac autonomic changes during standing may provide additional information on the association between HRV and performance, as well as training status, compared to recording exclusively during supine rest.

MATERIALS AND METHODS

The *Mammutmarsch*

The *Mammutmarsch* ultramarathon annually takes place near Berlin, Germany, at the end of May. With a total distance of 100 km, the course leads through the surroundings of Berlin mostly over paved streets, as well as forest trails or dirt tracks. The racetrack is mainly flat (maximal elevation gains $\leq$100 m), due to the specific geographical location of the city of Berlin. The time to complete the 100 km race is limited to 24 h, and it is mandatory for participants to perform the race by walking only, which is a feature that makes the *Mammutmarsch* considerably unique. Therefore, competitors undergo a very prolonged but low-intensity exercise. Regarding the starting time, all competitors are assigned to starting groups and thus enter the race between 3 and 5 p.m. The *Mammutmarsch* is an amateur ultramarathon, with a wide range of experience and training level among the competitors.

Study Design and Participants

This study was conducted as part of a larger investigation called *"Baseline characteristics, performance predictors and physiological changes in a 100 km walking ultramarathon."* A call among athletes competing in the *Mammutmarsch* in the years 2015, 2016, and 2018 was distributed through information material sent out by the race organizers as well as social networks. All competitors were eligible for study inclusion. Interested participants contacted the study team and, upon acceptance to partake in the study, were personally invited to the laboratory. All volunteers provided their written and informed consent to participate, and the study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Charité University of Medicine, Berlin, Germany (document number EA1/163/14).

A total of 56 competitors were enrolled in the larger investigation (2015: *n* = 20; 2016: *n* = 20; 2018: *n* = 16), and 25 of them were included also in our study (section "Statistical Analysis" and **Figure 1**).

Experimental Protocol

Study design is reported in **Figure 1**. Pre-race data collection was performed during the 7 days prior to the race, between 8 and 12 a.m., in a laboratory at the Charité Berlin. All participants had been advised to refrain from strenuous exercise, as well as consumption of coffee, alcohol, or other stimulants, during the 12 h before the measurement. All recordings took place in a quiet and comfortable room at constant ambient temperature (between 18 and 21°C).

After informed consent explanation and acceptance, anthropometric data were measured, then beat-to-beat HR was recorded for 12 min in supine (SUP), and, after verbal advice, for further 6 min in standing (STD) position (total duration of HR monitoring: at least 18 min). HR recordings were performed with a validated heart rate monitor (Polar Mod. RS800CX, Polar Electro Oy, Kempele, Finland) (Hernando et al., 2018). During HR recording, participants were requested to breathe normally and to avoid speaking and moving.

Moreover, on the day after the race, a questionnaire was sent to all competitors (to be returned within 30 days), to assess their individual performance in the *Mammutmarsch*, as well as training experience and subjectively rated training status (**Supplementary Figure S1** and section "Performance and Training Experience").

Outcome Measures

Heart Rate Variability

All RR interval series were first visually inspected by an experienced operator. Artifacts were removed and only recordings of at least 12 min during SUP and 6 min length during STD (eligibility cut-off $\leq$3 % artifacts), as well as recordings exhibiting sinus rhythm (verified by visual inspection of a preliminary 12-lead standard ECG), were deemed eligible for analysis. Using a commercial software (Kubios HRV vs. 2.1, Kuopio, Finland), validated indices of HRV were assessed, as markers of cardiac autonomic modulation of heart rate, in the time-, frequency-, and non-linear domain (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996). According to Tarvainen et al. (2014), the filter threshold was set at the "low" level. The correction was performed by replacing the identified artifacts with interpolated values using a cubic spline interpolation (Tarvainen et al., 2014).

To standardize the analysis, the last 10- and 5-min segments were selected from each eligible recording. In the time-domain, the root mean square of successive NN (normal-to-normal) differences (RMSSD) was computed, which represents vagal drive. As for frequency-domain, the spectral power of NN oscillation in the high frequency (HF: 0.15–0.40 Hz) band was assessed and reported also as normalized units (HF_{nu}) (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996). Like RMSSD, the HF power mainly depends on parasympathetic activity, being synchronous with respiratory sinus arrhythmia (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996). Additionally, the low frequency (LF: 0.04–0.15 Hz) band (which depends on both parasympathetic and sympathetic activity) was analyzed and similarly reported as normalized units (LF_{nu}) (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996). In the non-linear domain, assessment of the short-term (α1)

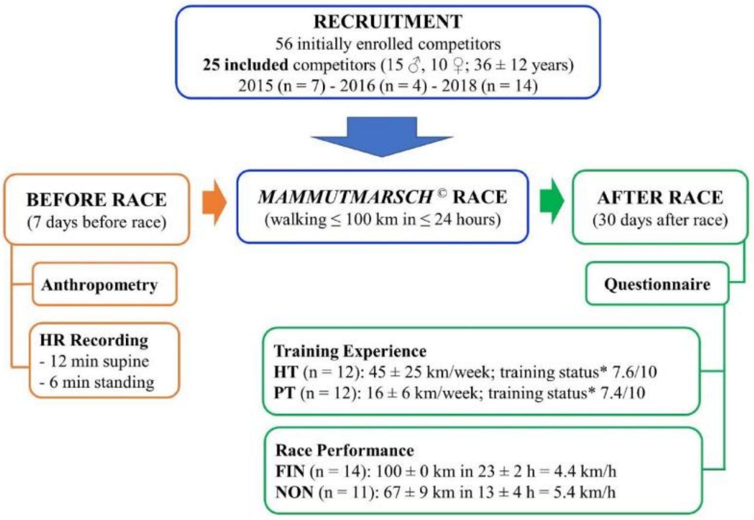

FIGURE 1 | Study design. Experimental procedures overview employed in all three *Mammutmarsch* competitions of 2015, 2016, and 2018 (m ± SD). *Self-rated training status on a 10-point Likert scale.

self-similarity coefficient of NN intervals was performed using detrended fluctuation analysis alpha 1 (DFA1) (Peng et al., 1995). The DFA1 indicates changes in the cardiac autonomic modulation, as it increases with a shift in sympathovagal balance toward increased sympathetic drive or lower vagal activity (Tulppo et al., 2001; Penttilä et al., 2003; Castiglioni et al., 2011). It should also be mentioned that DFA1 is not affected by respiratory frequency (Castiglioni et al., 2011) and may thus serve to confirm findings in time- and frequency-domain indices.

Performance and Training Experience

The race performance, as well as individual training experience, were assessed through a custom-made questionnaire (see **Supplementary Figure S1**). Participants were requested to state their total accomplished distance and time, as well as specific reasons for withdrawal before race completion, if applicable (see section "Performance" for details), and their subjectively rated greatest challenge during the *Mammutmarsch*.

Moreover, the questionnaire required participants to provide details about their training experience and fitness level in a standardized form, for example, by reporting the total distance and type of endurance training per week (mentioned here as km/week) in the 6 months prior to the competition. Only kilometers traveled by foot, i.e., walking or running, were considered for the analysis. This data was analyzed to characterize participants' training status: subjects were divided into two groups based on the median of the weekly training kilometers (i.e., 24.5 km/week) declared for the preceding 6 months. Accordingly, participants were either categorized as highly (HT: ≥24.5 km/week) or poorly trained (PT: <24.5 km/week).

The last section of the questionnaire required participants to subjectively rate their endurance training status at the time of the *Mammutmarsch* on a 10-point Likert scale, with 0 being "extremely poor" and 10 being "excellent."

Statistical Analysis

If not otherwise stated, all data are reported as mean ± standard deviation (m ± SD). In total, datasets from 25 competitors were eligible for our statistical analysis. The remaining 31 enrolled athletes were excluded from the analysis due to drop out, insufficient data quality, or loss to follow up (see **Figure 1**). A sample size of $n = 25$ was deemed appropriate, as research on HRV in ultra-endurance athletes usually accounts for 10–30 subjects (Gratze et al., 2005; Cottin et al., 2007; Foulds et al., 2014; Rundfeldt et al., 2018) and it must be considered that the *Mammutmarsch* as a walking ultramarathon presents a unique study environment.

The entire sample (ALL, $n = 25$) was divided into subgroups. The competitors who reached the 100 km finish line were categorized as "finishers" (FIN), whereas competitors who dropped out before race completion were categorized as "non-finishers" (NON).

Furthermore, standard median split technique was used to assign competitors into dichotomous variables (HT and PT) regarding training status, retrieved from the questionnaires.

The difference of HRV indices due to position change during HR recording (supine vs. standing) was calculated by subtracting per each index the value measured in supine from the value recorded in standing position. The calculated difference was then referred to as "delta" and represented by the correspondent Greek capital letter "Δ" before each HRV index label.

To test for normal distribution, the Shapiro-Wilk-Test was applied, whereas the Equal-Variance-Test served to test for variance. To achieve normal distribution, a log-transformation was administered to RMSSD values (RMSSD_{log}) (Castiglioni et al., 2011).

Differences between subgroups (e.g., FIN vs. NON) were analyzed using either an unpaired Student's *t*-test or, when normality check failed, a Mann-Whitney rank sum test.

The relationships between HRV and performance, HRV and training status, as well as between performance and training status were assessed by the analysis of simple linear regression, with the calculation of the Pearson-Product-Moment-Correlation coefficient (r) or, if normality was not passed, Spearman Correlation coefficient (r_s). Partial correlations controlling for the effect of sex and BMI were computed. All statistical analyses were performed using SigmaPlot 12.3 (Systat Software, San José, CA, United States), with the significance level set at $p < 0.05$.

RESULTS

Performance

A total of 25 athletes participating in the 2015 ($n = 7$), 2016 ($n = 4$), and 2018 ($n = 14$) *Mammutmarsch* competitions were included in the statistical analysis. Regarding ALL ($n = 25$), 14 athletes were able to complete the 100 km course (FIN) and 11 competitors withdrew during the race (NON), due to fatigue or musculoskeletal complaints. **Table 1** depicts demographic and anthropometric data pre-race. Race performance data is reported

TABLE 1 | Subject demographics.

	ALL (n = 25; 15 M; 10 W)	**FIN (n = 14; 10 M; 4 W)**	**NON (n = 11; 5 M; 6 W)**
Age, years			
Men	39 (13)	37 (14)	41 (11)
Women	31 (10)	31 (5)	31 (13)
All	36 (12)	35 (12)	36 (13)
Weight, kg			
Men	77 (7)	76 (9)	80 (1)
Women	62 (7)*	60 (6)	63 (8)
All	71 (10)	71 (11)	70 (10)
Height, cm			
Men	181 (7)	180 (9)	183 (3)
Women	170 (6)*	169 (7)	170 (6)
All	177 (9)	177 (10)	176 (8)
BMI, kg/m^2			
Men	23.4 (1.1)	3.2 (1.3)	23.7 (0.5)
Women	21.4 (1.2)*	21.1 (0.7)	21.6 (1.4)
All	22.6 (1.5)	22.6 (1.5)	22.6 (1.5)

*Subject demographics for all participants (ALL) and subgroups (FIN and NON). No significant differences between FIN and NON groups. *p < 0.05 between women (W) and men (M). [mean (SD)].*

TABLE 2 | Performance data.

	ALL	FIN	NON	*p*
Distance, km	85.4 (17.9)	100.0 (0.0)	66.8 (9.3)	< 0.001
Finish Time, h	18.6 (5.6)	22.7 (2.0)	13.4 (4.1)	< 0.001
Velocity, km/h	4.9 (1.2)	4.4 (0.4)	5.4 (1.7)	0.09

Performance data [mean (SD)] of all participants (ALL), as well as for subgroups (FIN and NON).

in **Table 2**. In FIN, we observed a slightly reduced velocity compared to NON ($p = 0.09$).

Heart Rate Variability

As expected, we found a depressed parasympathetic drive in STD vs. SUP for all participants, irrespective of sex, performance, and training level (**Figure 2** and **Supplementary Table S1**).

No significant differences between men and women were observed in both SUP and STD HRV values, although a tendency to higher vagal modulation was indicated by HF_{nu} values in female (SUP: $p = 0.07$; STD: $p = 0.06$ vs. male; **Supplementary Table S1**). Therefore, data were pooled regardless of sex for further analyses.

Figures 2A1–A3 show HRV indices in FIN and NON groups. No significant differences in FIN vs. NON for HRV parameters could be detected.

Interestingly, in FIN, a significant negative correlation between ΔHF_{nu} and velocity [velocity = 4.162 - (0.0108 × ΔHF_{nu}); $r = -0.65$, $p = 0.01$)] (i.e., the higher the decrease from SUP- to STD-HF_{nu}, the higher the velocity), as well as a significant positive correlation between ΔDFA1 [velocity = 4.132 + (0.652 × ΔDFA1); $r = 0.57$, $p = 0.04$)] and velocity (i.e., the higher the increase to STD-DFA1, the higher the velocity), were retrieved (**Figures 3A,B**). Conversely, none of the investigated HRV indices were significantly correlated with performance in NON. Furthermore, in FIN, there was a trend to a negative correlation between STD-$RMSSD_{log}$ and velocity [velocity = 5.919 - (1.017 × STD-RMSSD); $r = -0.48$, $p = 0.08$); **Figure 3C**]. **Supplementary Table S2** further depicts correlations between pre-race HRV and performance in FIN and NON groups. Similar results were obtained when computing partial correlations controlling for the effect of sex and BMI.

Training Experience

Training experience data was retrieved for 24 competitors, as one athlete was not available for post-race follow-up. Assessment of subjective records served to characterize the training volume (mean km/week during the past 6 months prior to competition), as well as self-rated endurance capacity, as "training status." In ALL, the mean distance walked or run per week amounted to 30 ± 23 km (ranging from 5 to 112.5 km/week). The subjectively perceived training status among competitors was rated at 7.5 ± 1.4 out of 10 (**Supplementary Table S3**).

Additionally, the relationship between training experience and pre-race HRV, as well as performance, was investigated in the entire sample. In ALL, a higher STD-HF_{nu} would significantly relate to greater km/week [STD-HF_{nu} = 15.750 + (0.0692 × km/week); $r_s = 0.50$, $p = 0.01$)] and a lower SUP-DFA1 was associated with higher self-rated endurance status (SUP-DFA1 = 1.616 - (0.0783 × training status); $r_s = -0.38$, $p = 0.07$)], although this did not attain significance (**Figure 4** and **Supplementary Table S4**). There were no significant correlations between training experience (both km/week and self-reported training status) and performance (velocity, total distance covered) in ALL.

As mentioned, split median analysis of reported training volume (km/week) served to assign competitors to highly (HT, $n = 12$) and poorly trained (PT, $n = 12$) subgroups. HT presented a significantly higher training volume (+29 km/week, $p < 0.001$). However, subjectively rated endurance capacity did not differ between groups (**Supplementary Table S3** for subgroup details).

Pre-race HRV values in HT and PT groups are depicted in **Figures 2B1–B3**. Greater parasympathetic drive in HT compared to PT was indicated by significantly higher STD-HF_{nu} (+12, $p = 0.01$), whereas STD-LF_{nu} was lower in HT vs. PT (-12, $p = 0.01$), in line with above-mentioned findings in ALL.

Furthermore, in HT, a higher DFA1 value in STD predicted a significantly greater velocity [velocity = 3.316 + (0.873 × STD-DFA1); $r_s = 0.57$, $p = 0.04$; **Figure 5**]. In PT, no significant correlations between HRV indices and performance could be observed. **Supplementary Table S5** depicts results of correlation analysis between pre-race HRV and performance in HT and PT groups.

DISCUSSION

This study was conducted to evaluate the relationship between short-term baseline HRV indices, and both performance and training experience, in a group of amateur athletes participating in a 100 km walking ultramarathon. Our findings emphasize the value of pre-race HRV assessment for performance prediction in ultra-endurance competitions. Moreover, regarding the use of HRV as a tool to monitor and predict performance, our results support the additional value of including an orthostatic challenge (supine-standing), compared to HRV assessment exclusively in supine position. We could not observe any sex-related differences in supine HRV. Current research in standard populations demonstrated women to account for greater parasympathetic drive compared to men (Koenig and Thayer, 2016). However, HRV may also depend on body composition (Esco et al., 2011; Koenig et al., 2014), physical activity (Rennie et al., 2003), and, in females, on the phase of the menstrual cycle (Schäfer et al., 2015), whose analysis is beyond the purpose of this study. Unfortunately, the proportion of female ultramarathoners is still smaller compared to male athletes, and a great number of studies investigated HRV parameters in male ultra-endurance athletes only, or accounted for very few female subjects (Gratze et al., 2005; Cottin et al., 2007; Ramos-Campo et al., 2019). Further studies with larger female samples are therefore warranted.

The hypothesis that successful competitors (FIN) would be characterized by higher parasympathetic activity in pre-race measurements compared to non-finishing athletes (NON) was not supported by the results. This can be due to several aspects, such as, for example, specific race characteristics. The *Mammutmarsch* must be performed by *walking* only and *running*

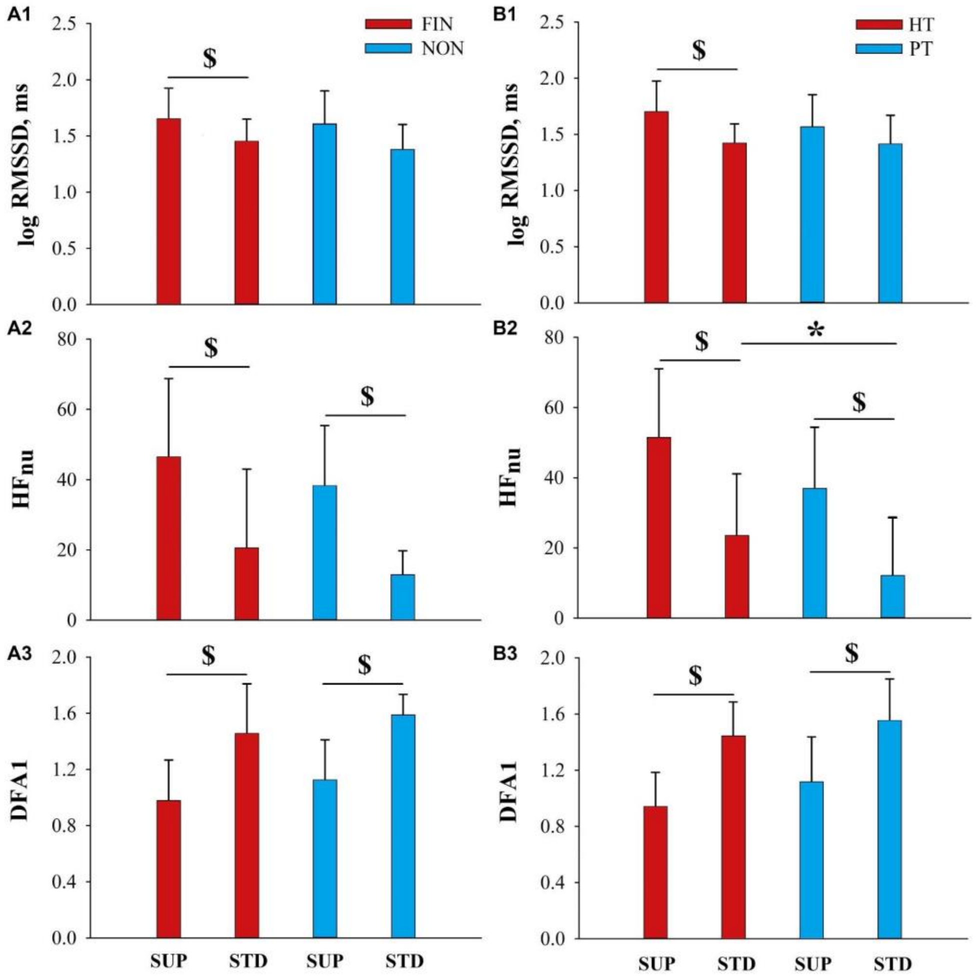

FIGURE 2 | HRV in FIN vs. NON, and HT vs. PT. HRV indices assessed before the race in FIN vs. NON **(A1–A3)**, and HT vs. PT **(B1–B3)**, respectively, in supine (SUP) and standing (STD) position. $ Indicates significant difference between SUP and STD ($p < 0.05$). * Indicates significant difference between HT and PT group for STD-HF_{nu} (HT +11.5, $p = 0.01$).

is not allowed. This creates a unique study environment and, to our knowledge, only one other study investigated cardiac autonomic modulation in a 24-h walking competition so far (Cottin et al., 2007). Also, in a walking amateur ultramarathon, extensive endurance training and preparation may not be required in order to compete. This is in contrast with other highly demanding ultramarathons, such as the Yukon Arctic Ultra, which is performed on a 690 km course in a subarctic climate (Rundfeldt et al., 2018).

Regarding performance, FIN accounted for a slower (by 1 km/h) velocity compared to NON. Even if this did not attain significance ($p = 0.09$), it may provide a valid argument to speculate about the characteristics of successful pacing, especially in this setting of a *walking ultramarathon*. Indeed, in ultramarathon, pacing may be essential (Knechtle et al., 2019). Improved performance has been associated with a reduction in initial speed (after reaching peak velocity, i.e., positive pacing) and lower variability in velocity (Bernard et al., 2009; Matta et al., 2019). Bossi et al. observed the fastest and most successful 24-h ultramarathon competitors to account for lower starting velocities, compared to less successful athletes (Bossi et al., 2017), this underlines our finding of lower overall velocity in FIN. Nevertheless, specific characteristics of pacing in a walking ultramarathon remain beyond the scope of this work and further studies are warranted.

To date, fewer investigations on the relationship between HRV and its change during orthostatic challenge, and ultra-endurance competition performance have been conducted (Bernardi et al., 1997; Gratze et al., 2005; Vescovi, 2019). Previous investigations reported the value and reliability of HRV assessment during standing in athletes (Boullosa et al., 2014). An orthostatic provocation maneuver may

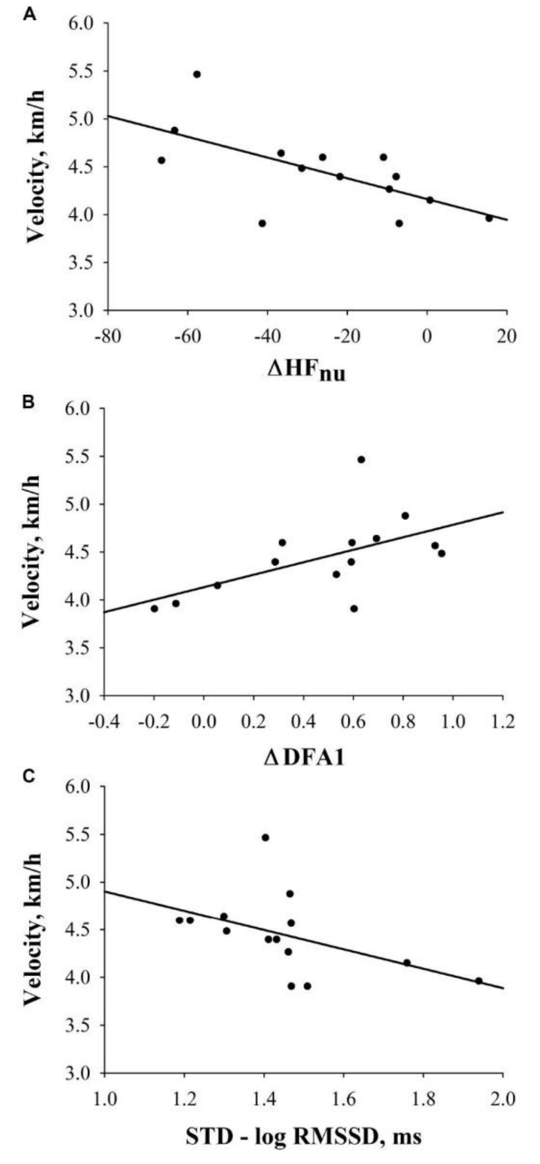

FIGURE 3 | Correlations between HRV indices and velocity in FIN. **(A)** Correlation between ΔHF_{nu} and velocity ($r = -0.7$; $p = 0.01$). **(B)** Correlation between ΔDFA1 and velocity ($r = 0.6$; $p = 0.04$). **(C)** Correlation between $STD\text{-}RMSSD_{log}$ and velocity ($r = -0.5$; $p = 0.08$).

reflect autonomic dynamic adaptation, with a shift in cardiac autonomic modulation toward increased sympathetic and decreased parasympathetic drive (Hynynen et al., 2011; Schäfer et al., 2015), as shown, respectively, by higher DFA1 (Tulppo et al., 2001) and decreased HF power (Gonçalves et al., 2015). Indeed, HRV may be described as "a psychophysiological measure of autonomic flexibility" (Williams et al., 2019), indirectly reflecting the ability to respond to various internal and external demands (An et al., 2020). Conversely, a reduced autonomic responsiveness to stressors, such as an excitatory stimulus during orthostatic challenge, has been proposed as the most common feature of pathophysiological conditions (Grant et al., 2012). Gratze et al. observed that, after ultra-endurance competition, the adaptability to orthostatism is dramatically reduced, as many athletes experience orthostatic intolerance (Gratze et al., 2005). Therefore, the ability of the autonomic and cardiovascular system to react to a marked and persistent reduction of blood pressure (BP), which occurred immediately after ultra-endurance performance (Gratze et al., 2005), is vital to limit the cardiac effort necessary to counteract the BP fall. Implementing an orthostatic challenge (during which BP is abruptly reduced) (Ueno and Moritani, 2003) may thus provide information on the autonomic ability to react to (and counteract) BP falls immediately after endurance exercise, which is currently considered one of the main reasons of cardiovascular fatigue in endurance athletes (Gratze et al., 2005). This may be mitigated by optimal training, as a more pronounced decrease in parasympathetic drive (and thus possible "preservation" of orthostatic tolerance) after standing up has been observed in women with higher training volumes compared with those with lower training volumes (Gilder and Ramsbottom, 2008). There is further evidence showing that the changes in cardiac autonomic modulation during orthostatic stress may better reflect the relationship between HRV (in terms of parasympathetic responsiveness) and physical capacity and activity (Ueno and Moritani, 2003; Grant et al., 2009; Gonçalves et al., 2015), as well as exercise volume (Gilder and Ramsbottom, 2008) and training load (or overtraining) (Hynynen et al., 2008), as orthostasis may sensitively depict exercise-induced changes in cardiac autonomic modulation in response to a stimulus (Grant et al., 2012; Schneider et al., 2019).

Only in FIN group, significant correlations between HRV and race performance were observed: a greater decrease in vagal drive upon position change would predict a greater velocity. Firstly, only the transition between supine and standing postures (i.e., Δvalues) could reflect the association between HRV and performance. Secondly, we obtained two similar significant regressions by examining two HRV indices from two different analysis domains: HF_{nu} is a spectral index (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996) and the DFA1 is a recursive index of signal complexity (Castiglioni et al., 2011). Therefore, these findings are unlikely to derive from a simply casual observation. Generally, aerobic training chronically increases vagal drive (Iellamo et al., 2004; Buchheit, 2014). However, in training periodization, parasympathetic drive may decrease pre-competition, while sympathovagal drive increases and predominates (Iellamo et al., 2002; Manzi et al., 2009; Hug et al., 2014). According to previous research, autonomic responsiveness (i.e., parasympathetic withdrawal reactive to orthostatic challenge, representing an essential marker of healthy autonomic reactivity) (Grant et al., 2012) may relate to both physical activity and training load (Grant et al., 2012) as well as physical capacity (e.g., VO_{2max}) (Gilder and Ramsbottom, 2008). In return, training volume (Knechtle et al., 2010) and physical capacity (Millet et al., 2011) have both been associated with

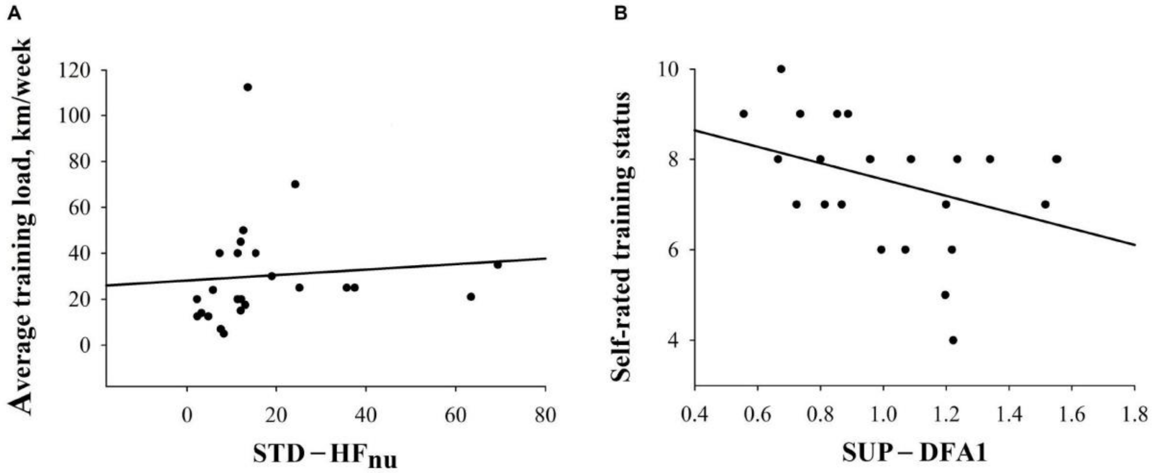

FIGURE 4 | Correlations between HRV indices and training experience in ALL. **(A)** Correlation between STD-HF$_{nu}$ and km/week ($r = 0.5$; $p = 0.01$). **(B)** Correlation between SUP-DFA1 and self-reported endurance training status ($r = -0.4$; $p = 0.07$).

greater endurance performance. It is likely that the capacity to override vagal predominance chronically acquired by long-term aerobic training may then facilitate race performance (Iellamo et al., 2002). This underlines our suggestion that FIN may account for a better dynamic ability (i.e., parasympathetic responsiveness) (Grant et al., 2012) to adapt cardiac autonomic modulation to a physiological challenge and therefore be more successful. Indeed, in NON, no correlations between HRV and performance could be observed. Although NON did account for similar resting HRV like FIN, they may not have been able to significantly overdrive parasympathetic predominance during orthostatic challenge, in order to allow successful prolonged cardiac performance. Still, the description of the physiological mechanisms underlying the observed phenomenon is beyond the scope of this paper and deserve further investigations.

Furthermore, the HT group showed greater vagal drive than competitors with lower training volume, the PT group (**Figure 2B3** and "Training Experience"), as confirmed by correlation analysis between HRV and training experience in the entire sample of athletes (ALL) (**Figure 4** and **Supplementary Table S4**). Specifically, we observed greater training volume and higher subjectively rated endurance capacity to be associated with higher indices of vagal modulation. As for subjective training status, we observed higher endurance capacity ratings in participants with lower DFA1 (i.e., greater vagal modulation) during supine rest, in line with previous findings (Ravé et al., 2020). Also, the observed positive correlations between greater vagal drive and higher training volume are in line with current findings of higher parasympathetic predominance and greater total HRV at rest in response to prolonged aerobic exercise (Hautala et al., 2001; Bellenger et al., 2016). Therefore, the subjective training experience assessment in amateur athletes through post-race questionnaires seems to be a valuable and feasible tool, as it may be difficult to monitor training over an extended time period in leisure-time ultramarathon competitors.

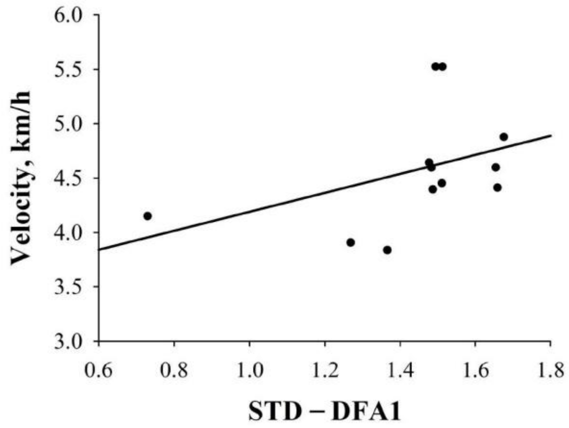

FIGURE 5 | Correlations between HRV indices and performance in HT. Correlation between STD-DFA1 and velocity (km/h) ($r = 0.6$; $p < 0.05$).

Limitations

Although we deemed the total sample size of $n = 25$ to be appropriate (see section "Statistical Analysis"), higher sample numbers are warranted. In addition, future studies should implement larger female sample proportions, as women are generally underrepresented in ultramarathon research (Gratze et al., 2005; Cottin et al., 2007; Ramos-Campo et al., 2019). As for HRV assessment methods, we did not record respiration rate or blood pressure. Both may influence HRV during supine and standing (Brown et al., 1993), while blood pressure may especially mediate HRV parameter changes in response to orthostatic challenge (Task Force of the European Society of Cardiology, and the North American Society of Pacing, and Electrophysiology, 1996; Ueno and Moritani, 2003).

Finally, we could not assess a possible influence of body composition on cardiac autonomic modulation (Esco et al., 2011; Koenig et al., 2014), as we did not measure body fat. Regarding training experience assessment, although post-race self-reported measures resulted as a valuable and feasible tool, controlled assessment of training experience may have generated more accurate data.

CONCLUSION

In trained and successful amateur ultramarathoners, higher pre-race vagal drive may be associated with better training experience and predict competition success. However, in poorly trained and unsuccessful competitors, supine HRV assessment may not serve for performance prediction. Additionally, HRV assessment throughout an orthostatic challenge may provide additional information on dynamic adaptability of the ANS to physiologic challenges and therefore be valuable for performance prediction. Self-reported training experience may be reliably associated with parasympathetic drive, therefore indirectly predicting performance.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin, Germany (document number EA1/163/14). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MM and LR contributed equally to the study by writing the manuscript and analyzing the data. GM contributed equally with MS by designing the study, structuring data results, and drafting the manuscript. MS planned and implemented the study with the help of MJ, LR, and MM. H-CG and GM provided critical expertise, feedback, and revised with all the co-authors the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.00731/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

CURRICULUM VITAE

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

LIST OF PUBLICATIONS

Lea C. Rundfeldt and Martina A. Maggioni, Robert H. Coker, Hanns-Christian Gunga, Alain Riveros-Rivera, Adriane Schalt and Mathias Steinach: **„Cardiac Autonomic Modulations and Psychological Correlates in the Yukon Arctic Ultra: The Longest and the Coldest Ultramarathon”**, *Frontiers in Physiology*, 2018; Impact Factor 4.134

Martina A. Maggioni, Paolo Castiglioni, Giampiero Merati, Katharina Brauns, Hanns-Christian Gunga, Stefan Mendt, Oliver S. Opatz, **Lea C. Rundfeldt**, Mathias Steinach, Anika Werner and Alexander C. Stahn: **„High-Intensity Exercise Mitigates Cardiovascular Deconditioning During Long-Duration Bed Rest”**, *Frontiers in Physiology*, 2018; Impact Factor 3.394

Lea C. Rundfeldt, Hanns-Christian Gunga and Mathias Steinach: **“Anabolic Signaling and Response in Sarcopenia as a Model for Microgravity Induced Muscle Deconditioning: A Systematic Review”**, *Reviews in Human Space Exploration*, 2019; Impact Factor not yet available

Martina A. Maggioni and **Lea C. Rundfeldt**, Hanns-Christian Gunga, Marc Joerres, Giampiero Merati and Mathias Steinach: **“The Advantage of Supine and Standing Heart Rate Variability Analysis to Assess Training Status and Performance in a Walking Ultramarathon”**, *Frontiers in Physiology*, 2020; Impact Factor 3.201

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