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Habilitationsschrift

Physiological Changes during an Ultramarathon in Extreme Cold: The Yukon Arctic Ultra, the Longest and Coldest Ultramarathon

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für das Fach **Physiologie**

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I. List of Main Abbreviations

BAT	Brown adipose tissue
CRP	C-reactive protein
D1	YAU checkpoint "During 1" at 277 km
D2	YAU checkpoint "During 2" at 383 km
ECG	Electrocardiography
FFM	Fat free mass
FGF	Fibroblast growth factor
FM	Fat mass
GX	Glycocalyx
HS	Heparan sulfate
HRV	Heart rate variability
HYA	Hyaluronan
MET	Metabolic equivalent
NPY	Neuropeptide Y
NT-Pro BNP	N-terminal prohormone of brain natriuretic peptide
POMS-SF	Profile of mood states, short form
POST	YAU checkpoint after the finish at 690 km
PRE	YAU checkpoint before the start
RPE	Ratings of perceived exertion
SYN	Syndecan CD-138
TBW	Total body water
TQR	Total quality of recovery
YAU	Montane® Yukon Arctic Ultra

1. Introduction

1.1 Study Background

While a great and growing number of people worldwide suffer from consequences of a sedentary lifestyle (1, 2), an increasing number of people engage in endurance exercise (3, 4) known to ameliorate and protect against these detrimental outcomes (5). Yet, the knowledge about physiological changes in athletes participating in very long endurance events is scarce. While up to tens of thousands of athletes compete in hundreds of classic marathons each year, only a few hundred do so in ultramarathons, i.e., over distances longer than 42.195 km. Nevertheless, the number of such athletes continually increased over the last decades (6) and furthermore, the number of athletes participating in extreme environments, such as cold or hot environments, and their achieved performances, are increasing as well (7, 8).

Several previous studies have shown tremendous physiological changes in participants of marathon and ultramarathon foot races, as in energy expenditure, body composition, vegetative control, cardiac markers, and markers of muscular strain; some of which were of pathological relevance (9-12). Yet, the performance levels during “short” ultramarathons may still be very high, e.g., at running speeds of up to 14 km/h over 80 km distance and of up to 13 km/h over 160 km distance (8). Current literature, however, lacks information regarding changes during ultramarathons of very long distances (i.e., several hundred kilometers) at very low intensity, i.e., walking instead of running. In addition, adverse conditions like very cold climate and sleep deprivation have not been investigated in such settings. Furthermore, the physiological processes linking functions like energy expenditure, metabolism, stress, and resilience, are still not well understood or lack investigation all together. The few existing studies, however, used different approaches, varied in distances and environments, investigated different parameters and are therefore hardly comparable, while it has been noted that studies undertaken in the filed cannot be easily replicated in a laboratory (13). In addition, many studies still recruited only male participants even though more women enter such extreme events (7).

As the number of ultramarathon foot races of very long distances – also in extreme climates – increases, as well as the number of athletes partaking in them, there is a growing need to investigate the physiological changes that take place among the athletes during such events. It has furthermore been surmised that investigating such events provide an excellent opportunity to increase our knowledge about human adaptability and resilience (14-16).

The Yukon Arctic Ultra (YAU) has been coined to be the longest and the coldest ultramarathon in the world, as it challenges athletes to complete the very long distance of 690 km under the extremely cold climate conditions of North-Canadian subarctic winter (17). The YAU athletes face the challenges of long-term endurance exercise in a very cold climate and under diminished resting conditions. Thusly, the YAU served as a model to investigate physiological changes among healthy athletes during an ultra-long endurance exercise in extremely cold climate. The approach was to conduct an integrative evaluation of various physiological parameters that are described further below and to develop this study into a broader investigation over several editions of this race in order to include a sufficient number of participants. Figure 1 depicts key features of the YAU and the conducted investigation.

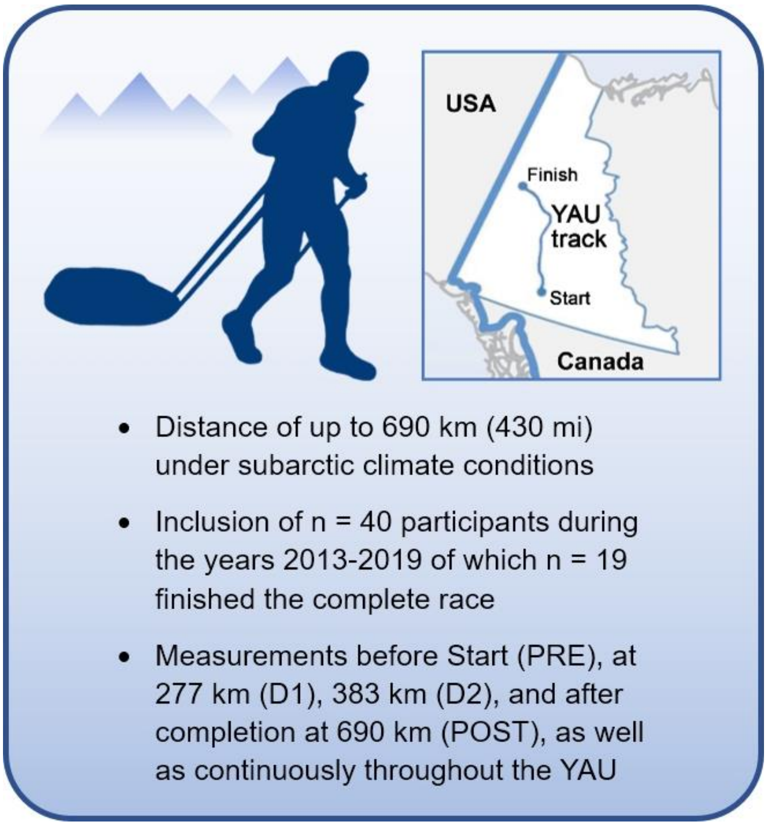

Figure 1: The YAU ultramarathon, taking place biennially over its longest distance in subarctic climate in the Yukon Territory (white map area) served as study model for the investigations during the years 2013-2019. (Graphic created by Mathias Steinach)

The YAU organizers were contacted regarding a possible collaboration in 2012 and the first feasibility study was performed in 2013. From that study arose a collaboration with the University of Alaska, Fairbanks that was strengthened and continued over the several YAU-studies. Other collaborations ensued as well, such as with the department of anesthesiology of the Ludwig-Maximilians-University Munich, and several doctoral students were able to write their doctoral theses within this topic, some of whom conducted their research on-site during the YAU editions of 2017, and 2019.

1.2 The Yukon Arctic Ultra

The Montane® Yukon Arctic Ultra is an ultramarathon-race, taking place in the Yukon Territory of North-Western Canada (17). It starts the first Sunday of each February and distances alternate biennially so that the longest distance of 690 km (430 mi) is conducted in every odd-numbered year. The YAU comprises of three race-categories in which athletes may enroll: a hiking footrace, cross-country skiing, and mountain biking. The duration of the YAU is limited to 14 days during which the race must be finished. The YAU trail is prepared by volunteers using snowmobiles making the surface even and relatively stable to hike upon and to pull sleds, which may weigh up to 20 kg containing the athletes gear, e.g., tent, sleeping bag, snowshoes, shovel, cooking utensils, clothing, food provisions, et cetera. Nevertheless, fresh snowfall or water overflow on the trail can lead to severe impairment of movement. To support the athletes' orientation, the trail is marked with poles about every 20 to 30 meters and athletes use GPS-devices and maps to navigate the trail. The trail-surface is mostly flat, however, for sections on the Yukon-river, piled-up ice floes may form cumbersome formations that are difficult to traverse. Furthermore, there are general gains in elevation of about 500 to 700 meters for the first and last sections of the YAU.

Each athlete carries a GPS-device (Spot®, Spot LLC, Virginia, USA), which allows being tracked by the race-organizers and athletes may use the device in cases of emergency to call for assistance. Nevertheless, athletes must partake in a mandatory training course regarding the possible hazards of the race and must demonstrate to the organizers their ability to handle the challenges presented by the environment, e.g., to use their equipment properly, to be able to make a fire in a certain time, demonstrate skills how to avoid hypothermia and frostbite, to manage minor injuries like blisters, et cetera. In addition, all athletes must present a certificate issued by their physicians confirming their health. Medical staff is on duty at ten checkpoints, which are about 50 km apart and either located indoors or within large tents, to administer medical support, and to screen for injuries like frostbite, in which case the concerning athlete would be disqualified from the race to ensure the athletes' safety and health.

Except for those few indoor resting possibilities, all athletes are required to conduct all activities, including preparation of meals, resting and sleeping, personal hygiene, et cetera, on the trail whilst being exposed to the environment of the Yukon's subarctic winter. The mean 24-hour temperatures in Whitehorse during the start of each YAU in 2013, 2015, 2017, and 2019 were: –17.0°C, –26.6°C, –21.1°C, and –30.5°C, respectively; the mean 24-hour temperatures in Dawson, each two weeks later at the finish were: –19.4°C, –12.6°C, –24.0°C, and –22.9°C, respectively, indicating comparable and very cold climates during each investigated year.

1.3 Study Implementation: Participants, Measurements, and Parameters

Participants

A total number of 117 endurance athletes enrolled in the 690 km YAU footrace during the investigated years 2013 (24), 2015 (19), 2017 (35), and 2019 (39) in order to compete in that category. From those 117 athletes, n = 40 (13 female and 27 male) volunteered to take part in the study (8 in 2013, 9 in 2015, 10 in 2017, and 13 in 2019), so that 34% of all 690 km YAU footrace competitors of the investigated years took part in the study. Most study participants (n = 39) were of Caucasian descent and one male athlete was of Asian origin.

From the 40 study participants, n = 19 (6 female and 13 male) were able to complete the entire distance of 690 km. In addition, a considerable number of participants reached the first measurement checkpoint after 277 km (n = 32) and the second after 383 (n = 20) and thusly yielded data and serum samples. In 2019, a control group of n = 7 (5 female, 2 male) was included in the investigation. That group consisted of the YAU's medical staff, race volunteers, and scientists during the YAU 2019, who were not conducting the race itself, but who were exposed considerably to the same environmental factors as the athletes (e.g., cold exposure, day-night cycle).

The recruitment for this investigation took place through the YAU organizers. A call for participants, with a description of all planned measurements and the overall study, was transmitted to the athletes enrolled in the 690 km footrace category as well as to the volunteers and medical staff for the control group in 2019. The organizers predominantly contacted experienced athletes, who had completed several prior ultramarathons and / or who had also completed the YAU before. Interested athletes then contacted the principal investigator through e-mail and received further detailed information regarding the investigation. The potential study participants had several weeks of time to pose any questions and to decide whether or not to take part in the study. All adult athletes enrolled in the YAU 690 km footrace category and adult volunteers in 2019 were eligible for the study; there existed no further inclusion or exclusion criteria. Four to seven days prior to the race-start, the potential study participants met with the investigators in Whitehorse, Yukon, Canada, and had the chance to personally discuss further questions and to finally give their informed written consent to take part in the study.

The study was approved by the Charité Ethics Board (IRB-number: EA4/109/12). All conducted procedures and measurements complied with the Declaration of Helsinki (7th revised version, 64th World Medical Association meeting, Fortaleza, Brazil) regarding the treatment of human subjects (18).

Measurements

During three to seven days preceding the race (PRE), baseline data were obtained in the city of Whitehorse, the capital of the Yukon Territory. The measurements comprised of weighing, determination of body composition through Bioimpedance analysis, collection of questionnaires, obtainment of ECGs, and collection of blood samples that were processed into serum samples and frozen for consecutive analysis. In addition, continuous measurements consisted of actigraphy throughout the YAU to evaluate the participants' energy expenditure. The conducted measurements during each edition of the YAU are explained in detail for each publication further below.

To evaluate the changes taking place whilst participating in the YAU, measurements and serum collections were conducted at two in-race checkpoints and at the finish. The checkpoints were chosen for accessibility by car, availability of running water and uninterrupted electrical power, being indoors with adequate space, as well as providing reasonable quietness and ambient temperature to perform all procedures under comparable and controlled conditions such as at during PRE. To this end, the first checkpoint during the race (During 1, D1) was located after 277 in the city of Carmacks, the second checkpoint (During 2, D2) was located after 383 km in the city of Pelly Crossing, and finally the post measurements (POST) were conducted after 690 km in Dawson City.

Only the above-mentioned locations met the criteria (e.g., in community centers) so that the first and final third stretch between measurements were rather long (between PRE and D1: 277 km; between D2 and POST: 307 km) while the second one was shorter (between D1 and D2: 106 km). Figure 2 illustrates the distances between the checkpoints.

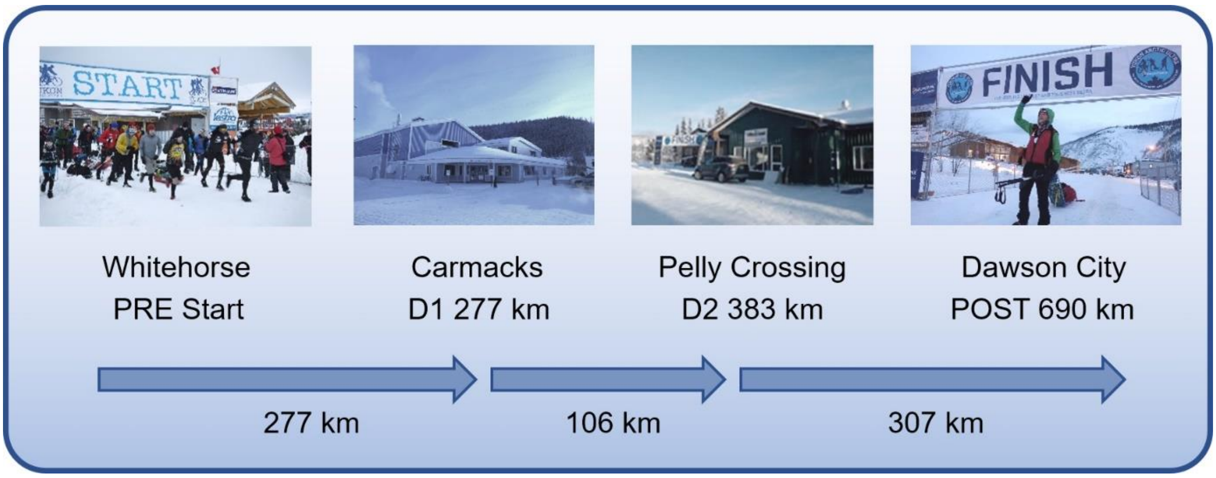

Figure 2: Depiction of the YAU start and finish line and of the study checkpoints at Carmacks and Pelly Crossing. (Photos taken by Mathias Steinach)

Parameters

The aim of the overall investigation was to attempt an integrative approach from the systemic to the molecular level into investigating physiological changes caused by ultralong endurance exercise at low intensities in an extreme environment. Key aspects were therefore the continuous measurements of energy expenditure through actigraphy (Sensewear®, Bodymedia, Pittsburgh, PA) (19) and the measurement of the athletes' body composition with the differentiation between fat mass (FM), fat free mass (FFM), and total body water (TBW), at the four checkpoints through bioimpedance analysis (BIA) (20). After the feasibility study in 2013, a collaboration with the University of Alaska (group of Prof. Coker, Institute of Arctic Biology) was formed that allowed analysis of serum samples so that various parameters of metabolism, stress, inflammation, and endothelial change could be evaluated during YAU 2015 to 2019.

In addition, further measurements were undertaken, such as of heart rate variability (HRV) through the use of miniaturized RR-sampling and electrocardiography (ECG) (Polar® Electro Oy, Kempele, Finland (21), and Faros®, Bittium, Oulo, Finland (22)), which allowed an estimation of both physiological and psychological aspects of human adaptation to stressors (23, 24). Furthermore, questionnaires were taken profile of mood states short form (POMS-SF), rating of perceived exertion (RPE), and of total quality of recovery (TQR) that allowed the evaluation of changes in mood, affect, and perceived exertion (25, 26) among the athletes during the YAU and which were evaluated in conjunction with other obtained parameters to form an integrative picture of the physiological changes that occur during the YAU. Figure 3 illustrates the interplay of the various measurements and evaluations of the investigation.

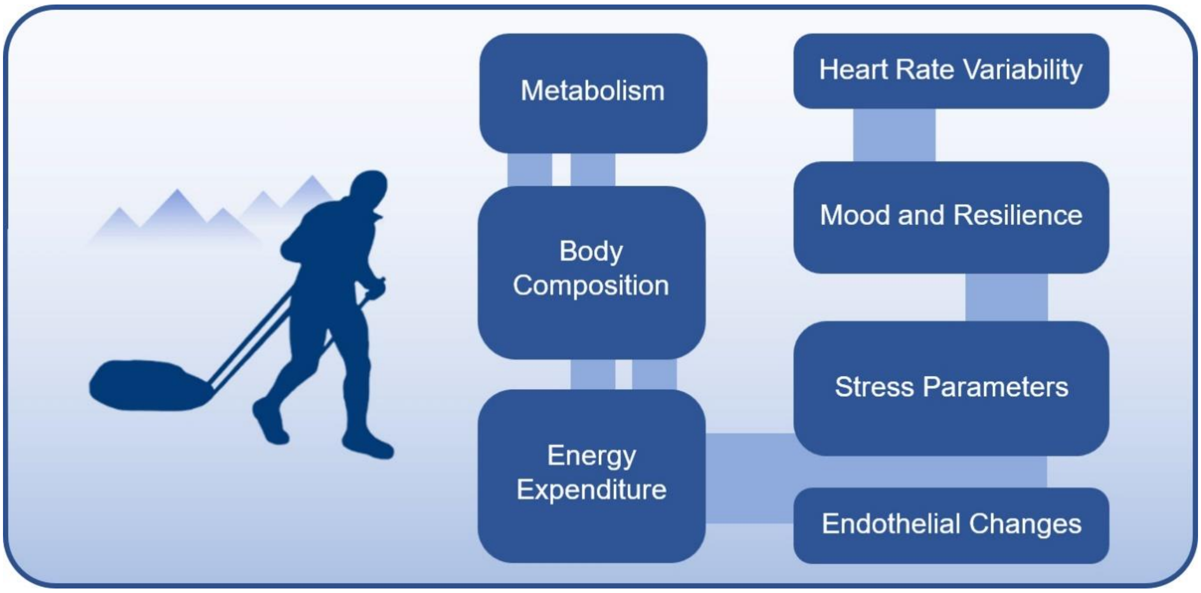

Figure 3: Study parameters and their integrated evaluation as combination of all published manuscripts where these parameters were analyzed. (Graphic created by Mathias Steinach)

2. Own Publications

The investigation was intended to allow integrated analyses of various physiological changes from the systemic to the molecular level among the YAU athletes. Naturally, a field investigation under circumstances as difficult as in subarctic northern Canada, must consider practical implications, such as the overall feasibility of the project, availability of measurement devices and their operational capability under the harsh field conditions, processing and transport of serum samples, the achievable number of successful participants per event, but also the availability and involvement of the various scientists and their working groups. It was therefore decided to start off with an evaluation of the impact on body composition, metabolism, and cold exposure-related myokines. Already, measurements were taken for the second evaluation regarding autonomous regulation, mood, and resilience. As more successful participants could be included, further evaluations were possible regarding energy balance and body composition, stress parameters and resilience, and finally a more specialized analysis of endothelial changes in context with stress parameters. Figure 4 depicts the time-course of the investigation and the main focus of each study block.

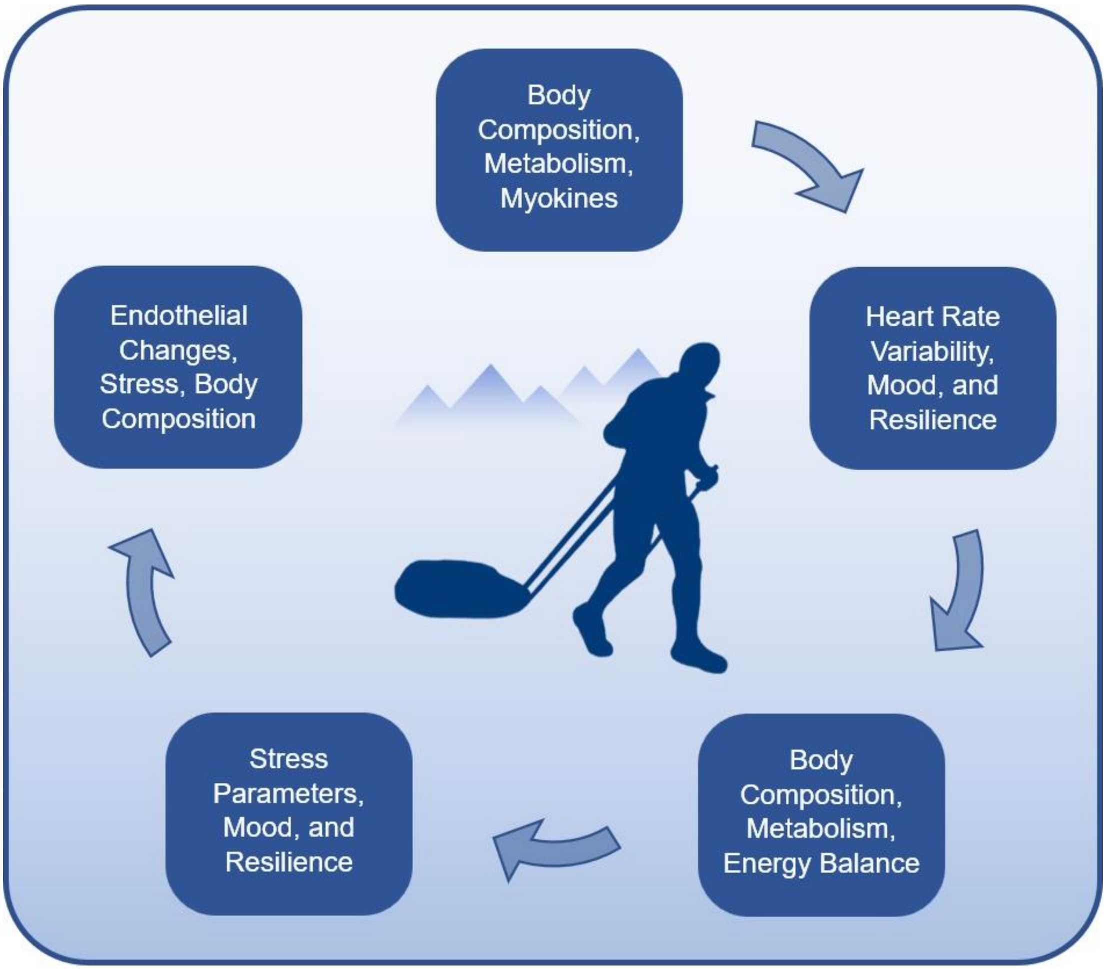

Figure 4: Chronological order of the integrated evaluation. (Graphic created by Mathias Steinach)

2.1 First paper: Body Composition and Metabolism

Coker, R.H., Weaver, A.N., Coker, M.S., Murphy, C.J., Gunga, H.C., **Steinach, M.**: *Metabolic Responses to the Yukon Arctic Ultra: Longest and Coldest in the World.* Med Sci Sport Exer., 2017

In this first paper we evaluated the changes in body composition along with several metabolic markers among the YAU study participants of 2015. Eight participants were included in this evaluation of which four reached the finish after 690 km. It was the focus of this work to assess the influence of the continuous cold exposure and ultralong low intensity endurance exercise of the YAU on myokines irisin and meteorin-like and on fibroblast-growth factor (FGF)-21 along with changes in fat free mass (FFM) and fat mass (FM). In addition, further metabolic markers such as creatinine, acetoacetate, acetate, valine, and isoleucine were also evaluated.

Cold exposure has been shown to promote an increase in serum irisin and meteorin-like as well as in FGF-21 (27-29), which in turn may change the profile of white-to-brown adipose tissue and lead to heightened non-shivering thermogenesis (27, 28, 30). Elevated activity of brown adipose tissue (BAT) can alter substrate metabolism (31) and may lead to a caloric deficit and changes in body mass and composition (32), which in combination with continuous endurance exercise might have implications on resilience and performance capacity of personnel working under such conditions (33, 34). It was therefore of interest to evaluate changes in these parameters under the field conditions of the YAU. Figure 5 depicts the evaluated parameters of this study in the context of the integrated evaluation.

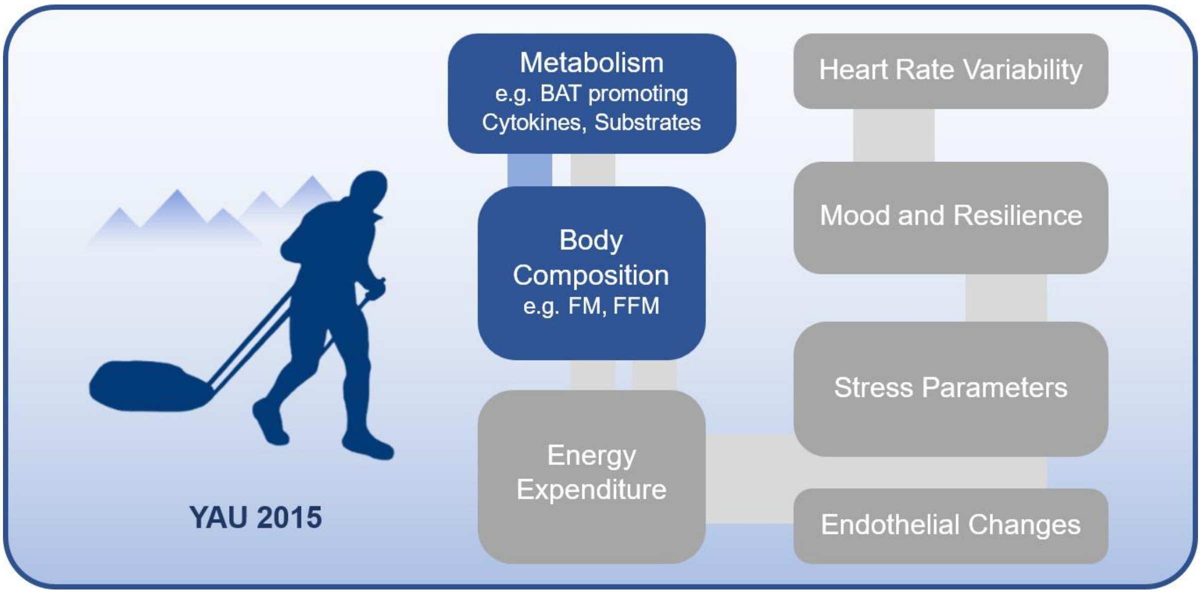

Figure 5: Evaluated parameters (dark blue boxes) in the first paper. (Graphic created by Mathias Steinach)

OPEN

Metabolic Responses to the Yukon Arctic Ultra: Longest and Coldest in the World

ROBERT H. COKER[1], ALYSSA N. WEAVER[1], MELYNDA S. COKER[1], CARL J. MURPHY[2], HANNS-CHRISTIAN GUNGA[3], and MATHIAS STEINACH[3]

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ABSTRACT

COKER, R. H., A. N. WEAVER, M. S. COKER, C. J. MURPHY, H.-C. GUNGA, and M. STEINACH. Metabolic Responses to the Yukon Arctic Ultra: Longest and Coldest in the World. *Med. Sci. Sports Exerc.*, Vol. 49, No. 2, pp. 357–362, 2017. **Purpose**: The Yukon Arctic Ultra is considered the longest and coldest ultraendurance event in the world. Cold exposure and exercise has been reported to influence circulating levels of myokines, adipokines, and hepatokines that may influence considerable alterations in the regulation of metabolism. The purpose of the study was to evaluate the influence of the Yukon Arctic Ultra (430-mile event) on potential activators of brown fat, metabolites, and body composition in healthy individuals. **Methods**: Eight male and female participants (mean ± SEM: age, 44 ± 3 yr; body mass index, 23.4 ± 0.9) were recruited for participation. Blood samples were collected at pre-event, mid-event, and post-event checkpoints. **Results**: The temperature during the event ranged from −45°C to −8°C. Because of these extremely challenging conditions, 50% of the participants withdrew from competition by the 300-mile mark, and those that surpassed 300 miles lost a significant (P = 0.002; P = 0.01) amount of body weight (76 ± 5 kg to 73 ± 4 kg) and fat mass (13 ± 1 kg to 12 ± 3 kg), respectively. With respect to serum irisin, there was a trend (P = 0.06) toward significance from pre-event (1033 ± 88 ng·mL^{-1}), mid-event (1265 ± 23 ng·mL^{-1}) to post-event (1289 ± 24 ng·mL^{-1}). Serum meteorin-like and fibroblast growth factor-21 remained stable throughout the event. There were no changes in creatinine, acetoacetate, acetate, and valine. Serum lactate decreased (P = 0.04) during the event. **Conclusions**: The influence of cold exposure and extreme physical exertion may promote substantial increases in serum irisin, and specific alterations in substrate metabolism that largely preserve skeletal muscle and physiological resilience. **Key Words:** COLD EXPOSURE, EXERCISE, METABOLISM, EXERTION

Long-term cold exposure promotes nonshivering thermogenesis (7). Recent studies have demonstrated the activation of brown adipose tissue due to cold stress in humans (6,24). Although the influence of nonshivering thermogenesis may have beneficial implications with respect to weight loss (31), the combination of chronic physical exertion and cold exposure could lead to sustained periods of negative caloric balance. Preservation of lean body mass may be stressed and diminish physiological resilience for military and/or emergency personnel who operate under such conditions (8,16,21,29). To understand the potential significance of these factors, physiological responses in a dynamic field setting must be directly measured under such arduous conditions (5,11,17).

Modest cold exposure (i.e., 12°C) and/or acute aerobic exercise have been reported to promote an increase in circulating irisin and meteorin-like (15,23,24). These myokines are primarily released from skeletal muscle but adipose tissue may also contribute to cold-induced and/or exercise-induced stimuli (15,23). The release of fibroblast growth factor (FGF)-21 from the liver under similar conditions may enhance mitochondrial metabolism (15). Collectively, irisin, meteorin-like, and FGF-21 may foster changes in white and/or brown fat that promotes elevations in the thermogenic profile (15,20,23).

Given the exciting data from preclinical studies that demonstrated a reversal of diet-induced obesity by increasing brown adipocyte-like cell abundance (22), the concomitant physiological challenge of cold stress and exercise may have even greater implications on the regulation of substrate metabolism (33). There is a paucity of field-derived data available in humans who have been exposed to extreme cold during continuous physical activity. Unlike well-controlled laboratory settings that do not include the combined stress

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of shelter requirements, nutrient provisions and logistical challenges, we choose to evaluate metabolic regulation during the Yukon Arctic Ultra (YAU). The YAU is considered the longest and coldest ultra in the world. Therefore, we hypothesized that participation in the 430-mile distance of the YAU would promote elevations in irisin, meteorin-like, and FGF-21 in conjunction with alterations in metabolic pathways as determined by metabolomics (19). Moreover, we anticipated that these alterations in metabolic regulation would have a significant influence on body composition, including the potential loss of skeletal muscle.

METHODS

Subjects. Eight healthy individuals (mean ± SEM; 44 ± 3 yr; body mass index [BMI] = 23.3 ± 0.9) participating in the 430-mile YAU were recruited for the study. The course for the YAU follows the first 430 miles of the noted Yukon Quest Trail that is considered to be the most difficult mushing event in the world. The race begins every year during the first week of February. Because of the adverse winter conditions caused by the time of year, the course itself and the overall length of the event, the YAU is consistently ranked as one of the most difficult events in the world. Athletes participating in the event for the first time are required to complete a training course that covers first aid, shelter (especially for snow, cold, and rewarming), gear discussions, nutritional recommendations, an overview of trail conditions, and is designed to prepare all athletes for the conditions of the event that require sufficient planning, proper provisions, and sufficient experience. All competitors must be self-reliant and either pull a sled (pulk) or attach necessary items to their bike. It should contain adequate food, water, clothing, cooking equipment, and emergency items. An adequate sleeping system is essential and typically contains a small tent or bivy sack along with an arctic sleeping bag and a mattress.

The event takes place in the Yukon Territory during the month of February. Temperature conditions range from daily lows of approximately −25°C to daily highs of −12°C. Combined with wind conditions of 5 to 25 mph, the risk of hypothermia and/or frostbite is extreme. Although it was beyond the scope of the present study to evaluate energy expenditure or total hours of activity, historical data from the event suggest an average speed of 2–3 mph and 2–6 h of sleep per night. Exposure to extreme conditions of cold stress is inevitable and continuous.

Four women and four men were initially recruited for the study. One woman and one man had dropped out by the 173-mile checkpoint. By the second checkpoint at 239 miles, two additional females had dropped out. Because of the harsh conditions, only 50% of the participants completed at least 300 miles of the event and only individual completed the entire distance. Blood samples under room temperature conditions at pre-event, checkpoint 1, checkpoint 2, and post-event (Fig. 1).

We provided comprehensive explanations of study details and implications, and the participants were given due time to clarify further questions and to express their desire to partake in the study. Each one of the participants provided their written informed consent. The study was approved by the local ethics committee at Charité Universitätsmedizin Berlin, and received an exemption from the University of Alaska Fairbanks Institutional Review Board. All procedures were conducted in accordance with the Declaration of Helsinki regarding human subjects.

Body weight and composition. We measured body mass, BMI, and body composition prerace, during-race, and postrace checkpoints. Anthropometric data of the study participants were gathered using standard equipment (medical scale and height meter; SECA, Germany) while participants were dressed with minimal clothing. We used an Akern BIA 101 (Florence, Italy) to measure fat mass, lean tissue mass, soft lean mass, and percent body fat using bioelectrical impedance analysis via the tetrapolar electrode method on all participants pre-event, at two checkpoints and post-event. Tests were conducted at indoor checkpoints under well-controlled and comfortable temperature conditions. Participants were instructed to lie supine for 10 min before the measurement, and the same physician performed the examination each time to maintain the consistency of the measurement. The BIA 101 measures resistance and a fixed constant sine current of 50 kHz for the determination of reactance in human tissue. The device and method has been clinically validated and provided a mobile platform of data collection during the YAU (12). The BIA 101 is a reference class instrument that has received certification in Europe (medical CE) and the United States (FDA) (27).

Collection and analysis of irisin, meteorin-like, and FGF-21. Serum samples were collected while participants

Participant Recruitment*	Pre-Event**	Checkpoint 1** (173 miles)	Checkpoint 2** (239 miles)	Post-Event**^ (300-430 miles)

*All individuals were briefed on the details of the study and informed consent was obtained.
**Serum samples were collected and body composition measurements were completed under ambient room temperature conditions.
^Only 50% of the participants completed at least 300 miles of the event and only individual completed the entire 430 mile distance.

FIGURE 1—Protocol schematic.

were seated in comfortable position by a physician at pre-event, checkpoint 1, checkpoint 2, and post-event locations under consistent room temperature conditions. These samples were analyzed using a solid-phase competitive enzyme-linked immunosorbent assay kit. The intra-assay %CV was <10% for irisin, <10% for meteorin-like and a sensitivity of <10 $pg \cdot mL^{-1}$ for FGF-21.

NMR analysis. Serum samples for the determination of creatinine, acetoacetate, acetate, valine, and isoleucine were collected pre-event, checkpoint 1, checkpoint 2, and post-event under room temperature conditions. These samples were introduced to deuterium oxide (D_2O, 99.8% Alfa Aesar, Ward Hill, MA) and transferred into 5-mm NMR tubes (Wilmad Lab Glass, Buena, NJ). ^{1}H-NMR spectra were acquired at 17°C (based on Methanol calibration) with a 600-MHz Bruker Avance-III system running TopSpin 3.2 software (Bruker Biospin, Fremont, CA) using a dual resonance high-resolution SmartProbe with single axis Z-gradient (18). The water signal was suppressed using NOESY presaturation followed by CPMG relaxation editing for suppression of macromolecules ("PROF_CPMG" parameter set in TopSpin 3.2). A standard, trimethylsilyl propionic-2,2,3,3-tetradeuteropropionic acid (TMSP) (3.87 mM in D_2O) contained in a sealed insert and placed in the NMR tube was used for metabolite quantification of fully relaxed ^{1}H-NMR spectra and as a ^{1}H chemical shift reference (0.0 ppm). The ^{1}H-NMR peaks for single metabolites were identified and referred to published chemical shift or a metabolite chemical shift library. After Fourier transformation, phasing, and baseline correction in TopSpin, each ^{1}H peak was integrated (2). The absolute concentration of each metabolite was then referred to the TMSP integral and calculated according to the equation adapted from Serkova et al (28): $C_x = (I_x{:}N_x \times C)/I{:}9$, where C_x is metabolite concentration ($\mu mol \cdot mL^{-1}$), I_x is integral of metabolite ^{1}H peak, N_x is number of protons in metabolite ^{1}H peak, C is TMSP concentration, and I is integral of TMSP ^{1}H peak at 0 ppm (this is nine as TMSP contains nine protons). An additional correction factor of 10.041 was applied to adjust for the differences in diameters between the NMR tube and the insert (experimental determined from reference samples) (28). The final metabolite concentrations were expressed as millimolar per liter.

Statistical comparisons over time were made using one-way ANOVA, and Bonferroni *post hoc* tests were applied to significant group–time in interactions. Data are reported as means ± SEM.

RESULTS

Subjects. Eight individuals (four women and four men) who were participating in the 430-mile distance of the YAU were recruited and enrolled in the study. Due to the especially challenging environmental conditions during the first 100 miles of the event (ie., −45°C), four of the participants had dropped out shortly before or after the midpoint of the event. Only one study-participant completed the entire 430-mile distance. The only remaining woman withdrew at the 300-mile mark, skipped 30 miles then reentered and eventually completely withdrew at the 400-mile mark. The third and fourth participants withdrew at 300 and 320 miles, respectively. We report post-event data as those individuals who completed at least the 300-mile distance. Finish times for all participants in the event ranged from 174 to 298 h. Body composition and blood samples were acquired immediately at the terminus of their effort under room temperature conditions.

Body weight. Pre-event body weight and BMI was 68.1 ± 3.8 kg and 23.4 ± 0.9 $kg \cdot m^{-2}$, respectively, for all eight individuals at the start of the event. The individuals with a lower pre-event body weight ($P = 0.03$) dropped out before midpoint (Table 1). In the individuals who completed at least 300 miles of the event, there was a significant decrease in overall body weight ($P = 0.002$) (Table 1). Surprisingly, the individuals (three men, one woman) that completed at least 300 miles retained almost all of their lean body mass but lost almost 2 kg of fat mass as derived from bioelectrical impedance (Table 1).

Irisin, meteorin-like, and FGF-21. Serum irisin was 1033 ± 88 $ng \cdot mL^{-1}$ at pre-event and rose ($P = 0.06$) up to 1289 ± 24 $ng \cdot mL^{-1}$ by the end of the event (Fig. 2). Serum meteorin-like remained stable 7.7 ± 0.1 $ng \cdot mL^{-1}$ (pre-event) to 7.4 ± 0.3 $ng \cdot mL^{-1}$ (post-event) (Fig. 3). Despite an absolute increase in FGF-21 due to a marked elevation in one participant (i.e., $\Delta + 110$ $pg \cdot mL^{-1}$), there was no change from pre-event (46 ± 4 $pg \cdot mL^{-1}$) to post-event (79 ± 36 $pg \cdot mL^{-1}$) (Fig. 4).

Metabolites. Serum lactate was within normal values at pre-event and decreased ($P = 0.04$) at post-event (Table 2). Serum creatinine, acetoacetate, acetate, valine, and isoleucine were within normal values at pre-event and remained stable at post-event (Table 2).

DISCUSSION

We studied the influence of chronic exposure to extreme cold and physical activity during the YAU on serum adipokines, hepatokines and myokines, and alterations in metabolic pathways using a metabolomic approach. It was our assertion that if preclinical studies or those performed

TABLE 1. Clinical characteristics.

	Pre-Event	Checkpoint 1	Checkpoint 2	Post-Event
Age, yr	44 ± 3 (38 ± 4)	41 ± 3	38 ± 4	38 ± 4
Sex	4 M, 4 F (3 M, 1 F)	3 M, 3 F (3 M, 1 F)	3 M, 1 F	3 M, 1 F
Height (cm)	171 ± 3 (170 ± 3)	152 ± 12 (170 ± 3)	170 ± 3	170 ± 3
Weight (kg)	68 ± 4 (76 ± 5*)	69 ± 6 (76 ± 5*)	74 ± 3	73 ± 4**
BMI ($kg \cdot m^{-2}$)	23 ± 1 (25 ± 1)	23 ± 1 (25 ± 1)	25 ± 1	25 ± 1
Fat-free mass (kg)	55 ± 4 (62 ± 2)	57 ± 5 (63 ± 5)	61 ± 2	61 ± 3
Fat mass (kg)	13 ± 1 (14 ± 2)	12 ± 1 (13 ± 2)	13 ± 1	12 ± 3***

Data presented without parentheses represent to mean ± SEM of all individuals in the event at that time. Parentheses provided in the first two columns represent the mean ± SEM for those individuals who finished at least 300 miles of the YAU.
*Significant difference between individuals who completed the event versus those who did not ($P = 0.03$).
**Significant decrease from pre-event to post-event ($P = 0.007$).
***Significant decrease from pre-event to post-event ($P = 0.03$).
F, female; M, male.

APPLIED SCIENCES

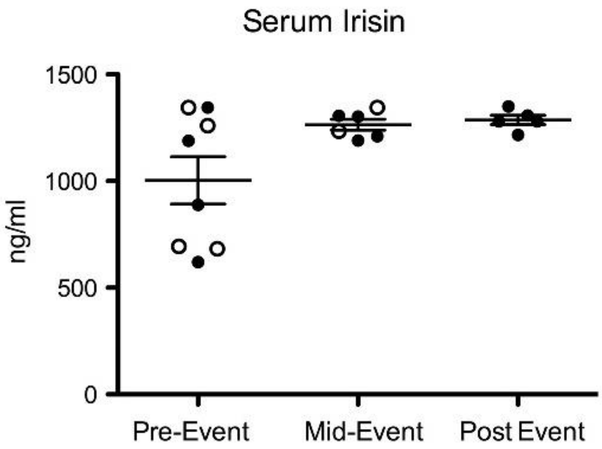

FIGURE 2—Serum irisin concentrations during the pre-event, mid-event, and post-event checkpoints. Data presented reflect the number of individuals participating in the event at that time point. Closed circles reflect data from participants that completed at least 300 miles. (*open circles*) Data from those individuals who dropped out before the 300-mile mark. Data are presented as means ± SEM.

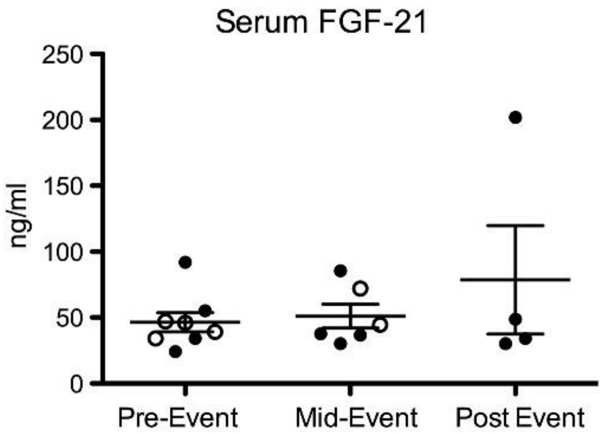

FIGURE 4—Serum FGF-21 concentrations during the pre-event, mid-event, and post-event checkpoints. Data presented reflect the number of individuals participating in the event at that time point. (*closed circles*) Data from participants that completed at least 300 miles. (*open circles*) Data from those individuals who dropped out before the 300-mile mark. Data are presented as means ± SEM.

under modest laboratory conditions supported the role of cold exposure and/or exercise on these factors, the extreme stress (i.e., physiological, temperature, and mental) of the YAU should provide truly remarkable data. The results of the present study have demonstrated that although serum meteorin-like and FGF-21 did not increase during the YAU, irisin was significantly elevated before the event and tended to rise toward the end of the event. Lactate levels decreased significantly by the end of the event.

Body weight was reduced during the event, and the pre-event body weight of the participants who completed the majority of the event was significantly greater than those who dropped out before the midpoint of the event. Although the only man that did not complete at least 300 miles of the YAU had the lowest body weight for a man, the only woman that completed at least 300 miles of the YAU had a relatively average weight for a woman in this study. Even so, these data suggest that body weight may play a role in the successful completion of the YAU that combines extreme cold, isolation, and sustained physical activity.

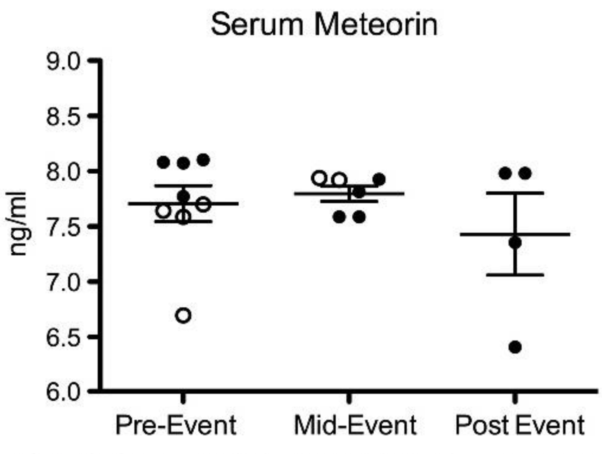

FIGURE 3—Serum meteorin-like concentrations during the pre-event, mid-event, and post-event checkpoints. Data presented reflect the number of individuals participating in the event at that time point. (*closed circles*) Data from participants that completed at least 300 miles. (*open circles*) Data from those individuals who dropped out before the 300-mile mark. Data are presented as means ± SEM.

The conditions of the YAU promoted definitive elevations in pre-event serum irisin that modestly increased throughout the event. In other clinical studies, irisin levels were modestly increased (i.e., 1.2-fold) with aerobic and resistance exercise training in overweight, untrained individuals but still did not reach the high levels found in YAU athletes (20). In the original manuscript that described the influence of exercise on irisin levels in humans, a 10-wk exercise training program promoted substantial increases in irisin that were linked to improvements in oxygen consumption, decreased body weight and improved glucose tolerance in pre-clinical models (3). Remarkably, irisin levels were already much higher than those reported in these previous studies but there are some important differences (15), including wide variations in fitness levels, length of training, acclimatization to cold and extreme cold. Notably, most athletes that participate in the YAU arrive in Whitehorse, YT, at least 1 wk before the start of the event. The demands of travel from countries ranging from Austria, Brazil, Czech Republic, Denmark, England, Germany, Italy, Scotland, South Africa,

TABLE 2. Serum metabolites.

	Pre-Event	Checkpoint 1	Post-Event
Tyrosine (μmol·L^{-1})	22 ± 1	29 ± 2	21 ± 1
Creatinine (μmol·L^{-1})	36 ± 2	34 ± 3	37 ± 3
Acetoacetate (μmol·L^{-1})	51 ± 6	121 ± 40	57 ± 9
Acetate (μmol·L^{-1})	71 ± 4	73 ± 34	40 ± 7
Valine (μmol·L^{-1})	130 ± 10	270 ± 120	290 ± 120
Isoleucine (μmol·L^{-1})	48 ± 3	111 ± 46	49 ± 3
Lactate (mmol·L^{-1})	1.5 ± 0.2	1.2 ± 0.1	0.8 ± 0.1*

Data are presented as means ± SEM. Four men and four women were included in the pre-event data, three males and three females were included in checkpoint 1 data, and three men and one woman were included in post-event data.
*Significant difference pre-event and post-event (P = 0.02).

Switzerland, and Taiwan to the far reaches of the Yukon Territory in the middle of winter are significant. The dynamic and extreme nature of the event and the physiological condition of the athletes may be responsible for the marked pre-event elevations in irisin. All athletes must complete a pre-event training session that is necessary to "prove" their ability to perform tasks that are necessary for survival under the frigid conditions, and this requirement may have influenced pre-event data.

Although the sustained elevations in circulating irisin were remarkable in the YAU athletes, it is unclear why serum meteorin-like and FGF-21 were not influenced by the physiological conditioning of the athletes and/or the extreme nature of the event. To our knowledge, the studies by Rao et al. (23) provide the only data on changes in meteorin-like elicited by exercise in humans. Baseline meteorin-like levels were also twofold higher than the levels reported in our current study and increased with a combination of aerobic and resistance exercise training (23). It has been delineated that the PGC-1α4 gene responsible for the secretion of meteorin-like is largely influenced by resistance exercise and that was not a major part of the physical activity in the YAU. Even though FGF-21 has been posited as a key element that induces a thermogenic response in brown adipose tissue in response to cold exposure, our data did not provide a consistent response and more work is needed to understand the regulation of FGF-21 in response to cold exposure. Unfortunately, little if any data exists on the effects of cold exposure on meteorin-like and FGF-21 levels, making the interpretation of these data even more challenging.

Previous studies have recently noted a significant alteration in gene expression specific to glucose uptake, glycolysis, and glycogen metabolism in the brown adipose tissue of rodents expose to a cold environment for 2–4 d (13). Recent preclinical studies have demonstrated the importance of cold-induced glucose uptake and how mammalian rapamycin complex 2 is activated in brown adipocytes. Although we did not study substrate kinetics *per se*, our data that demonstrate stable concentrations of other metabolites that remained within normal limits throughout the event demonstrate a general lack of overall ketosis (32). Normal levels of acetoacetate also demonstrate that there were no limitations with regard to carbohydrate intake or the athletes' ability to metabolize carbohydrates. The reduction in lactate levels may indicate a potential increase in gluconeogenic demand but we did not assess changes in substrate kinetics during this study. From data collected during the 2013 YAU, energy intake likely supplied less than 50% of the overall energy demand (12).

Although we were able to determine an impressive level of metabolic resilience under challenging conditions with regard to physical exertion and cold exposure, our results with respect to the retention of skeletal muscle under these conditions were even more surprising. These values derived from bioelectrical impedance methodology were consistent with no change in serum creatinine that can represent alterations in skeletal muscle (1). Because of the preexisting elevations in serum irisin that persisted during the entire event, we would have anticipated increased "browning" of adipose tissue, increased uncoupled ATP synthesis in conjunction with exercise-induced caloric expenditure (10). Even though we have reported the preservation of skeletal muscle during exercise-induced weight loss in middle-age overweight individuals with low fitness levels (4), we were not sure if skeletal muscle would be sufficiently resilient under the demands of extreme cold and prolonged exercise. The Tors de Géants, physical exertion (i.e., 322 km with a total positive and negative elevation change of ~24,000 m) is considered by many to be the longest and hardest "Mountain Ultra Marathon" and is relatively similar in terms of overall physical exertion. The Tors de Géants has a shorter overall distance (~230 miles shorter), greater changes in elevation (24,000 m in positive and negative elevation) that ultimately results in similar rates of attrition of around 50%–60%. Unlike the YAU, there are 43 "refreshment stations" where participants can eat and sleep, and seven "life bases" where they can obtain medical care in the Tors de Géants. Finish times range from a minimum of 80 h to a maximum of 150 h (roughly 25%–50% of the duration encountered in the YAU). Recent investigations conducted on athletes finishing the Tors de Géants have reported up to almost 100% muscle preservation (25). However, the temperature conditions of the Tors de Géants are quite comfortable with relatively no heat or cold stress and multiple aid stations compared with the extreme cold and isolated conditions of the YAU. Other ultramarathons with shorter distances completed without concomitant environmental stress have reported muscle preservation as well (9,14,30). Even with a limited number of participants, our data provide the first report of almost complete muscle preservation under the extreme conditions of prolonged cold exposure experienced in the YAU.

In conclusion, serum irisin, meteorin-like, and FGF-21 did not respond in a similar fashion to the conditions of the YAU. Irisin was elevated before the event, increased somewhat, and remained high in the individuals that finished the event. Lactate levels were reduced and potentially implicated the importance of gluconeogenesis in sustaining physical exertion under the additional stress of extreme cold. Even under extreme conditions of physiological, temperature, and mental stress promoted by participation in the YAU, a tremendous degree of resilience was exhibited by the athletes, including the preservation of lean body mass. Future studies are planned to obtain precise measurements of energy expenditure, sleep quality, and dietary intake along with the molecular components of protein metabolism that may be potentially responsible for the relative preservation of lean body mass in these athletes competing at all distances (26, 100, 300, and 430 miles) of the YAU.

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2.2 Second paper: Heart Rate Variability and Mood Changes

Rundfeldt, L.C., Maggioni, M.A., Coker, R.H., Gunga, H.C., Riveros-Rivera, A., Schalt, A., **Steinach, M.**: *Cardiac Autonomic Modulations and Psychological Correlates in the Yukon Arctic Ultra: The Longest and the Coldest Ultramarathon*. Front Physiol., 2018

In this second paper we evaluated the changes in heart rate variability as well as in psychometric measures among the YAU study participants of 2013, 2015, and 2017. 16 participants were included in this evaluation of which ten reached the finish after 690 km. It was the focus of this work to assess the influence the YAU on vegetative control expressed through changes in heart rate variability (HRV) in conjunction with changes in mood and perceived exertion.

It has been shown that endurance training effects and performance level can be evaluated through HRV assessment (35, 36), leading to an increased HRV and predominance of the parasympathetic drive at rest (37), while periods immediately after exercise were shown to be associated with decreased HRV and parasympathetic withdrawal (38, 39), which has also been described for ultramarathon athletes (9). On the other hand, ultra-endurance exertion has been associated with mental fatigue and disturbances in mood (40, 41). It was therefore of interest to evaluate changes in these parameters through measurements of HRV, e.g., root mean square of successive RR differences (RMSSD), low frequency to high frequency-ratio (LF/HF-ratio), along with psychometric measures using questionnaires, e.g., profile of mood states short form (POMS-SF), total quality of recovery (TQR) under the field conditions of the YAU, especially since mood changes may reflect in changes of HRV (42). Figure 6 depicts the evaluated parameters of this study in the context of the integrated evaluation.

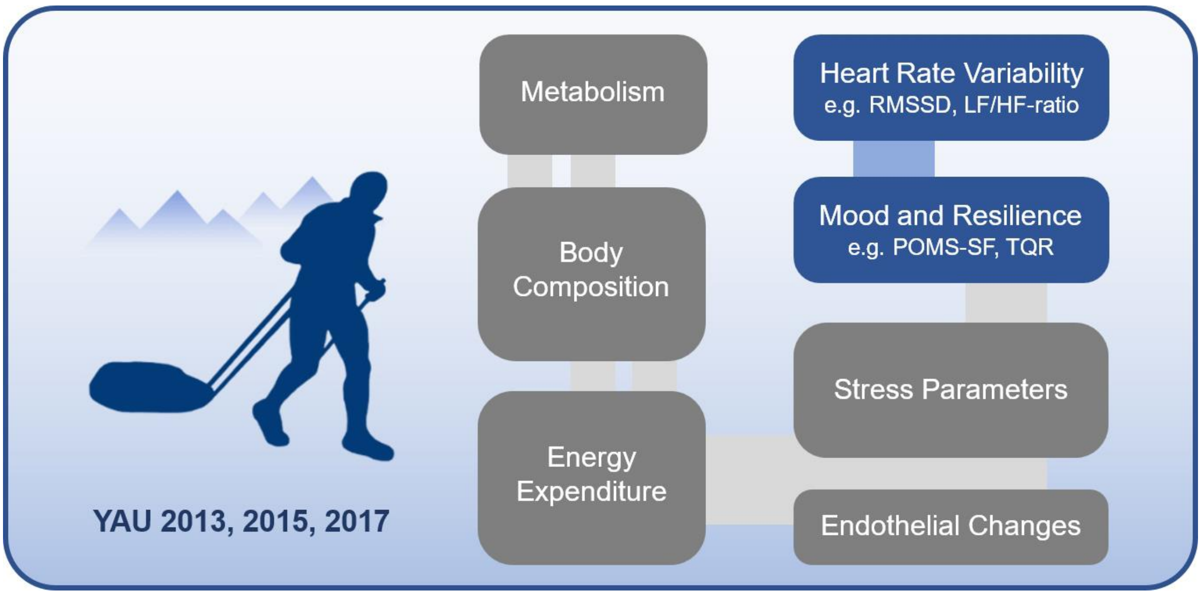

Figure 6: Evaluated parameters (dark blue boxes) in the second paper. (Graphic created by Mathias Steinach)

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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

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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**.

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.

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.

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

[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).

[2]http://climate.weather.gc.ca/historical_data/search_historic_data_e.html.
[3]http://arcticultra.de/en.

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

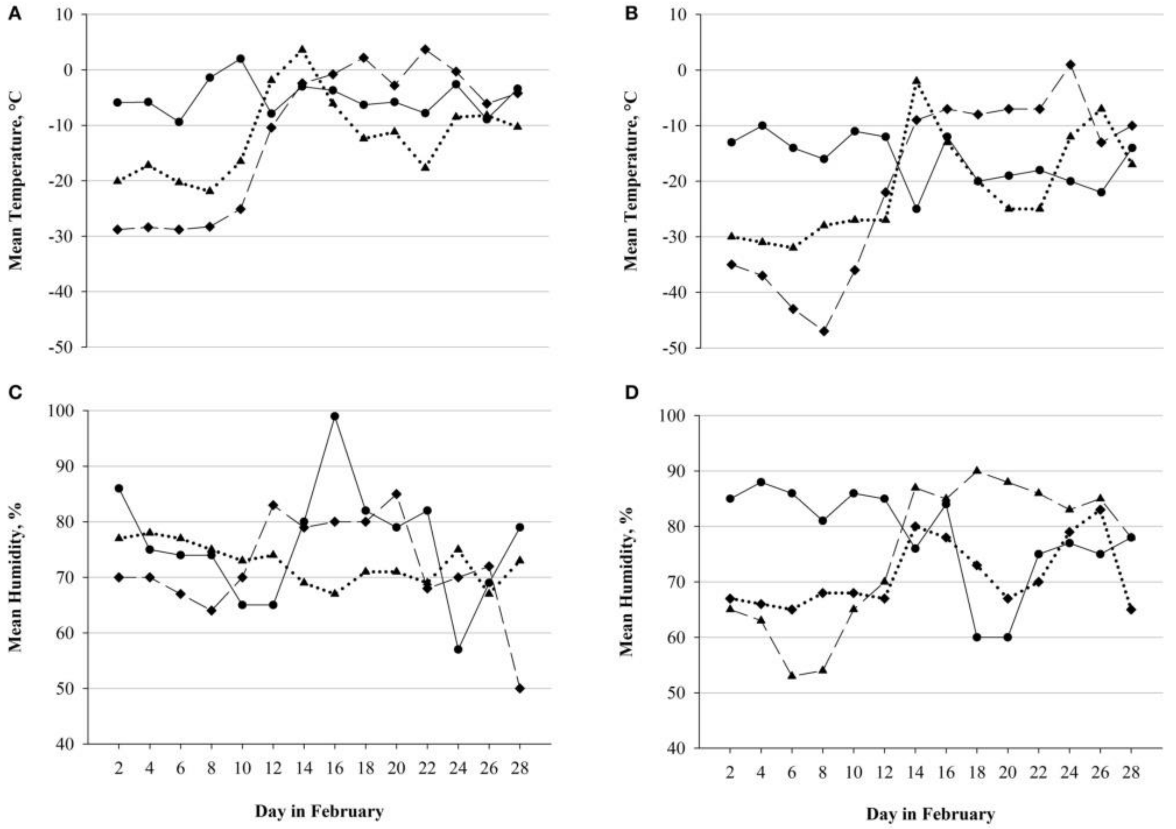

FIGURE 1 | Weather conditions across editions. Mean temperature and mean humidity as measured in Whitehorse and Dawson City in February 2013 (solid line), February 2015 (long dashed line), and February 2017 (dotted line). **(A)** Mean temperature in Whitehorse. **(B)** Mean temperature in Dawson City. **(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,

[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).

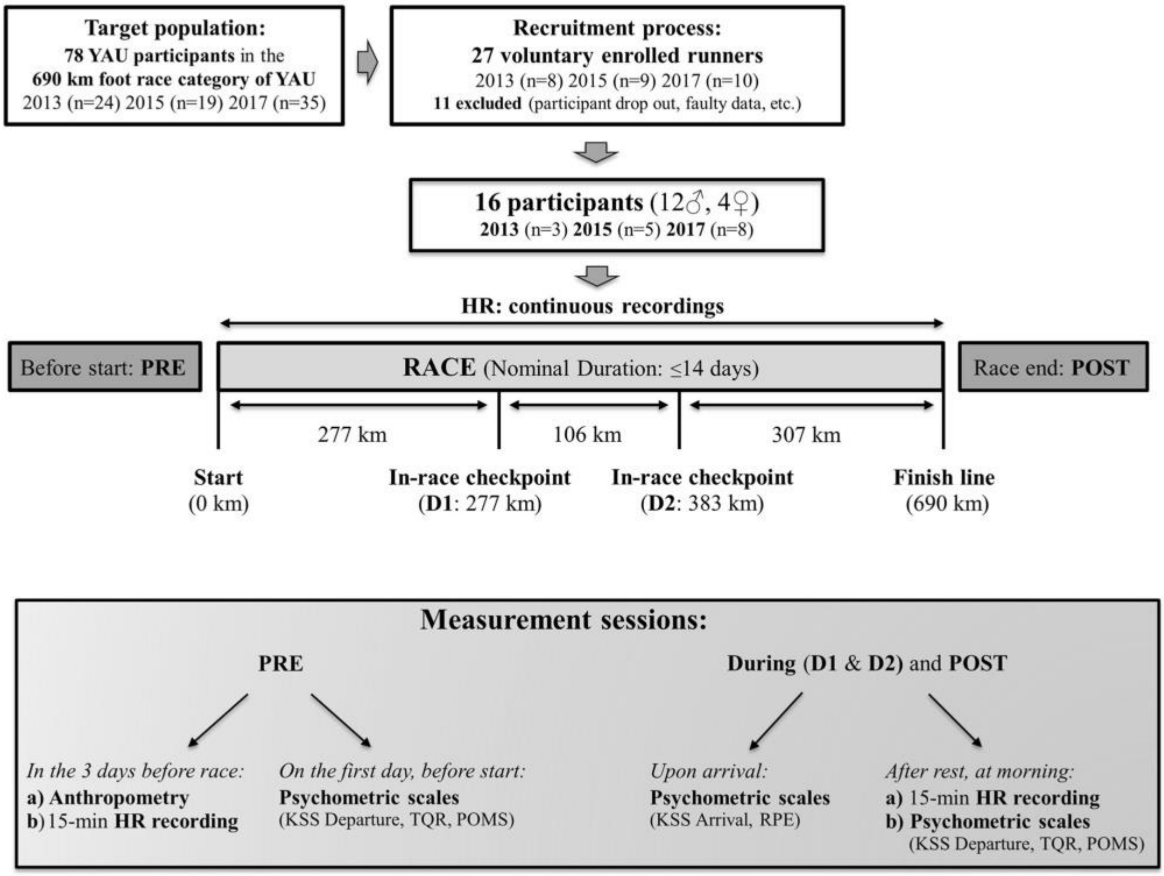

FIGURE 2 | Experimental protocol. Schematic representation of the study protocol and experimental procedure employed in all three YAU races of 2013, 2015, and 2017, except for psychometric assessment (included only in the 2015 and 2017 races).

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 (Elenttä 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).*

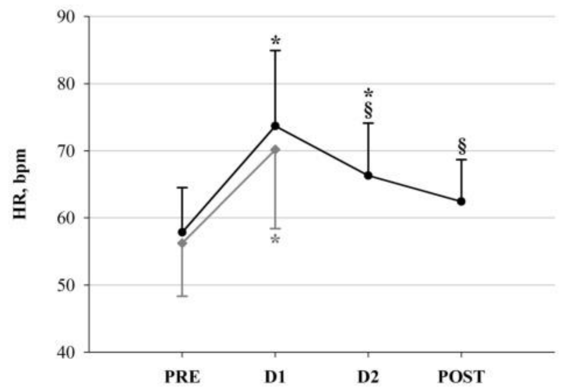

FIGURE 3 | Heart Rate (HR) in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup (One-Way RM ANOVA). §$p < 0.05$ vs. D1 (One-Way RM ANOVA).

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**).

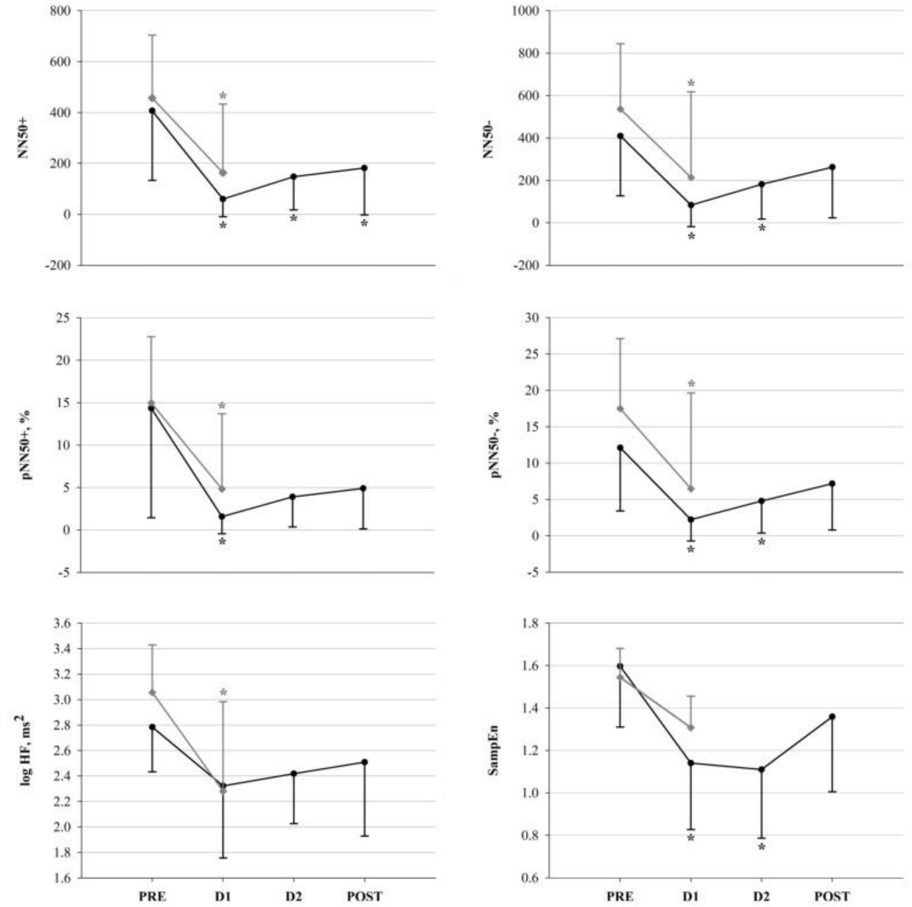

FIGURE 4 | Vagal modulation indices in FIN (black line) and in NON (gray line) across time-points. $^{*}p < 0.05$ vs. PRE within subgroup (all variables: One-Way RM ANOVA; pNN50+: Friedman ANOVA).

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

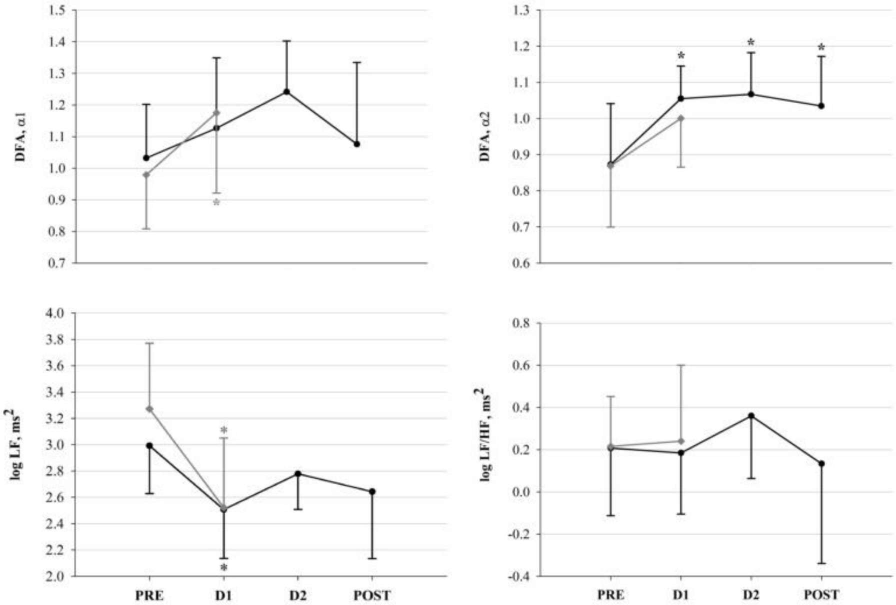

FIGURE 5 | Sympathovagal balance indices in FIN (black line) and in NON (gray line) across time-points. *$p < 0.05$ vs. PRE within subgroup (One-Way RM ANOVA).

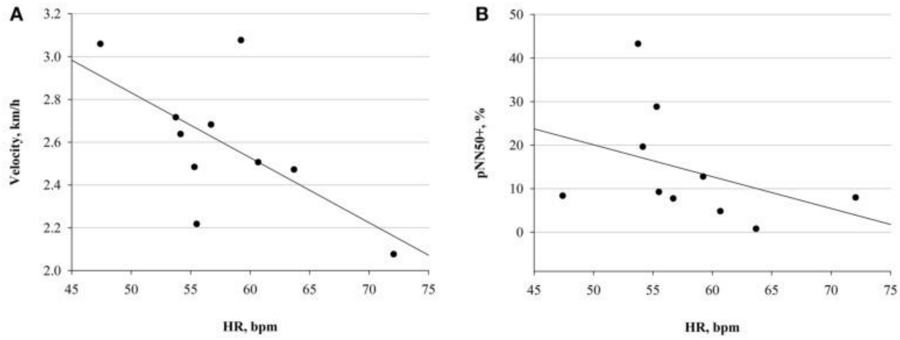

FIGURE 6 | Correlations at PRE in FIN group. **(A)** Correlation between HR and mean velocity ($r = -0.63$; $p = 0.04$). **(B)** Correlation between HR and pNN50+ ($r = -0.67$; $p = 0.03$).

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

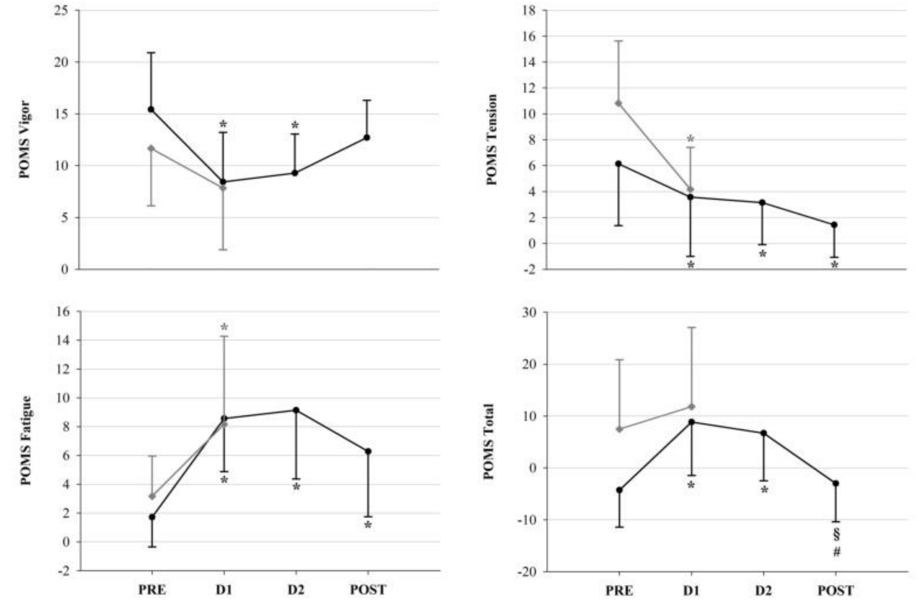

FIGURE 7 | POMS mood state scores in FIN (black line) and in NON (gray line) across time-points: $^*p < 0.05$ vs. PRE within subgroup; $^{\S}p < 0.05$ vs. D1; $^{\#}p < 0.05$ vs. D2 (One-Way RM ANOVA).

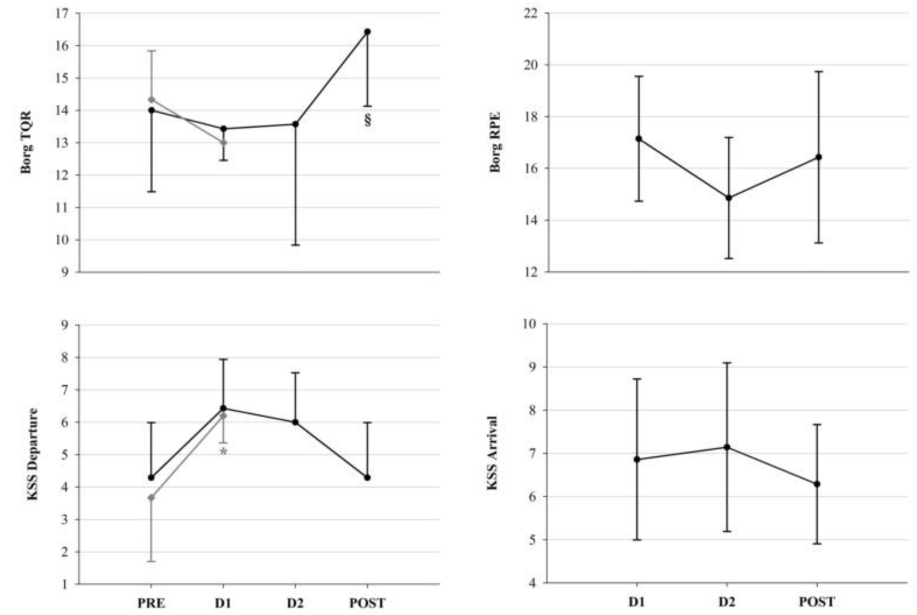

FIGURE 8 | Fatigue scale scores. Borg and KSS scores in FIN (black line) and in NON (gray line) across time-points. $^*p < 0.05$ vs. PRE within subgroup; $\S p < 0.05$ vs. D1 (One-Way RM ANOVA).

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

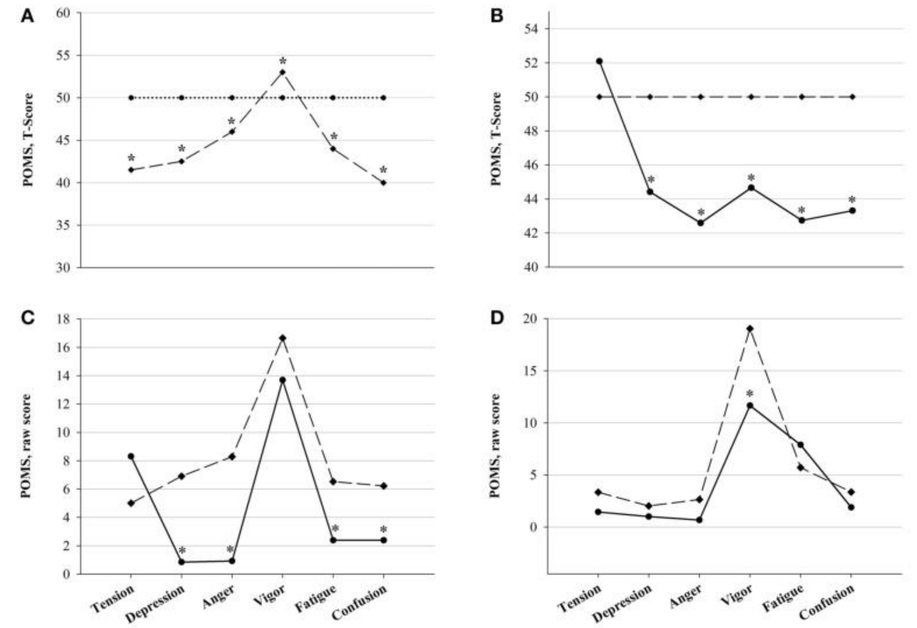

FIGURE 9 | Comparison of POMS results with normative data (Unpaired Student's *t*-Test). **(A)** *Iceberg profile* of mood states in an athletic sample (dashed line) plotted against sedentary subject norms (dotted line), **(B)** Mood states in all YAU subjects (ALL, solid line) plotted against normative 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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2.3 Third paper: Energy Expenditure, Body Composition, and Metabolism

Schalt, A., Johannsen, M., Kim, J., Chen, R., Murphy, C.J., Coker, M.S., Gunga, H.C., Coker, R.H., **Steinach, M.**: *Negative Energy Balance does not alter Fat Free Mass during the Yukon Arctic Ultra – the Longest and the Coldest Ultramarathon.* Front Physiol., 2018

In this third paper we evaluated changes in energy expenditure, body composition, and metabolism among the YAU study participants of 2017. Ten participants were included of which five reached the finish after 690 km. It was the focus of this work to assess energy balance in conjunction with changes in FM, FFM, and total body water (TBW). Furthermore, muscle proliferation markers myostatin and follistatin were evaluated as well as serum markers of carbohydrate, protein, and fat metabolism (e.g., lactate, alanine, fatty acids, acetoacetate).

It has been shown that athletes traversing ultra-long distances in very cold climate relied on a high fat metabolism, with a great amount of expended energy, while retaining positive protein synthesis and preserving fat free mass (43, 44). However, the preservation of fat free mass under the circumstances of a chronic energy deficit in conjunction with muscle proliferation markers has not been thoroughly investigated. Myostatin and follistatin have been shown to play a role in muscle growth and proliferation where myostatin inhibits and follistatin enhances muscle growth (45, 46). Since it was shown that myostatin is inhibited through cold exposure (47) and exercise (48), it was of interest to evaluate muscle proliferation markers during the YAU while also evaluating metabolism substrates and estimating energy balance through the measurement of energy expenditure using actigraphy and energy input through dietary reports. Figure 7 depicts the evaluated parameters of this study in the context of the integrated evaluation.

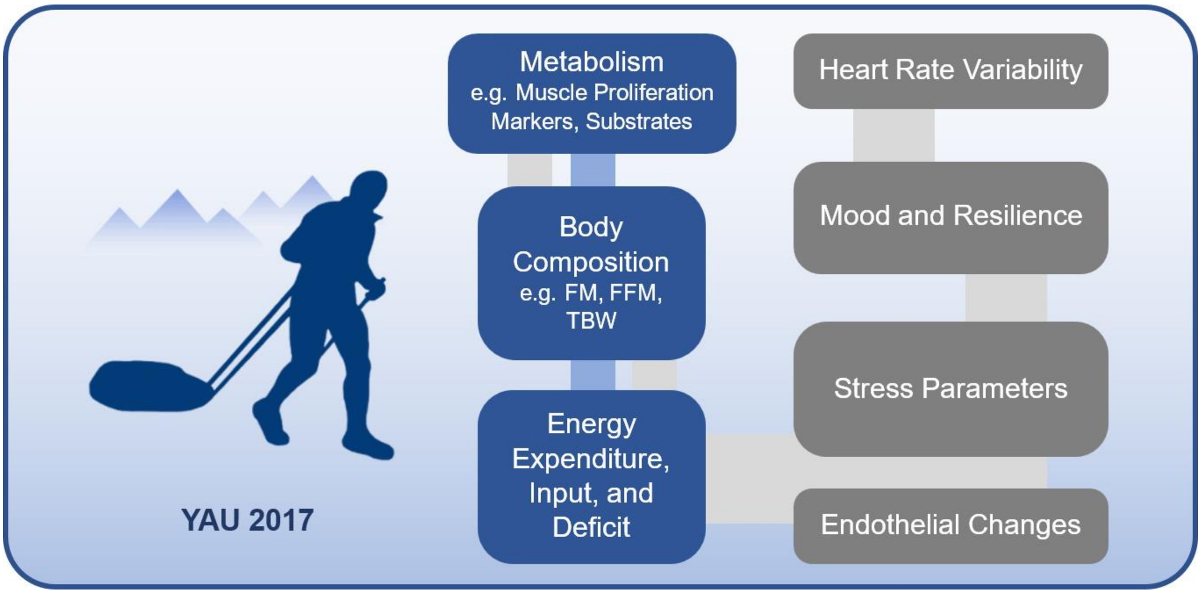

Figure 7: Evaluated parameters (dark blue boxes) in the third paper. (Graphic created by Mathias Steinach)

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ORIGINAL RESEARCH
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Negative Energy Balance Does Not Alter Fat-Free Mass During the Yukon Arctic Ultra—The Longest and the Coldest Ultramarathon

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Purpose: The objective of this study was to determine alterations in caloric balance, body composition, metabolites, and cytokines in athletes participating in the Yukon Arctic Ultra.

Methods: Ten participants traveling on foot in the 2017 692-km event were recruited for the study. Measurements and samples were obtained at pre-event, 278 km (C1), 384 km (C2), and post-event. Body composition measurements were obtained using bioelectrical impedance analysis. Accelerometer devices were utilized to provide an estimation of caloric expenditure and dietary recalls provided assessments of caloric intake. Blood serum samples were collected, processed, and analyzed using enzyme-linked immunosorbent assays or nuclear magnetic resonance. Results were analyzed using linear mixed model, presented as means ± SD, and considered significant at $p < 0.05$.

Results: Participants (8 males, 2 females; age: 37 ± 10 years; body mass index: 24.4 ± 2.5 kg/m^2) were recruited. Four males and one female completed the entire event in 260 ± 19 h. Caloric intake/expenditure was 4,126 ± 1,115 kcal/day and 6,387 ± 781 kcal/day, respectively, indicating a caloric deficit of 2,261 ± 1,543 kcal/day. Total mass, body mass index, and fat mass were reduced at each time point of the event. Fat-free mass (FFM) was unchanged throughout the event. Follistatin was increased at C1 (1,715 ± 876 pg/ml) in comparison to baseline. Acetoacetate increased significantly at post-event (6.1 ± 1.5 mg/ml).

Conclusions: Despite a pronounced caloric deficit and sustained activity under extreme cold conditions, FFM was preserved with an increase in serum follistatin and acetoacetate. Future studies should be directed at the role of nutrient strategies and/or training methods on the retention of FFM under these conditions.

Keywords: body composition, cold exposure, cytokines, extreme environment, ultramarathon

INTRODUCTION

The Yukon Arctic Ultra (YAU) has been advertised as the "World's Toughest and Coldest Ultra" (Montane, 2017). Participants must travel 692 kilometers (430 miles) from Whitehorse to Dawson City, Yukon Territory. The event takes place in the winter months when temperatures can easily reach as low as −48°C. Historical data show individuals average a speed of 3–5 km/h, sleep only 2–6 h per night, and it typically takes participants 7–12 days to complete the event. Due to the strenuous nature of the YAU, it is not uncommon for 50% or more of the individuals to drop out of the event. Rigorous safety standards may also disqualify individuals from continuing if officials deem that it is unsafe (e.g., signs of frostbite).

Over 20 years ago, the physiological resilience of two men who walked 2,300 km across Antarctica completely unsupported was described (Stroud et al., 1996). Utilizing a high-fat diet, these individuals adequate to elevated levels of protein synthesis that fostered the preservation of lean tissue (Stroud et al., 1996). In previous studies with the athletes participating in the YAU, we evaluated changes in body composition, serum cytokines, and metabolites (Coker et al., 2017). Remarkably, fat mass (FM) was reduced but fat-free mass (FFM) was preserved in a very limited number of participants with only one athlete completing the entire event (Coker et al., 2017). Additional studies performed with athletes in the Alaska Mountain Wilderness Ski Classic under similar Arctic winter conditions also demonstrated the preservation of lean tissue mass despite high levels of energy expenditure, as measured by dual-energy X-ray absorptiometry (Johannsen et al., 2018). While all of these data are interesting (Stroud et al., 1996; Coker et al., 2017; Johannsen et al., 2018), alterations in FM relative to FFM in conjunction with the estimation of caloric balance during exercise under chronic, extreme cold conditions have not been thoroughly investigated.

Myostatin and follistatin have been posited to exert a direct role in skeletal muscle growth and proliferation (Lee et al., 2010; Bragg et al., 2014). Studies have described the short-term influence of modest exercise and/or limited cold exposure on these glycoproteins (Wolfe et al., 1984; Haman, 2006). For example, myostatin mRNA is suppressed by cold exposure (Ijiri et al., 2009) and exercise (Zak et al., 2018). Given that myostatin plays an inhibitory role in muscle growth (Bragg et al., 2014) and follistatin may enhance muscle growth (Lee et al., 2010), it is important to evaluate whether they may be altered during the YAU or similar events/scenarios.

The objective of the present study was to determine the impact of the participation in the YAU on: (a) caloric balance and metabolites, (b) body composition, and (c) follistatin and myostatin that have not been comprehensively evaluated under these conditions. We hypothesized that participants would sustain a chronic net negative caloric balance, develop a metabolite profile indicative of ketogenesis, and maintain FFM. We further hypothesized that myostatin would decrease, and follistatin would increase in conjunction with the retention of FFM. The mechanisms responsible for survival in the cold environment are heavily dependent upon the preservation of lean tissue mass (Haman 2006; Haman and Blondin, 2017). Therefore, it is important to completely understand these mechanisms that might be applied to circumstances involving military or space personnel, bush pilots, and other minimally supported individuals who may face similar occupational conditions. The loss of FFM is also important in space exploration as the dietary intake of astronauts falls below positive caloric balance and further increases the loss of FFM, and may compromise the overall mission (Stein et al., 1999).

MATERIALS AND METHODS

General Information

Participants of the 2017 YAU 692-km (430-mile) event were recruited for this study (n = 10, 8 males, 2 females; 37 ± 10 years of age). Nine of the participants were of Caucasian descent, one of Asian descent. Five were able to finish the complete distance (one female, four males). All subjects gave 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 procedures complied with the Declaration of Helsinki (54th Revision 2008, Korea) regarding the treatment of human subjects.

These individuals were at chronic risk for the development of frostbite and/or hypothermia. First-time athletes were required to complete a training course that detailed first aid, shelter, equipment, nutrition, trails, and other necessary information necessary to sufficiently prepare the individuals for the arduous conditions. All competitors were required to pull their own sleds to carry equipment. Vital equipment included food, water, clothing, cooking utensils, sleeping preparations, and emergency preparedness items. In spite of these precautions, 50% of the study participants in 2017 were unable to complete the race for a variety of reasons ranging from severe muscle cramps to frostbite. We added an additional checkpoint in comparison to our work on the 2015 event so that data and samples were collected at pre-event (Whitehorse), checkpoint 1 (C1) at 278 km (Carmacks), checkpoint 2 (C2) at 384 km (Pelly Crossing), and post-event (Dawson City).

Body Composition

Body composition was measured using bioelectrical impedance analysis (BIA) *via* the tetrapolar electrode method (Sun et al., 2003), which is an established method to determine body composition (Gudivaka et al., 1999). These measurements were taken with an Akern BIA 101 (Florence, Italy) at the four checkpoints of the event: pre-event, C1, C2, and post-event. The BIA measurements provided values for FFM, and total body water (TBW) using equations appropriate for this cohort:

FFM Males (kg) = $9.33285 + 0.0006636$ Height (cm)^2 − 0.02117 Resistance (Ω) + 0.62854 Weight (kg) − 0.1238 Age (years);

FFM Females (kg) = $10.43485 + 0.00064602$ Height (cm)^2 − 0.01397 Resistance (Ω) + 0.42087 Weight (kg) (Segal et al., 1988).

TBW Males (l) = 1.203 + 0.499 Height (cm)^2/Resistance (Ω) + 0.176 Weight (kg);

TBW Females (l) = 3.747 + 0.45 Height (cm)^2/Resistance (Ω) + 0.113 Weight (kg) (Sun et al., 2003).

FM was calculated by substracting FFM from total mass (TM). Fat-free mass index (FFMI) was calculated as (FFM/height^2), as was fat mass index (FMI), respectively (FM/height^2). At each of the checkpoints, measurements were conducted indoors under similar room temperature conditions, just after awakening from an overnight rest, with a voided bladder, prior to breakfast, and dressed with light underwear.

Dietary Intake

Dietary recall information was obtained from the participants *via* a "Food & Energy Intake Form" and confirmed by a nutritional specialist. Participants were required to fill out the form in advance, naming the food items and including information such as kcals, weight, quantity packed, and quantity consumed. The caloric content of meals prepared by race organizers at the checkpoints was estimated using the type of dish on the menu as reference. Estimation of caloric content from the completed forms was based on information provided in "Food and Nutrition Tables" (Souci et al., 2016). All values were then verified by a professional dietitian from the Charité Health Academy.

Energy Balance

Energy expenditure was estimated using the SenseWear Pro3 Armband (Bodymedia, Pittsburgh, PA). The SenseWear Professional software was used to analyze and intepret the raw data. (King et al., 2004; Welk et al., 2007; Almeida et al., 2011). This particular device has been demonstrated to provide a reliable and accurate method for the measurement of physical activity and the assessment of energy expenditure (King et al., 2004; Welk et al., 2007; Koehler and Drenowatz, 2017). Data for dietary recall and energy expenditure were used to calculate net caloric balance for the participants.

Blood Parameters

Blood serum samples were collected by a physician under consistent room temperature conditions at each checkpoint and centrifuged to separate whole blood from serum. Serum samples were then pipetted into cryovials and stored in a liquid nitrogen dewar for transport at −80°C and later analysis.

Myostatin and follistatin were measured by enzyme-linked immunosorbent assay according to manufacturers' instructions (R & D systems, Minneapolis, MN). All samples were analyzed in triplicate. Serum samples for NMR-derived metabolite analysis were thawed and vortexed to ensure homogeneity and transferred into 5-mm NMR tubes (Wilmad Lab Glass, Buena, NJ). ^{1}H–NMR spectra were acquired at 17°C (based on methanol calibration) with a 600-MHz Bruker Avance-III system running TopSpin 3.2 software (Bruker Biospin, Fremont, CA), and using a dual-resonance high-resolution SmartProbe with single axis Z-gradient (Nicholson et al., 1995). The water signal was suppressed using NOESY presaturation followed by CPMG relaxation method, editing for suppression of macromolecules ("PROF_CPMG" parameter set in TopSpin 3.2). A standard, trimethylsilyl propionic-2,2,3,3-tetradeuteropropionic acid (TMSP, 3.87 mM in D_2O) contained in a sealed insert and placed in the NMR tube was used for metabolite quantification of fully relaxed ^{1}H–NMR spectra and as a ^{1}H chemical shift reference (0.0 ppm). After Fourier transformation, phasing, and baseline correction in TopSpin, each ^{1}H peak was integrated (Bogren et al., 2014). The ^{1}H-NMR peaks for single metabolites were identified and referred to published chemical shift or a metabolite chemical shift library. The absolute concentration of each metabolite was then referred to the TMSP integral and calculated according to the equation: $C_x = (I_x/N_x \cdot C)/I/9$, where Cx is the metabolite concentration (μmol/ml), Ix is the integral of metabolite ^{1}H peak, Nx is the number of protons in metabolite ^{1}H peak, C is the TMSP concentration, and I is the integral of TMSP ^{1}H peak at 0 ppm (this is nine as TMSP contains nine protons) (Serkova et al., 2005). An additional correction factor of 11.304 was applied to adjust for the differences in diameters between the NMR tube and the insert (determined using reference samples). The coefficient of variation for alanine, fatty acids, lactate, acetoacetate, ß-glucose, histidine, and formate was 0.2, 0.3, 0.4, 0.5, 0.4, 0.1, and 0.7, respectively. The final metabolite concentrations were expressed as mg/ml.

Statistical analysis was performed using R Studio version 1.1.422 software. Since 50% of the participants were unable to complete the event, missing data points existed and data were analyzed using a linear mixed model approach. The mixed-effect approach works in essence the same as a traditional ANOVA, but accounts for missing data as well as the repeated measures study design when determining if differences exist between the four time points of the event (Gelman and Hill, 2007; Mallinckrod et al., 2008). This allows for the use of our entire sample of participants, and avoids arbitrary selection and the potential for the biased interpretation of our data. A *post hoc* analysis using the Tukey's honest significant difference test was then used to determine if differences were statistically significant (Gelman and Hill, 2007; Mallinckrod et al., 2008). Values are reported as means ± SD and were considered significant with a $p < 0.05$.

RESULTS

Characteristics

Eight males and two females were initially recruited for the study ($n = 10$; age = 37 ± 10 years). Due to the harsh environmental conditions, only 5 of the 10 participants were able to complete the 2017 event. One male and one female dropped out before C1. By C2, two males had dropped out. Between C2 and the finish, another male dropped out of the event. Of the five participants that were able to complete the event, the average completion time was 260 ± 19 h. Therefore, four males and one female completed the entire event.

Body Composition

Total body mass (TM) and FM were reduced at each time point (**Table 1**). There were no significant changes in body mass index (BMI), FFM, or FFMI (**Table 1**).

Hydration Status

Total body water (TBW) remained stable at each time point (**Table 1**).

TABLE 1 | Body composition.

	Pre-event (*n* = 8)	Checkpoint 1 (*n* = 8)	Checkpoint 2 (*n* = 6)	Post-event (*n* = 5)
BMI (kg/m^2)	24.3 ± 2.9	23.7 ± 2.4	23.8 ± 3.0	22.8 ± 2.7
TM (kg)	75.9 ± 12.1	74.0 ± 10.5*	75.7 ± 10.9	70.7 ± 9.3*
FFM (kg)	62.6 ± 9.8	62.0 ± 9.2	62.8 ± 9.4	59.3 ± 7.4
FM (kg)	13.3 ± 3.2	12.0 ± 2.6*	12.9 ± 2.6	11.4 ± 2.9*
FM (%)	17.5 ± 2.6	16.2 ± 2.8*	17.1 ± 2.6	16.1 ± 2.8*
FFMI (kg/m^2)	20.1 ± 2.3	19.9 ± 2.0	19.7 ± 2.4	19.7 ± 2.2
FMI (kg/m^2)	4.3 ± 0.9	3.9 ± 0.8*	4.1 ± 0.9	3.7 ± 1.0*
TBW (liters)	44.6 ± 5.2	46.5 ± 5.4	47.2 ± 4.5	46.9 ± 2.6

*Data are presented as mean ± SD. *Different from previous time point. p < 0.05.*

TABLE 2 | Alterations in serum metabolites.

Metabolite	Pre-event (*n* = 8)	Checkpoint 1 (*n* = 8)	Checkpoint 2 (*n* = 6)	Post-event (*n* = 5)
Alanine (mg/mL)	5.21 ± 0.34	3.63 ± 0.29*	3.48 ± 0.15*	3.28 ± 0.29*
Fatty Acids (mg/mL)	8.24 ± 1.62	3.81 ± 0.41*	4.45 ± 0.64	4.09 ± 0.69*
Lactate (mg/mL)	37.33 ± 5.38	20.76 ± 1.92*	23.43 ± 2.03*	26.39 ± 3.60
Acetoacetate (mg/mL)	0.82 ± 0.13	3.08 ± 1.08	2.97 ± 0.67	6.09 ± 1.56*
ß-glucose (mg/mL)	13.47 ± 1.52	20.35 ± 1.82*	18.14 ± 1.12*	19.03 ± 2.27*
Histidine (mg/mL)	1.00 ± 0.05	0.80 ± 0.046	0.71 ± 0.06*	0.80 ± 0.12
Formate (mg/mL)	0.07 ± 0.02	0.13 ± 0.01*	0.13 ± 0.02*	0.12 ± 0.03

*Data are presented as means ± SD. *Different from pre-event value. p < 0.05.*

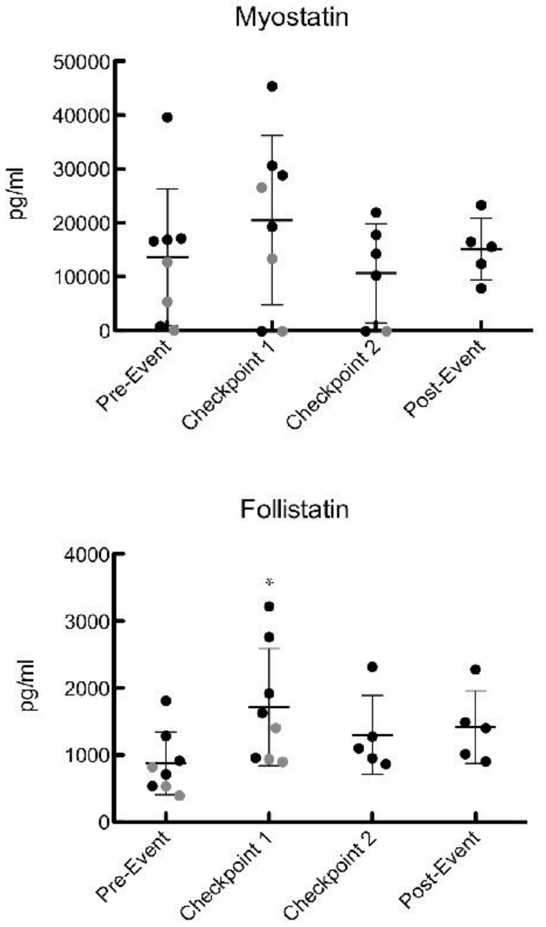

FIGURE 1 | Serum follistatin and myostatin during pre-event, C1, C2, and post-event. Data points colored in black represent finishers and data points represented in gray represent dropouts. For samples below the limit of detection of the assay (270 pg/ml for myostatin), the samples were assigned a value of half the LOD to avoid biasing the results. Values are expressed as mean ± SD. *Represents significant difference from baseline.

Caloric Balance

Complete dietary recall data were provided by all five participants who finished the event. The average caloric intake for these participants was 4,126 ± 1,115 kcals/day. Their corresponding average energy expenditure was 6,387 ± 781 kcals/day, indicating a negative caloric balance of 2,261 ± 1,543 kcals/day.

Metabolites

Fatty acids and derivatives and alanine and ß-glucose were decreased initially at C1 and remained reduced throughout the event (**Table 2**). There was a transient decrease in serum lactate at C1 and C2 and a reduction in serum histidine at (C2) (**Table 2**). Serum acetoacetate was increased substantially at post-event (**Table 2**).

Cytokines

Blood serum was analyzed for myostatin and follistatin concentrations at each of the aforementioned time points (**Figure 1**). There were no significant changes in myostatin (pre-event: 13,676 ± 12,617 pg/ml, C1: 20,528 ± 15,595 pg/ml, C2: 10,656 ± 8,915 pg/ml, post-event: 15,172 ± 5,669 pg/ml), but the values at C1 and C2 were below detectable limits for one participant ($p = 0.35$) (**Figure 1**). Follistatin was significantly higher at C1 than any other time point of the event (pre-event: 871 ± 470 pg/ml, C1: 1,715 ± 876 pg/ml, C2: 1,355 ± 544 pg/ml, post-event: 1,417 ± 542 pg/ml) (**Figure 1**).

DISCUSSION

The primary objective of this study was directed at the assessment of caloric intake and caloric expenditure, body composition, and alterations in serum metabolites in athletes traversing the entire 692-km distance of the YAU. We were also interested in potential alterations in myostatin and follistain in conjunction with this event. Similar to our previous investigations in athletes exercising in the extreme cold for extended periods (Coker et al., 2017; Johannsen et al., 2018), there was a sustained amount of negative energy balance, significant reductions in FM but no change in FFM. Exceptionally challenging cold conditions in the 2017 YAU were noted, considerably worse during the first half of the event, and this made food preparation extremely difficult. In spite of this situation, energy intake was quite high at over 4,000 kcal/day. These findings seem to suggest that successful athletes may utilize a combination of training, nutrient, and pacing strategies that enable adaptive responses to the physical, psychological, and environmental stresses of the event.

Energy balance plays a significant role in the modulation of protein turnover and maintenance of FFM (Pasiakos et al., 2010), and the severity of negative energy balance results in detrimental reductions in physical performance (Murphy et al., 2018). In an example of sustained negative energy balance from physical exertion and dietary restriction over an extended period (−1,000 kcal/day over an 8-week period), the investigators reported simultaneous muscle atrophy and decreased physical function (Nindl et al., 2007). This work highlights one of the more dramatic scenarios describing negative caloric balance over a significant period of time. Moreover, the interactive influence of dietary restriction and physical exertion across the duration of this type of physiological stress plays a major role in the risk of muscle atrophy (Tassone and Baker, 2017). It is unclear whether the duration of these physiological challenges emerged as a primary factor in the deterioration of physical function.

Recent research has shown that participants of ultraendurance events tend to maintain FFM even with the added stress of cold exposure (Stroud et al., 1996; Saugy et al., 2013; Coker et al., 2017; Johannsen et al., 2018). Caloric expenditure and intake were not examined in conjunction with body composition analysis in our previous work with this particular cohort (Coker et al., 2017). We have now demonstrated that athletes were able to maintain FFM even with an estimated caloric deficit of approximately 2,200 kcals/day. While this may seem quite remarkable, pre-clinical studies have highlighted the interorgan transfer of amino acids that may contribute to the conservation of muscle and other tissues, even during short-term exercise (Williams et al., 1996). This assertion has been further supported by the results of clinical studies that utilized stable isotope methodology to demonstrate that protein degradation was not increased by acute exercise and the diversion of amino acids toward the exercised muscle (Devlin et al., 1990).

In addition to the influence of exercise on the sequestration of amino acids by the working muscle (Devlin et al., 1990), nutrient intake and/or progressive, exertion-linked changes in fuel selection may play a significant role in the sparing of amino acids that facilitate the maintenance of FFM (Burke, 2015). Macronutrient intake data were not available in this particular study, but similar expeditions have reported variable dietary intake of approximately ~35–66% carbohydrate, 29–55% fat, and 17–20% protein in ultra-athletes (Wolfe et al., 1984; Stroud et al., 1993, 1996; Gannon et al., 2001; Praz et al., 2015). We know from our own anecdotal observations during the 2015 and 2017 YAU that many of these athletes may consume more fat than described in earlier studies. This seems plausible, as these particular athletes were required to carry the majority of their dietary provisions in their pulks during the event and dietary fat represents a more efficient method of caloric delivery relative to the amount of weight pulled. In our study, plasma acetoacetate increased progressively, eightfold relative to baseline by the end of the event. This indicates an increasing reliance on ketones as a fuel during prolonged exercise (Galvin et al., 1968) and that these YAU participants may modify their use of fat as fuel (Pitsiladis et al., 1999) through potentially beneficial responses in PGC1α mRNA (Slivka et al., 2012). Reductions in lactate and alanine suggest a gluconeogenic shift in the provision of circulating glucose, but they are not conclusive due to the inability to assess glucose kinetics in this environmental setting (Coker and Kjœr, 2005). Future studies in this cohort will focus on whether athletes "train" their ability to convert to ketosis and/or the molecular adaptations that play a role in their resilience.

We measured myostatin and follistatin because of their widely known roles in regulating muscle growth and metabolism (Lee et al., 2010; Bragg et al., 2014; Druet et al., 2014), and yet limited information with regard to their adaptive responses to sustained exertion and cold exposure in a "real-world" field setting. It has been demonstrated that these cytokines are influenced by factors other than exercise including cold exposure, diet, and sleep deprivation (Coker and Kjœr, 2005; Allen et al., 2011; Hansen et al., 2011; Vamvini et al., 2011). Previous work by Zak et al. (2018) also demonstrated rapid alterations in myostatin mRNA in response to exercise performed even under moderately cold conditions. It is highly likely that participants in this event experienced all of these physiological stressors and to a greater absolute and relative extent than comparisons made to well-controlled laboratory conditions. While the primary mediators responsible for alterations in these cytokines remain unclear, numerous factors play a role in protein metabolism under conditions of physiological stress. For example, interleukin-6 has been also demonstrated to initially increase during strenuous exercise, promote alterations in substrate utilization that could be beneficial to skeletal muscle (Pedersen et al., 2001), and then decline in a fashion similar to the alterations in follistatin.

One of the limitations of our study involves the use of the SenseWear device to estimate energy expenditure. The device may underestimate energy expenditure during exercise performed at a high intensity (i.e., >10 METs) (Drenowatz and Eisen, 2011). However, due to the nature of the event lasting over 10 days, it is quite likely that the predominance of physical exertion ranged only from low to moderate exercise intensity. Therefore, our results are very likely consistent with other studies that have demonstrated the SenseWear device as a reliable method for the estimation of energy expenditure in a variety of settings (King et al., 2004; Welk et al., 2007; Koehler and Drenowatz, 2017). We also recognize that the presentation of dietary intake data that only includes an estimation of energy intake without information about macronutrient intake may be somewhat limiting. Many ultramarathon athletes may manipulate macronutrient intake such that the fat intake increases in proportion to carbohydrate and protein (Peters, 2003). This may have a beneficial influence on fat oxidation (Leckey, 2018), but the lack of macronutrient data in this study limits our ability to provide inferences in this regard. Furthermore, we acknowledge the limitations of the single-frequency bioimpedance analysis used in this investigation to estimate FM, FFM, and TBW. BIA represents an indirect method to estimate body composition that is not only dependent on the appropriate regression formula but also on procedural consistency of its application. In addition, alterations among various cohorts may also distort the results, for example, kidney diseases, cardiovascular conditions, etc., as well as ethnic group affiliation (Kyle et al., 2004). However, we have employed formulas appropriate for this cohort of healthy trained adults to estimate FM and FFM (Segal et al., 1988) and TBW (Sun et al., 2003) that have been shown to perform better than other formulas with smaller biases and better agreement (Seoane et al., 2015). In addition, great care was taken to perform the measurements under the same conditions by the same operator.

In this study, we were able to extend our previous findings suggesting the preservation of FFM during the Yukon Arctic Ultra. These findings are limited by the use of methodologies such as the estimation of caloric balance and body composition by indirect assessment. Serum concentrations of metabolites and progressively increasing levels of acetoacetate posit alterations in metabolic regulation of a potential shift toward and increasing reliance on ketones as fuel. We have also demonstrated that follistatin increases initially and then returns to baseline. Future studies should be directed at the assessment of macronutrient intake and additional cytokines that may be involved in the maintenance of physiological resilience throughout the event.

AUTHOR CONTRIBUTIONS

MJ assisted in sample transport, conducted analysis on cytokines and metabolites, and contributed to manuscript; AS conducted research, recruited participants, collected all samples, calculated energy expenditure and energy intake, and contributed to the manuscript; JK and RC conducted analysis on cytokines and contributed to manuscript; CM conducted analysis on metabolites and was responsible for the oversight of molecular analysis; MC assisted in sample transport and contributed to the manuscript; H-CG contributed to research plan and manuscript; RC contributed to research plan and cytokine analysis, was responsible for sample transport, and the final draft of the manuscript; MS contributed to research plan, recruited participants, collected all samples, served as study physician, calculated energy expenditure and energy intake, and contributed to the manuscript.

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Conflict of Interest Statement: Dr. Coker is a managing partner and co-owner of Essential Blends, LLC that has received funding from the National Institutes of Health to develop clinical nutrition products. The data presented in this manuscript are unrelated. We declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

2.4 Fourth paper: Stress, Mood, and Resilience

Kienast, C., Biere, K., Coker, R.H., Genov, N.N., Jörres, M., Maggioni, M.A., Mascarell-Maricic, L., Schalt, A., Genov, M., Gunga, H.C., **Steinach, M.**: *Adiponectin, leptin, cortisol, neuropeptide Y and profile of mood states in athletes participating in an ultramarathon during winter: An observational study.* Front Physiol., 2022

In this fourth paper we evaluated changes in stress and resilience related parameters along with psychometric measures among the YAU participants of 2015, 2017, and 2019. 32 athletes were evaluated of which ten reached the finish after 690 km and seven control. It was the focus of this work to assess stress and resilience parameters along with mood and perceived exertion.

It was shown that ultramarathons can induce mood changes (49), while releasing stress hormones and negatively affect physiological functions such as in musculature, heart and kidneys (50, 51). Very cold environments may impair cognitive ability (52), which in combination with sleep loss and disturbed thermoregulation further cognitive impairment (53, 54). In addition, gastrointestinal symptoms common during ultramarathons may lead to mood disturbances (55), which are enhanced through consequential reduced nutritional intake (50). On the other hand, peptides such as the orexigenic neuropeptide Y (NPY) are being released during stress that may strengthen mental and physical performance (56), while its counterplayer, the adipocyte-derived anorexigenic hormone leptin decreases (57). In addition, exercise has been shown to increase the release of NPY (57) as well as adiponectin (58). It was therefore of interest to evaluate these peptides known to be affecting mood (56) as well as stress hormones along with psychometric measures of mood (POMS-SF). Figure 8 depicts the evaluated parameters of this study in the context of the integrated evaluation.

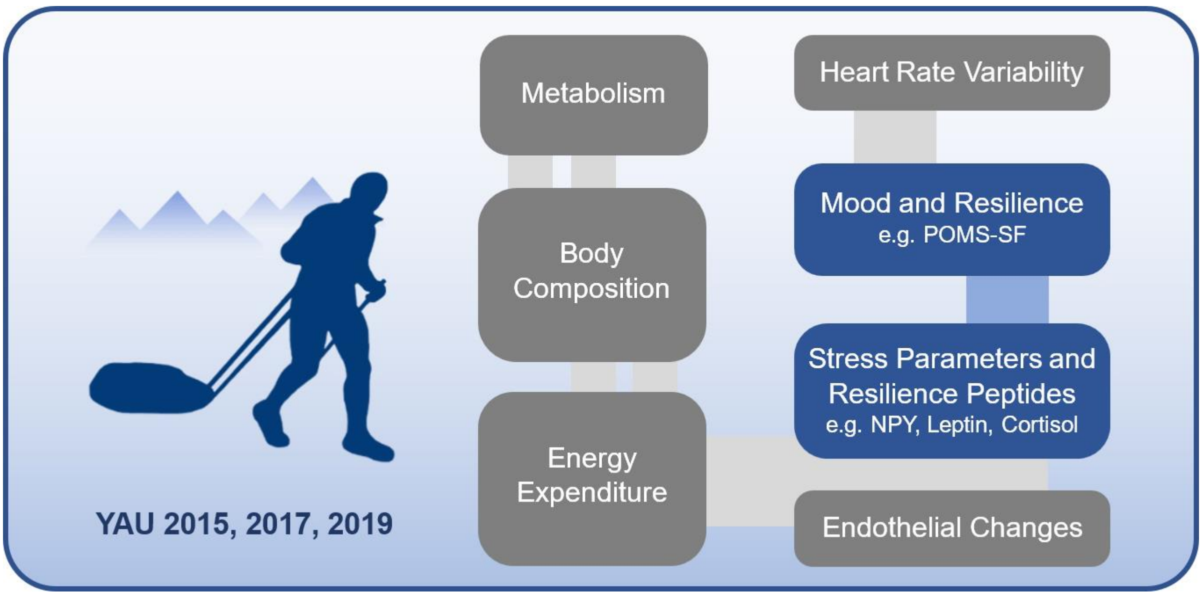

Figure 8: Evaluated parameters (dark blue boxes) in the fourth paper. (Graphic created by Mathias Steinach)

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Adiponectin, leptin, cortisol, neuropeptide Y and profile of mood states in athletes participating in an ultramarathon during winter: An observational study

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Background: The Montane® Yukon Arctic Ultra (YAU) is one of the longest (690 km) and coldest (+10.6°C–43.9°C) ultramarathons worldwide. Taking part in an ultramarathon is associated with great physiological and psychological stress, which can affect one's mood, level of hormones, and peptides. The current study aimed to identify relationships between peptides, hormones, and mood states in participants during this ultramarathon.

Methods: The study cohort consisted of 36 participants (19 men, 17 women, 38.64 ± 9.12 years) split into a finisher (n = 10), non-finisher (n = 19), and control group (n = 7). Data were collected at four time points: baseline (PRE), during (D1 after 277 km, D2 after 383 km), and after the race (POST). Questionnaires were used to assess ratings of perceived exertion (RPE), total quality of recovery (TQR), and profile of mood states (POMS-SF). Serum NPY, leptin, adiponectin, and cortisol were measured.

Results: Among non-finishers, scores for confusion, anger, depression, and tension-anxiety (PRE vs. D2, $p < 0.05$) increased, while vigor decreased (PRE vs. D1, $p < 0.05$). In contrast, finishers' tension-anxiety scores decreased (PRE vs. D1, $p < 0.05$). Fatigue increased in finishers (PRE vs. POST, $p < 0.05$) and non-finishers (PRE vs. D1, $p < 0.05$). In non-finishers, depressive mood correlated positively with leptin, anger, and confusion at several time points ($p < 0.001$). In

Abbreviations: BIA, bioelectrical impedance analysis; BMI, body mass index; NPY, Neuropeptide Y; POMS, Profile of Mood States; SF, Short Form; YAU, Yukon Arctic Ultra.

finishers, NPY correlated with TQR at PRE ($p < 0.05$), while leptin correlated negatively with TQR at POST ($p < 0.05$). Tension-anxiety correlated highly with perceived exertion in non-finishers ($p < 0.001$) and with cortisol in finishers ($p < 0.05$) and non-finishers ($p < 0.001$). In finishers, confusion correlated negatively with NPY ($p < 0.01$).

Conclusion: The study reveals an essential interplay between hormones and mood states affecting performance: Leptin was associated with anger and a depressive mood state in non-finishers and worse recovery in finishers. In contrast, NPY appeared linked to a lower confusion score and heightened recovery in finishers. A simultaneous increase in depressed mood, anger, tension-anxiety, and confusion might harm performance and lead to race failure.

KEYWORDS

ultramarathon, profile of mood states (POMS), leptin, adiponectin, NPY (neuropeptide Y), cold climate

1 Introduction

In recent years, extreme sports such as ultramarathons have been gaining popularity (Scheer et al., 2020). Ultramarathons are all running distances that are longer than the classic marathon distance of 42.195 km. The spectrum of running distances in ultramarathons can last hundreds to thousands of kilometers on any terrain or surface (Scheer et al., 2020). The present study deals with the Montane® Yukon Arctic Ultra (YAU), designated “the world’s coldest and toughest ultramarathon.”

Following the position statement of the Ultra Sports Science Foundation by Scheer et al. (2020), the Montane® YAU can be described as follows: a race distance of 690 km, a race time limited to 14 days, belonging to the multi-day race category, the running surface is off-road mainly, and there is a total cumulative elevation gain of 6,680 m and loss of 7,001 m. The Montane® YAU belongs to the self-sufficient races, meaning the athletes must carry all equipment and sustenance on their own. In particular, the participants of the Montane® YAU use a sled that they pull behind them. Furthermore, a GPS tracker is attached to this sled, which sends a signal in an emergency. The Montane® YAU puts the participant into an extreme situation. Lièvre and Gautier (2009) describe a situation as extreme when it has three characteristics: (1) it is evolving, (2) uncertain, and (3) risky. Similar to a polar expedition, participation in the Montane® YAU requires complex and multidimensional adaptation defined by the dynamic influence of environmental and personal constraints and resources (i. e., physical and psychological) on adaptation (Nicolas et al., 2019). The Montane® YAU might cause participants to push beyond their limits at the expense of their resources.

So far, there exists little quantitative data on ultramarathon runners. The number of ultramarathon participants is small, and the challenges for researchers in collecting data under sometimes extreme conditions are complicated. Besides, very few studies have examined the effects of ultramarathons in cold environments. Research shows that ultramarathons can lead to sleep and mood disturbances (Rundfeldt et al., 2018; Brager et al., 2020). In addition, there are infections, gastrointestinal symptoms, dehydration, skeletal muscle and dermatological injuries with effects on the kidneys, liver, and heart (Costa et al., 2010, 2016; Knechtle and Nikolaidis, 2018). Ultramarathon also affects the stress hormone level, increasing cortisol release (Knechtle and Nikolaidis, 2018). While most of these effects are transient, some of them can lead to severe and long-term damage.

In 2018, one of the Montane® YAU participants, Roberto Zander, lost both legs and his right hand due to frostbite. Confusion due to lack of sleep and energy led to a series of detrimental decisions that eventually caused the athlete to take off his gloves and shoes and walk unprotected for 14 h, after which a search-and-rescue team found him. Especially fatigue and dehydration are predisposing factors for frostbite (McArdle et al., 2015). Dehydration and gastrointestinal problems can lead to confusion or dizziness (Glace et al., 2002). Sleep deprivation can affect cognitive performance (Hurdiel et al., 2015) and thermoregulation (Dewasmes et al., 1993). Also, acute cold exposure can impair cognitive performance (Falla et al., 2021), which might affect decision-making. This means overexposure to cold temperatures and the failure to heed warning signs in time can increase the risk of frostbite and leave permanent damage (i. e., long-term consequences). But also, dermatological injuries such as foot blisters might make the tissue more susceptible to frostbite.

Costa et al. (2016) showed that gastrointestinal symptoms and dermatological injuries (e. g., foot blisters) might lead to reduced nutritional intake, and can affect the participant’s performance and mood. Gastrointestinal symptoms were found to be negatively correlated with recovery and positively with anxiety (Urwin et al., 2021). Murray and Costa (2012) described a general perturbed immune function during an ultramarathon, which increases the risk of illness, infection, and wound healing abilities. Additional whole-body cooling affects cellular components of the immune system

(Costa et al., 2010). Ultra-endurance exercise and cold exposure may evoke suboptimal host defense, increasing the risk of infection and decreasing effective wound healing.

Thusly, an ultramarathon runner has to deal with the race's challenges and possible effects on his health, underscoring the importance of mental strength. Along this line, Schütz et al. (2012) described that "finishing an ultra race is more a matter of mind than a matter of the body." Especially extreme situations can exacerbate stress states (Nicolas et al., 2019). Most athletes describe an increase in fatigue and a reduction in vigor (Rundfeldt et al., 2018; Graham et al., 2021). In such extreme situations, mood management seems to be of particular concern. Additionally, the survival instinct activates systems dedicated to satisfying essential needs, such as thirst, hunger, and thermoregulation. Hormones and peptides are released, acting on hypothalamic receptors to regulate energy balance, affecting behavior (e. a., increasing food-seeking) and mood (e. a., anxiety) (Kienast et al., 2019). The hypothalamus releases the orexigenic peptide neuropeptide Y (NPY) in response to stress (e. g., cold exposure, exercise), which might strengthen mental and physical performance (Kienast et al., 2019).

Research shows that during endurance exercise, adiponectin and NPY levels increase (Karamouzis et al., 2002; Czajkowska et al., 2020). By contrast, the counterplayer of NPY, the adipocyte-derived hormone leptin, decreases (Karamouzis et al., 2002). Leptin belongs to the anorexigenic hormones (Kienast et al., 2019). These peptides and hormones, regulating energy expenditure and food intake, might exhibit a specific interplay of unknown importance affecting mood states in ultramarathon athletes.

Therefore, the current study aims (1) to identify changes in the profile of mood states, (2) and to examine the relationships between hormonal/neurotransmitter variables (adiponectin, leptin, cortisol, and NPY) and different mood states during an Arctic ultramarathon. On the one hand, the study's design permits hormonal and neuropeptide assessment during a stable low-stress and high-stress phase, ensuring a uniform observation of various stressors across participants. On the other hand, the Montane® YAU provides an ethologically realistic model of acute, uncontrollable stress that closely approximates stress that military and astronauts may perceive during similar occupational conditions.

2 Materials and methods

2.1 The race

The Montane® YAU is an ultramarathon in the extreme environment of Canada's Arctic north-western Yukon Territory. The race occurs during February each year with different distances, where the longest distance of 690 km (430 miles) takes place only every 2 years. The race starts in Whitehorse and finishes at Dawson City. The trail is prepared and marked with wooden sticks with fluorescent tops. The surface is uneven and snow-covered, which can be knee-deep and hard to travel. The first part of the trail goes onto the frozen Yukon River, then over watersheds and several gradual, sometimes steep climbs. Overflow can be slowly rising water, possibly snow-covered, which may be difficult to navigate since the surface is not flat and very slippery. Among moose and bison, the athletes can also encounter wolves. After the first 160 km, the trail frequently goes on open fields and marsh, followed by a tight bush trail through the forest with parts of fallen trees on the path. The trail crosses several frozen lakes, which can have "soft spots" where the athletes can break through and end up in the water. The last part of the trail goes on the frozen Klondike River into Dawson City.

At the beginning of the race, the altitude is 610 m in Whitehorse, goes up to 1157 m at the highest point (King Solomon Dome), and ends at 319 m in Dawson City.

The duration of the race is limited to 14 days and must be completed during this time. This represents a minimum daily running distance of 49.28 km, more than one marathon (42.195 km) daily. The race has ten checkpoints that athletes should reach in a certain time. However, it is also possible not to reach a stage in the given time and to make up the time until the next stage. The ten checkpoints (stages) are in the following order: (1) Muktuk Adventures (42.195 km), (2) Dog Grave Lake (95 km), (3) Braeburn (160 km), (4) Mandanna Lake (235 km), (5) Carmacks (277 km), (6) McCabe (340 km), (7) Pelly Crossing (383), (8) Pelly Farm (438 km), (9) Pelly Crossing (300-mile finish) (483 km), and (10) Scroggie Creek (542 km). At each checkpoint, medical examinations are conducted by a medical team (including a medical doctor). The athletes are particularly examined for frostbite, in which case they are immediately excluded from the race. Each athlete can get one hot meal and hot water at the checkpoints; otherwise, the race is self-sufficient. The athletes must carry their survival equipment, tent, clothes, and food independently, using a carriage sled that they pull behind while walking (accounting for 20–30 kg). Running out of food between checkpoints is a reason for disqualification. The athletes are allowed to store and change up to three bags at the checkpoints. Except for two checkpoints (Carmacks at 277 km and Pelly Crossing at 383 km), athletes sleep and perform all activities outside.

For safety regulations, each athlete has to participate in survival training the week before the race. The training course includes the preparation for the extremely demanding conditions. For example, athletes must prove they can change clothes when wet or make a fire in a certain amount of time to stay warm and melt snow. For the race, special gear is mandatory (e. a., an avalanche shovel). Forgetting mandatory gear may result in a time penalty of up to 12 h.

The athletes are tracked with a GPS device (Spot®, Spot LLC, Virginia, United States) that is mounted on a carriage sled or pulk.

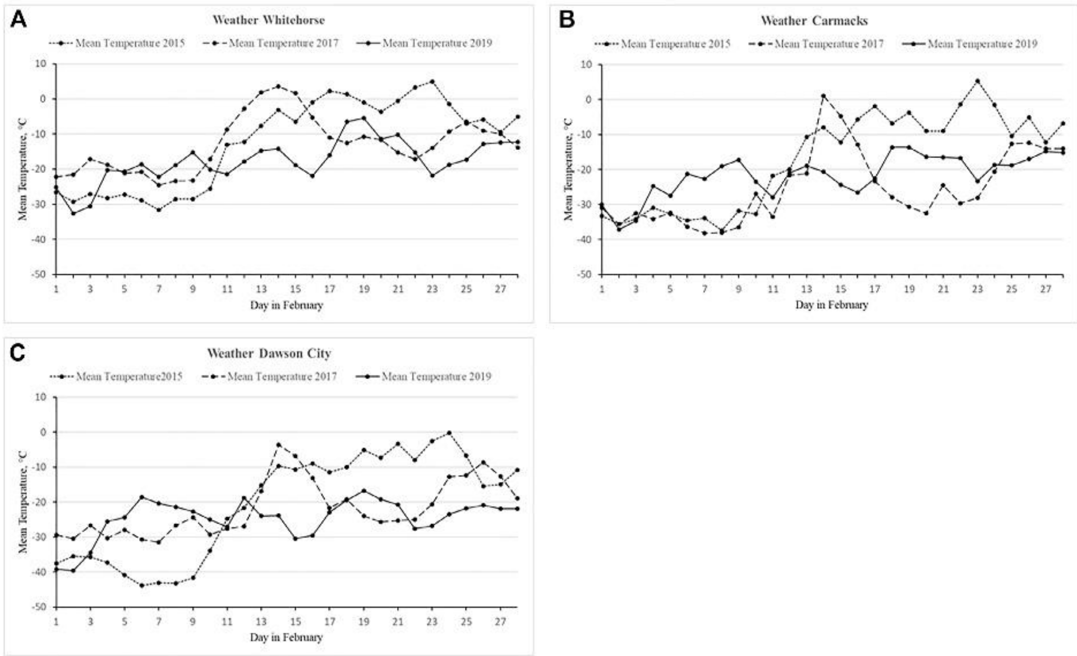

FIGURE 1
Weather conditions across editions. Mean air temperature was measured in Whitehorse **(A)**, Carmacks **(B)** and Dawson City **(C)** in February 2015 (dotted line), February 2017 (long dashed line), and February 2019 (solid line). No significant differences between editions or locations.

In case of an emergency, they can use it to call for assistance. When athletes start to bivvy (sleep), they must push the "Custom Message Button" on their Spot to inform the race organizers. It is not allowed to sit on their sleds on the mountain descent. Any acceleration is registered by the Spot and can lead to disqualification.

The athletes are exposed to adverse weather conditions, including hail and snow storms. Air temperatures recorded at the closest meteorological stations within the course during racing ranged from + 10.6°C and − 43.9 C (Figure 1) (weather data of the Government of Canada, 2020). More detailed information about the race is provided on the official website of the Montane® YAU (https://arcticultra.de/).

2.2 Subjects and study implementation

This study is part of continuing research since 2013: "Physiological changes of participants of the Yukon Arctic Ultra - an ultramarathon in an extremely cold climate". In our previous analyses, we evaluated (1) the metabolic responses of participants in the YAU (Coker et al., 2017), (2) the influence of energy balance on fat-free mass (Schalt et al., 2018), (3) cardiac autonomic modulations in conjunction with psychological correlates (Rundfeldt et al., 2018), and influences on glycocalyx shedding during ultra-endurance exercise (Steinach et al., 2022). In 2019, we continued our investigation of athletes participating in the 690-km race. This is an observational study because ultramarathon is a difficult-to-analyze topic, and study participants could not be randomized efficiently. Generating a large sample size is highly challenging because few people participate in an ultramarathon under such extreme conditions. To further strengthen the significance of our analysis, we combined the number of participants from 2015, 2017, and 2019 (n = 32). We included a control group (n = 7) based on the on-site volunteers for the first time. During the years 2015, 2017, and 2019, 32 athletes (13 female, 19 male) enrolled in the study: nine in 2015 (4 female, five male), 10 in 2017 (2 female, eight male), and 13 in 2019 (7 female, six male). In 2019, 7 (5 female, two male) volunteers not partaking in the 690-km race served as a control group. Of the 32 participants and the seven control (n = 39), only 36 (19 men, 17 women) were included in the data analysis (Figure 2).

Compared to the athletes, the volunteers in the control group were moderately active. They traveled by car or snowmobile up north towards Dawson City and took care of the preparation of the route. Their main tasks were maintenance of the route markings, luggage packing, and the support team's catering

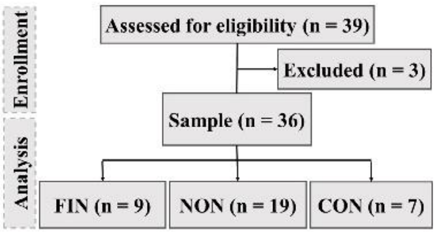

FIGURE 2
Overview of recruitment procedure. FIN = Finisher, NON = Non-finisher, CON = Control group.

(food preparation). Compared to the athletes, they spent less time outside and, thus, less exposed to cold weather conditions. They only slept indoors.

The recruitment for this study was conducted with the support of the event organizers. A call for participants, with a brief description of the research and planned measurements, was transmitted to the athletes. The organizers were encouraged to contact experienced athletes who had already participated in ultramarathons. Since such a study is difficult to reproduce, it was advantageous at this point to include already experienced athletes. Athletes and volunteers 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 decide whether to participate in the study. There were no further inclusion or exclusion criteria: all 690-km race category athletes were eligible to enter the study. This procedure was necessary because, due to the small number of participants in such races, it would otherwise have been difficult to gather sufficient study participants. All athletes were required to present a health certificate issued by their home physician to the event organizers to partake in the race. During a meeting in Whitehorse, Yukon Territory, Canada, 4–5 days before the race started, the potential study participants met with the investigators in person, had the chance to ask further questions, and finally gave their informed written consent to partake in the study. The Charité Universitätsmedizin Berlin Ethic Committee approved the study (review number EA4/109/12), all measurements and procedures complied with the Declaration of Helsinki (6th Revision 2008; Korea) regarding the treatment of human subjects.

2.3 Experimental protocol and measurements

The measurements were performed before and after the race and at two in-race checkpoints: (1) before the start of 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 2, D2) and (4) immediately after completion of the race in Dawson City at 690 km (POST) (Figure 3). The measurement checkpoints were all indoors and were chosen for accessibility, available electricity, water supply, sufficient space, comfortable ambient temperature, and low noise to perform all measurements and the blood collection under controlled conditions. The GPS data of the Spot were used to access the velocity of each athlete for further analysis (Table 2).

2.4 Anthropometric data

Anthropometric data were collected at the four measurement points after rest. Height was measured by the participants prior to the race and collected *via* e-mail by the study scientist. The body weight was measured at each measurement point with a calibrated scale (Seca, Germany, accuracy +/−100 g) on an even surface, and the participants were dressed in light underwear. Afterward, the body mass index (BMI) was calculated from these two parameters for each measurement point. In addition, body composition was determined by bioelectrical impedance analysis (BIA) using an Akern BIA 101 after rest, when the bladder was empty, and before breakfast. BIA is an established method to determine body composition that our department has already used in other studies (Schalt et al., 2018; Jörres et al., 2021). The BIA measurements provided values for fat mass using equations appropriate for this cohort, based on Segal et al. (1988) (Segal et al., 1988) and Sun et al. (2003) (Sun et al., 2003) calculation methods.

2.5 Blood parameters

Blood was taken at each measurement checkpoint after rest and before food intake. In order to achieve the required amount of serum volume, 15 ml of whole blood were drawn from the cubital vein into a serum tube (Sarstedt S-Monovette®, 50 units/ml) and immediately stored in a refrigerating unit at +2 to +8°C. Afterward, the samples were centrifuged (10 min at 2,000 x g) to separate serum from the cellular compounds, and the serum was pipetted into cryovials. Finally, the samples were stored in a liquid nitrogen Dewar for transport (−196°C). Without interrupting the cold chain, the serum samples were transported to Berlin. In the clinical and certified laboratory in Berlin (Germany) [Labor 28 GmbH, Berlin, Germany, accredited at the “DAkkS” (Deutsche Akkreditierungsstelle GmbH), the national accreditation body for the Federal Republic of Germany, according to regulation (EC) number 765/2008 and the accreditation body act of Germany] the samples were thawed to

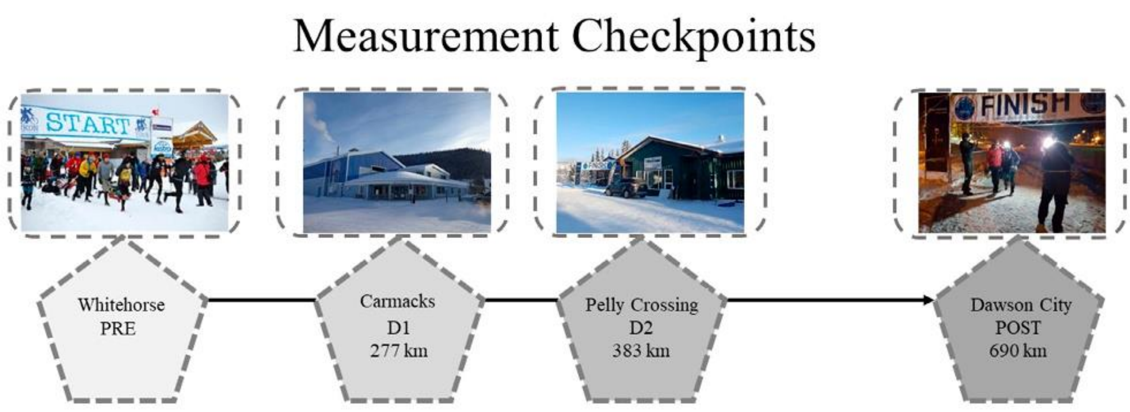

FIGURE 3
The measurement checkpoints: before the race in Whitehorse (PRE), during the race in Carmacks at 277 km (D1), and Pelly Crossing at 383 km (D2), after the race in Dawson City at 690 km (POST).

analyze cortisol (μg/dl), adiponectin (μg/dl), and leptin (ng/ml). The Ludwig-Maximilians-University of Munich (Germany) laboratory conducted further serum analyses for NPY (pg/ml).

2.6 Psychometric assessment

At each measurement checkpoint, a psychometric measurement was assessed. Standardized questionnaires were used to rate perceived exertion directly after arrival at the checkpoints (before rest) and the quality of recovery after rest. The assessment of mood states was conducted after rest.

2.6.1 Profile of mood states - Short form

Mood states were measured using the Shacham (1983) shortened 'right now' version of the Profile of Mood States (POMS) questionnaire (Shacham, 1983). The test requires the participant to indicate the state of emotions experienced in the past hours. The POMS-SF contains 37 single-word mood descriptors, each with a 5-point Likert response scale, from which six mood subscale scores for tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment could be calculated. Finally, the test yields five negative mood states (tension-anxiety, depression, anger, fatigue, confusion) and one positive mood state (vigor) (Shacham, 1983). To minimize response bias effects, participants were briefed to complete the POMS-SF based on how they felt now rather than attempting to memorize their previous mood. Every time a participant completed a POMS-SF, they could not see any previous questionnaire they had completed before.

2.6.2 Borg ratings of perceived exertion

Ratings of Perceived Exertion is a one-item self-report measure of perceived physical exertion. The content of this scale is a numerical indication of 6–20 of exertion. A score of six is comparable to a resting activity, and a score of 20 characterizes exhaustive exercise (Borg, 1962, Borg, 1970).

2.6.3 Borg total quality of recovery

As well as the perceived exertion scale, the Total Quality of Recovery scale contains a numerical indication of 6–20. By contrast, the total quality of recovery monitors the recovery process. A score of six means a very, very poor recovery and 20 a very, very good recovery (Kenttä and Hassmén, 1998).

3 Statistics

3.1 Data analysis and software used

The data were analyzed with R version 4.0.3 and R Studio 1.3.1093. The visualizations were created with the ggplot2 R package and with the support of the ggpubr R package for the addition of statistical tests. The statistical testing between the groups was performed with the t-test for two groups, e. g., finisher vs. non-finisher. The paired t-test was used to compare the effect of the same values in one group at different measurement points, e.g., PRE vs. POST. The correlation analysis and visualization were performed with the PerformanceAnalytics R package. The correlation analyses were conducted to find a relationship between different attributes in the three subgroups overall measurement points. Statistically, Pearson's co-efficient correlation was quantified. Peak detection in

TABLE 1 Subject demographics at baseline for all participants and in subgroups. FIN = Finisher, NON = Non-finisher, CON = Control group, FM = fat mass, BMI = body mass index, m = mean, SD = Standard Deviation.

Parameter	All (n = 36)		All athletes (n = 29)		FIN (n = 10)		NON (n = 19)		CON (n = 7)	
		m ± SD		m ± SD		m ± SD		m ± SD		m ± SD
Age (years)										
Men	38.32	9.29	38.71	9.45	42.60	11.59	37.08	8.45	35.00	9.90
Women	39.00	9.20	40.00	7.89	37.40	6.19	41.86	8.88	36.60	12.54
All	38.64	9.12	39.24	8.71	40.00	9.18	38.84	8.69	36.14	11.04
p-value (women vs. men)	0.826		0.701		0.548		0.259		0.880	
Weight (kg)										
Men	79.61	9.16	79.89	9.63	77.08	8.88	81.07	10.06	77.20	3.68
Women	64.97	9.76	65.57	11.37	64.40	10.34	66.41	12.79	63.52	4.69
All	72.70	11.90	73.97	12.46	70.74	11.28	75.67	13.01	67.43	7.84
p-value (women vs men)	<0.001		0.001		0.095		0.013		0.015	
Height (cm)										
Men	178.84	7.01	177.82	6.69	175.40	6.54	178.83	6.77	187.50	0.71
Women	167.94	8.00	167.33	7.94	168.60	6.58	166.43	9.18	169.40	8.88
All	173.69	9.22	173.48	8.83	172.00	7.15	174.26	9.69	174.57	11.43
p-value (women vs. men)	<0.001		0.001		0.140		0.007		0.042	
FM (%)										
Men	16.37	2.67	16.79	2.48	18.05	2.63	16.26	2.32	12.83	1.36
Women	25.04	4.41	24.95	4.94	24.15	3.92	25.52	5.79	25.25	3.24
All	20.46	5.64	20.17	5.46	21.10	4.50	19.67	5.96	21.70	6.64
p-value (women vs. men)	<0.001		<0.001		0.020		<0.001		0.004	
BMI (kg/m²)										
Men	24.90	2.57	25.24	2.47	25.10	3.09	25.30	2.33	21.96	1.21
Women	23.17	4.08	23.53	4.50	22.60	2.80	24.20	5.54	22.30	3.08
All	24.08	3.43	24.53	3.49	23.85	3.08	24.89	3.72	22.21	2.57
p-value (women vs. men)	0.133		0.199		0.217		0.526		0.891	

the data was performed using the R package pracma, with a minimum of seven increasing steps before reaching a peak set as the parameter for the 'findpeaks' function. A minimum peak spacing of 50 was used. Statistical summary tables containing arithmetic mean ± and standard deviations (m ± SD) values were created with the qwraps2 R package. The scales R package was used to work with the timestamps included in the data. The results of the statistical tests were considered significant if a p-value was below 0.05.

4 Results

4.1 Anthropometric characteristics

Participants were divided into three groups, which were also split by sex (Table 1). The three groups consisted of the finishers, non-finishers, and a control group. The finishers completed a total distance of 690 km. Those who did not finish the race gave up at an earlier stage of the race, or they were excluded from the race by the organizers for health reasons (e. g., frostbite) or because they were too slow. Too slow, in this case, meant that the organizers could estimate in what time span an athlete should be at one of the first checkpoints based on experience from previous races. In this case, the athlete was taken to the next checkpoint with a snowmobile. This allowed our study team to investigate these athletes as well directly after they were excluded from the race. The 690-km ultramarathon was completed by nine of 29 entrants, 20 withdrew from the competition at earlier points. Most of the study participants were of Caucasian descent, and one was of Asian origin. Their anthropometric data are presented in Table 1. There were no significant differences between the groups neither between women and men regarding age. The mean age of all participants was 38.64 (± 9.1). Women had lower weight and height (p < 0.001). They exhibited a

TABLE 2 Women vs. men's performance data for finishers and non-finishers. The overall completed distance, finishing time, and the total and moving average velocity. FIN = Finisher, NON = Non-finisher, m = mean, SD = Standard Deviation.

Parameter	All athletes (n = 29)		FIN (n = 10)		NON (n = 19)	
	m ± SD		m ± SD		m ± SD	
Distance (km)						
Men	367.32	237.34	690.00	0.00	232.86	122.00
Women	472.85	239.69	690.00	0.00	317.38	194.83
All	410.98	239.91	690.00	0.00	264.13	153.35
p-value (women vs. men)	0.278		1.000		0.256	
Finishing Time (hrs:min)						
Men	143:23	89:45	249:28	22:51	95:10	60:43
Women	198:28	100:56	278:58	19:57	131:23	90:25
All	165:49	96:34	264:13	25:30	107:57	71:57
p-value (women vs. men)	0.098		0.062		0.451	
Total Average Velocity (km/h)						
Men	2.89	0.62	2.79	0.34	2.93	0.72
Women	2.43	0.20	2.36	0.16	2.48	0.22
All	2.70	0.54	2.58	0.34	2.76	0.62
p-value (women vs. men)	0.023		0.031		0.128	
Moving Average Velocity (km/h)						
Men	4.45	0.44	4.57	0.29	4.39	0.50
Women	3.69	0.49	3.57	0.59	3.77	0.44
All	4.11	0.60	4.07	0.68	4.14	0.56
p-value (women vs. men)	<0.001		0.009		0.018	

higher fat mass ($p < 0.001$), except for the finisher group. In finishers were no differences in weight and height between the sexes. The BMI did not differ between the sexes across all groups.

4.2 Race performance

The average time to complete the course was 264:13 (± 25:30) hours. Men took less time to complete the race with 249:28 (± 22:51) hrs:min than women, who took 278:58 (± 19:57) hrs:min (Table 2). In finishers, the moving average velocity was 4.07 (± 0.68) km/h, whereas, in non-finishers, the overall moving velocity was 4.14 (± 0.56) km/h (Table 2). The non-finishers reached distances between 53.11 and 643.73 km. Thus, some participants from the non-finisher group ($n = 3$) were also examined at the study endpoint (POST).

4.3 Blood parameters

Leptin, NPY, adiponectin, and cortisol levels are depicted in the Supplementary Tables.

4.4 Profile of mood states—short form

Supplementary Figure S1 shows the six mood dimensions of the POMS-SF in the three groups at the four measurement points. For a simplified presentation and description of the results, the six dimensions of mood are written out in abbreviated form with the first word before the hyphen except for tension-anxiety. Tension-anxiety scores were significantly higher in all athletes than in the control group at baseline (t-test, $p < 0.05$). The non-finishers had higher tension-anxiety scores than the finishers at D1 (t-test, $p < 0.05$). And tension-anxiety increased in non-finishers (paired t-test, PRE vs. D2 $p < 0.05$). In contrast, tension-anxiety decreased in finishers throughout the race (paired t-test, PRE vs. D1, PRE vs. D2, and PRE vs. POST $p < 0.05$). At D2, non-finishers had a significantly higher depressive mood score than the control group (t-test, $p < 0.05$). The level of depressive mood score increased significantly PRE vs. D1 in non-finishers (paired t-test, $p < 0.05$) but not in finishers. We found no significant difference in anger scores between the three groups, even though anger increased in non-finishers significantly during the race (paired t-test, PRE vs. D1, $p < 0.05$). There was no significant difference in vigor at baseline between the three groups. After the race (POST), the level of

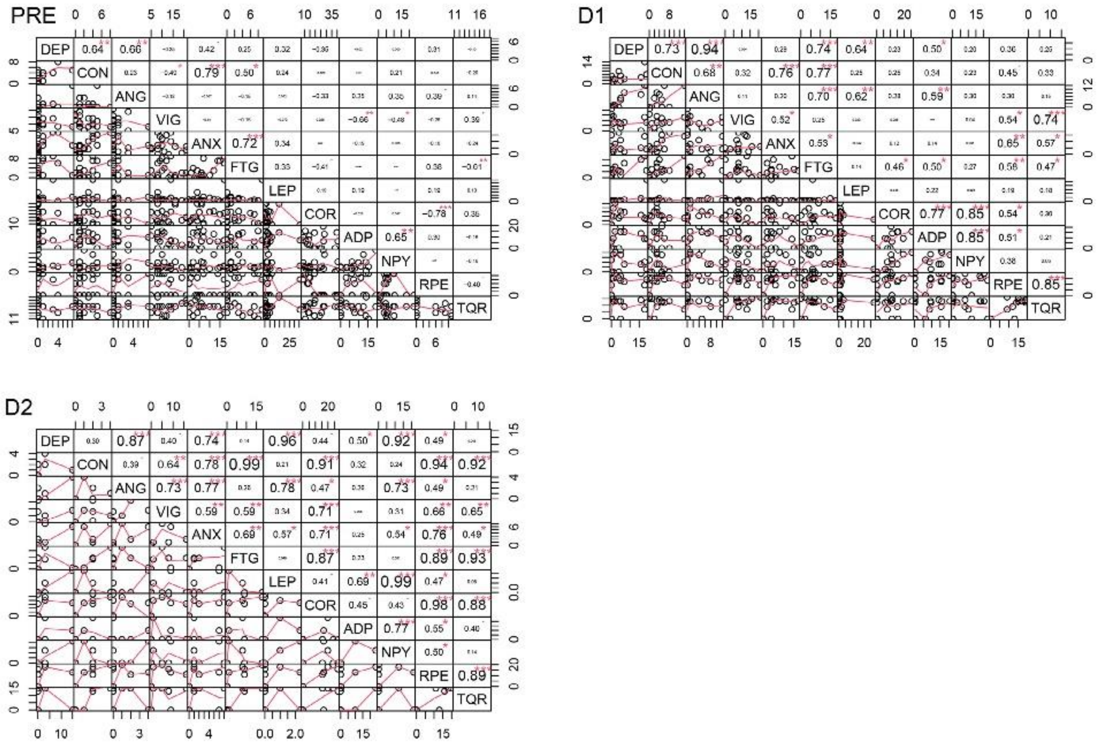

FIGURE 4
Correlation analyses for the non-finisher group at the four time points. DEP = Depression, CON = Confusion, ANG = Anger, VIG = Vigor, ANX = Tension-ANXiety, FTG = Fatigue, LEP = Leptin, COR = Cortisol, ADP = Adiponectin, NPY = Neuropeptide Y, RPE = Ratings of Perceived Exertion, TQR = Total Quality of Recovery.

vigor was higher in the finisher group than in the control group (t-test, $p < 0.05$). In non-finishers and finishers, vigor decreased. But only in non-finishers the reduction in vigor scores was significant (paired t-test, PRE vs. D1, $p < 0.05$). Across the race, fatigue increased in finishers (paired t-test, PRE vs. D1, $p < 0.05$, PRE vs. D2, $p < 0.001$, PRE vs. POST, $p < 0.05$) and in non-finishers (paired t-test, PRE vs. D1, $p < 0.05$). No significant difference was found between non-finishers and finishers in fatigue scores. Before the start of the race, confusion scores were rated higher in all athletes together vs. the control group (t-test, D1 $p < 0.05$). But there was no significant difference between the three groups at baseline, during and after the race. In non-finishers, confusion increased during the race (paired t-test, PRE vs. D1, $p < 0.05$) but not in finishers.

4.5 Correlation analyses

Figures 4–6 depict the correlation analysis results of the psychometric assessment, BORG scales, peptides and hormones. Figure 4 shows the result of the correlation analyses for non-finishers and Figure 5 for finishers. Figure 6 shows the result of the control group. In the non-finisher group, the data collected at the POST measurement point were not sufficient to perform a correlation analysis. Therefore, in Figure 4, the POST measurement point is not shown as it can be found in the figures and tables of the other values. A wide variety of correlations between the peptides and hormones were emerging. Of interest are the significantly lower correlations in the control group compared to the athlete subgroups. At baseline, leptin correlated positively with adiponectin (PRE Pearson 0.93, $p < 0.01$) and NPY (PRE Pearson 0.85, $p < 0.05$) in the control group. In the non-finisher group, this positive correlation was also found, but by the end of the race (D2 Pearson 0.69, $p < 0.01$). At D1, cortisol correlated positively with NPY in finishers (Pearson 0.57, $p < 0.05$), and in non-finishers with adiponectin (Pearson 0.77, $p < 0.01$) and NPY (Pearson 0.85, $p < 0.001$). There was no correlation between cortisol, NPY and adiponectin at any measurement point in the control group. In the control group, adiponectin correlated positively with NPY

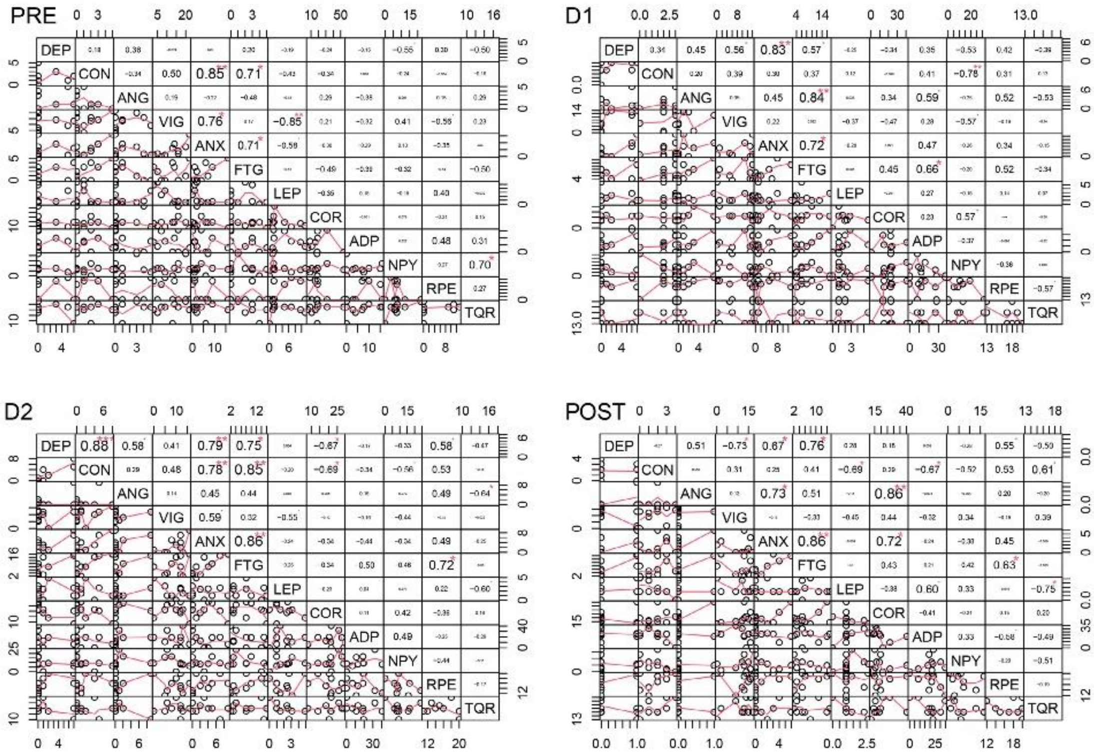

FIGURE 5
Correlation analyses for the finisher group at four time points. DEP = Depression, CON = Confusion, ANG = Anger, VIG = Vigor, ANX = Tension-ANXiety, FTG = Fatigue, LEP = Leptin, COR = Cortisol, ADP = Adiponectin, NPY = Neuropeptide Y, RPE = Ratings of Perceived Exertion, TQR = Total Quality of Recovery.

(PRE Pearson 0.71, $p < 0.05$). There was an apparent correlation between adiponectin and NPY, at all time points in non-finishers (PRE Pearson 0.65, $p < 0.01$; D1 Pearson 0.85, $p < 0.001$; D2 Pearson 0.77, $p < 0.001$). In contrast, this correlation was missing in finishers.

For a better overview, other results of the correlation analysis are described starting with one of each of the six mood dimensions. Only significant results are described in this section.

4.5.1 Tension-anxiety

In non-finishers and finishers, tension-anxiety correlated with fatigue at all measurement points (Non-finishers: PRE Pearson 0.72, $p < 0.001$; D1 Pearson 0.53, $p < 0.05$; D2 Pearson 0.69, $p < 0.01$ and finishers: PRE Pearson 0.71, $p < 0.05$; D1 Pearson 0.72, $p < 0.05$; D2 Pearson 0.86, $p < 0.01$; POST Pearson 0.86, $p < 0.01$). This correlation was highly significant in non-finishers at baseline. By contrast, this correlation was not found in the control group.

In non-finishers, tension-anxiety correlated high with perceived exertion (D1 Pearson 0.65, $p < 0.01$; D2 Pearson 0.76, $p < 0.001$) and low with total quality of recovery (D1 Pearson 0.57, $p < 0.05$; D2 Pearson 0.49, $p < 0.05$). Tension-anxiety correlated positively with cortisol in finishers (POST Pearson 0.72, $p < 0.05$) and non-finishers (D2 Pearson 0.71, $p < 0.001$).

4.5.2 Depressive mood

At baseline and during the first part of the race, the depressive mood score was positively correlated with confusion in non-finishers (PRE Pearson 0.64, $p < 0.01$; D1 Pearson 0.73, $p < 0.001$). This correlation was only found at D2 and not at the other measurement points in finishers (D2 Pearson 0.88, $p < 0.001$) and the control group (D2 Pearson 0.72, $p < 0.05$). Furthermore, the depressive mood score correlated positively with anger in non-finishers at each measurement point (PRE Pearson 0.66, $p < 0.01$; D1 Pearson 0.94, $p < 0.001$; D2 Pearson 0.87, $p < 0.001$) and in the control group at D2 (D2 Pearson 0.77, $p < 0.05$). The depressive mood score correlated positively with tension-anxiety (Non-finishers: D2 Pearson 0.74, $p < 0.001$; Finishers: D1 Pearson 0.83, $p < 0.01$; D2 Pearson 0.79, $p < 0.01$; POST Pearson 0.67, $p < 0.05$; Control group: D2 Pearson 0.78, $p < 0.05$),

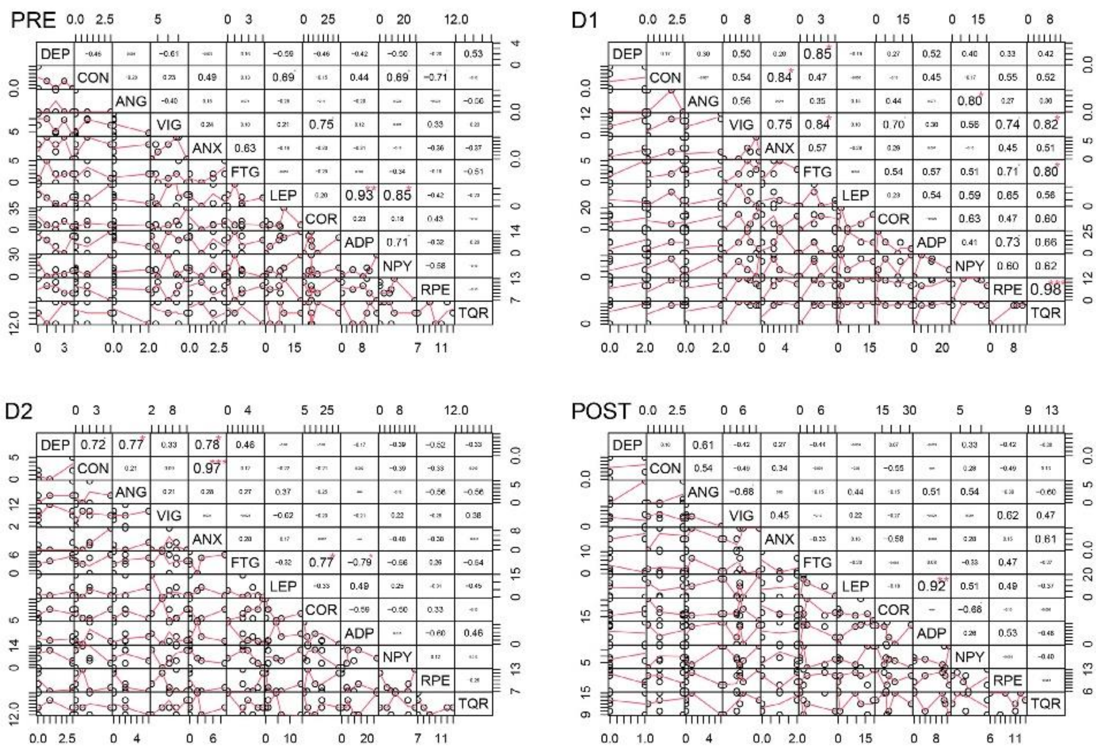

FIGURE 6
Correlation analyses for the control group at the four time points. DEP = Depression, CON = Confusion, ANG = Anger, VIG = Vigor, ANX = Tension-ANXiety, FTG = Fatigue, LEP = Leptin, COR = Cortisol, ADP = Adiponectin, NPY = Neuropeptide Y, RPE = Ratings of Perceived Exertion, TQR = Total Quality of Recovery.

and fatigue in all groups (Non-finishers: D1 Pearson 0.74, $p < 0.001$; Finishers: D1 Pearson 0.57, $p < 0.05$, D2 Pearson 0.75, $p < 0.05$; Control group: D1 Pearson 0.85, $p < 0.05$). The relationship between the depressive mood score, tension-anxiety, and fatigue was highly significant in non-finishers. There was a positive correlation between the depressive mood score and leptin at D1 (Pearson 0.64, $p < 0.01$) and D2 (Pearson 0.96, $p < 0.001$) in non-finishers. But also between the depressive mood score and adiponectin (Non-finishers: D1 Pearson 0.50, $p < 0.05$) and NPY (Non-finishers: D2 Pearson 0.92, $p < 0.001$). The depressive mood score correlated negatively with cortisol at D2 in finishers (Pearson -0.67, $p < 0.05$). These findings were not present in other groups.

4.5.3 Anger

In finishers and non-finishers, anger correlated positively with fatigue (Non-finishers: D1 Pearson 0.70, $p < 0.001$; Finishers: D1 Pearson 0.84, $p < 0.01$) and tension-anxiety (Non-finishers: D2 Pearson 0.77, $p < 0.001$; Finishers: POST Pearson 0.73, $p < 0.05$). In finishers, anger correlated positively with cortisol (POST Pearson 0.86, $p < 0.01$), and was also negatively correlated with total quality of recovery (D2 Pearson –0.64, $p < 0.05$). In the control and non-finisher groups, anger correlated positively with NPY (Non-finishers: D2 Pearson 0.73, $p < 0.01$; Control group: D1 Pearson 0.80, $p < 0.05$). In non-finishers also with leptin (D1 Pearson 0.62, $p < 0.01$; D2 Pearson 0.78, $p < 0.001$), adiponectin (D1 Pearson 0.59, $p < 0.01$), cortisol (D2 Pearson 0.47, $p < 0.05$), perceived exertion (D2 Pearson 0.49, $p < 0.05$) and vigor (D2 Pearson 0.73, $p < 0.001$).

4.5.4 Vigor

Vigor was positively correlated with total quality of recovery in non-finishers (PRE Pearson 0.39, $p < 0.05$, D1 Pearson 0.74, $p < 0.001$, D2 Pearson 0.65, $p < 0.01$) and the control group (D1 Pearson 0.82, $p < 0.05$). By contrast, in finishers, it was NPY that correlated positively with total quality of recovery (PRE Pearson 0.70, $p < 0.05$). And leptin correlated negatively with total quality of recovery (POST Pearson –0.75, $p < 0.05$) and vigor (PRE Pearson –0.85, $p < 0.01$) in finishers. In non-finishers, vigor correlated negatively with adiponectin (PRE Pearson –0.66, $p < 0.01$) and NPY (PRE Pearson –0.45, $p < 0.05$).

In non-finishers and the control group, vigor was positively correlated with cortisol (Non-finishers: D2 Pearson 0.71, $p <$

0.001; Control group: PRE Pearson 0.75, $p < 0.05$, D1 Pearson 0.70, $p < 0.05$). Also, vigor was positively correlated with perceived exertion in the control and non-finisher groups (Control group: D1 Pearson 0.74, $p < 0.05$; Non-finishers: D1 Pearson 0.54, $p < 0.05$, D2 Pearson 0.66, $p < 0.01$).

4.5.5 Fatigue

At baseline, fatigue correlated negatively only in non-finishers with total quality of recovery (Pearson −0.61, $p < 0.01$). By contrast, during the race, it correlated positively with the total quality of recovery in the same group (D1 Pearson 0.47, $p < 0.05$, D2 Pearson 0.93, $p < 0.001$). At D1, fatigue correlated positively with adiponectin in finishers (Pearson 0.66, $p < 0.05$) and non-finishers (Pearson 0.50, $p < 0.05$). At D2, fatigue correlated negatively with adiponectin in the control group (Pearson −0.79, $p < 0.05$). In non-finishers and finishers, fatigue correlated positively with perceived exertion (Non-finishers: D1 Pearson 0.58, $p < 0.01$, D2 Pearson 0.89, $p < 0.001$; Finishers: D2 Pearson 0.72, $p < 0.05$, POST Pearson 0.63, $p < 0.05$), but not in the control group. This finding was highly significant in non-finishers at D2. Fatigue correlated positively with cortisol in the non-finisher and control groups (Non-finishers: D1 Pearson 0.46, $p < 0.05$, D2 Pearson 0.87, $p < 0.001$; Control group: D2 Pearson 0.77, $p < 0.05$). Fatigue correlated positively with confusion in non-finishers and finishers (Non-finishers: PRE Pearson 0.50, $p < 0.05$, D1 Pearson 0.77, $p < 0.001$, D2 Pearson 0.99, $p < 0.001$; Finishers: PRE Pearson 0.71, $p < 0.05$, D2 Pearson 0.85, $p < 0.01$).

4.5.6 Confusion

Confusion correlated positively with tension-anxiety in non-finishers and finishers (Non-finishers: PRE Pearson 0.79, $p < 0.01$, D1 Pearson 0.76, $p < 0.001$, D2 Pearson 0.78, $p < 0.001$; Finishers: PRE Pearson 0.85, $p < 0.01$, D2 Pearson 0.78, $p < 0.01$). Whereas in the control group, only tension-anxiety, not fatigue, correlated positively with confusion (D1 Pearson 0.84, $p < 0.05$, D2 Pearson 0.97, $p < 0.001$).

In non-finishers, confusion correlated positively with perceived exertion (D1 Pearson 0.45, $p < 0.05$, D2 Pearson 0.94, $p < 0.001$). Also, confusion correlated positively with cortisol in non-finishers (D2 Pearson 0.91, $p < 0.001$). By contrast, only in finishers, confusion correlated negatively with cortisol (D2 Pearson −0.69, $p < 0.05$), adiponectin (POST Pearson −0.67, $p < 0.05$), and NPY (D1 Pearson −0.78, $p < 0.01$).

5 Discussion

This study examined the relationships between hormonal variables and mood states associated with participation in one of the coldest and longest ultramarathons in the world. The findings show that tension-anxiety scores decreased in finishers while tension-anxiety increased in non-finishers during the race. In both groups, finishers and non-finishers, fatigue scores increased. Noticeable was a decrease in vigor scores and an increase in confusion, anger, and depressive mood scores in non-finishers, not in the other groups. Leptin was associated with anger and a depressive mood state in non-finishers and worse recovery in finishers. In contrast, NPY appeared to be linked to reduced confusion and heightened quality of recovery in finishers.

Tharion et al. (1990) described that confusion significantly increased among athletes partaking in an ultramarathon. In the present study, the level of confusion was higher among all athletes than in the control group at baseline. This suggests an anticipatory affective state that was most likely a consequence of the race participants' conscious thoughts about their circumstances before the race and the upcoming start. By contrast, increased levels of confusion seemed to be disadvantageous during the race. Only in non-finishers did confusion increase during the race and correlated positively with perceived exertion and cortisol. At the same time, cortisol contributed to a lower score of confusion in the finisher group. However, NPY also might have reduced finishers' confusion scores during the race. Thus, these parameters notably appear to influence mood and probably contribute to the athletes' endurance and success. It seems important how high a hormone or peptide rises and at which point of the race to achieve one of these effects on mood.

Lane et al. (2009) suggest that optimal performance depends on an interplay between emotional intelligence and mood states. They indicated that appraisal of own emotions was associated with low anger scores, and utilizing emotions was associated with low confusion and tension-anxiety scores (Lane et al., 2009). Among non-finishers, the level of anger increased during the race, while this effect did not occur among finishers. It is possible that finishers were more aware of their emotions perceived during the race and able to anticipate the potential effect of their emotions on performance. Non-finishers had higher depressive mood scores than the control group. In all three groups, the depressive mood score correlated positively with tension-anxiety and fatigue. Lane and Terry (2000) proposed a model suggesting that depressed mood is a moderating factor in the relationship between anger and tension-anxiety with performance. While anger and tension-anxiety can benefit performance when a depressed mood is absent, both are linked with poor performance when an athlete is experiencing a depressed mood. In their studies, Lane and Terry 2000; Lane, 2001 also showed that anger was associated with poor performance in a concentration grid test when sports students scored high on depressive mood. At the same time, tension-anxiety scores showed no significant relationship with performance in either group (Lane et al., 2001). Specifically, among non-finishers, depressive and anger mood scores increased during the race. Moreover, the depressive mood score correlated positively with anger in the non-finishers. This is in line with Lane and Terry's observation (2000; 2001). There is evidence that an increase in these mood states - depression and anger - during racing can lead to failure in endurance sports.

Anger was associated with fatigue and tension-anxiety in all athletes, whether being finishers or non-finishers. In non-finishers, anger even correlated negatively with the quality of recovery. Tension-anxiety scores were significantly higher in all athletes than in the control group at baseline. Increased tension-anxiety scores in these athletes might be due to the high risks associated with failure and the loss of years of training and preparation. Since non-finishers scored higher on tension-anxiety, they might have perceived higher tension-anxiety than the finisher group during the first part of the race. The athletes described the first part of the route as particularly challenging and physically demanding. Tension-anxiety scores increased in non-finishers throughout the race, while they decreased in finishers. This contradicts one of the results of Lane and Terry (2000) and shows that poorer performance is related to an increase in tension-anxiety scores. The non-finishers seemed to struggle more with the race and their individual physical and psychological stress. This might have negatively influenced them in view of their own failure in this race. Graham et al. (2021) found that mental toughness correlated negatively with tension-anxiety during an Arctic ultramarathon. Tharion et al. (1990) compared finishers and non-finishers of an ultramarathon but did not find higher scores of tension-anxiety in one of these groups. The only significant difference between the groups was that finishers showed higher fatigue scores than non-finishers after the race (Tharion et al., 1990). In the present study, the fatigue score increased in all athletes more than in the control group. Similar to Tharion et al. (1990), no significant difference was found between non-finishers and finishers in fatigue scores during the race. In all athletes, tension-anxiety and fatigue scores correlated positively. It was interesting to note that perceived fatigue negatively affected recovery in non-finishers, while this was not the case in finishers. The finishers possibly found it easier to deal with fatigue without it harming recovery. They might have been mentally tougher. In non-finishers and the control group, vigor scores correlated positively with total quality of recovery. This correlation did not occur within the finishers. The finishers seemed not to depend on their vigor level to recover sufficiently. As has been shown in other studies, the level of vigor decreases during ultramarathon (Tharion et al., 1990; Lane and Wilson, 2011; Graham et al., 2021). Vigor decreased in both groups, finishers and non-finishers, but only in non-finishers was the decrease significant. This decline in vigor potentially affected the performance of non-finishers much more than finishers. Especially vigor is a crucial psychological attribute referring to physical strength, emotional energy, and cognitive liveliness (Shirom, 2011). Better performance seems to be associated with lower vigor scores, while poorer performance with higher confusion and depressive mood scores. Lane et al. (2009) revealed that optimism was associated with a high vigor score (Lane et al., 2009). In the present study, mainly fatigue scores increased during the race while vigor scores decreased in athletes. In other studies, similar effects of ultramarathons affecting mood states have been observed (Tharion et al., 1990; Graham et al., 2012; Rundfeldt et al., 2018; Graham et al., 2021). Slimani et al. (2018) showed that fatigue impairs body and cognitive performance in endurance athletes (Slimani et al., 2018). It is possible that both groups, finishers and non-finishers, are more optimistic than the control group. During the race, decreases in vigor probably affected optimism, but finishers handled the race better. Thus, they were more able to regulate their behavior to counteract negative mood states and emotions. Another study showed that vigor displayed a moderately strong correlation to sleep (Graham et al., 2012). Sleep is especially recognized as an essential component of recovery (Bird, 2013). In non-finishers and the control group, vigor correlated positively with the total quality of recovery, suggesting that vigor is also crucial for recovery. Furthermore, leptin correlated negatively with vigor and total quality of recovery in finishers, whereas NPY correlated positively with total quality of recovery. This shows the importance of these two transmitters in regulating effective recovery in athletes.

Brydon (2011) reported that higher basal leptin displayed greater stress-induced increases in heart rate and decreased heart rate variability (Brydon, 2011). Additionally, cortisol increases the level of leptin (Leal-Cerro et al., 2001). In the present study, we could not establish a relationship between cortisol and leptin. However, in this context, it is interesting to note that Rundfeldt et al. (2018) discovered an increased heart rate variability in finishers, indicating a reduced sympathetic activity. Thus, the finishers were less stressed than non-finishers. During a 25 km swim race, the level of NPY increased and corresponded with the successful completion of the competition (Karamouzis et al., 2002). Karamouzis et al. (2002) found that the increase in NPY was associated with a decrease in leptin. In the present study, leptin was negatively correlated with NPY. This finding occurred only in non-finishers and the control group.

Physical activity has been shown to reduce symptoms of anxiety and depression (Carek et al., 2011). Mainly running affects mood by reducing depression and confusion (Weinberg et al., 1988). In the present study, NPY appeared to decrease confusion scores in finishers, while leptin appeared to increase depressive mood scores. Morgan et al. (2000) showed that higher NPY levels were associated with improved higher performance under interrogation stress in Special Forces soldiers than non-Special Forces soldiers. The Special Forces soldiers' group had a higher dissociation score if the level of NPY increased during interrogation (Morgan et al., 2000). In line with the present study, higher levels of NPY might help to cope with extremely challenging situations, as dissociation is part of our deeply ingrained survival system (Sar and Ross, 2006). Morgan et al. (2001; 2002) suggested that elevated NPY levels could improve self-confidence and performance, thus enhancing stress resistance. NPY might serve as a homeostatic buffer to attenuate stress responses. Genetic NPY alterations with a lower level of NPY are associated with PTSD (Yehuda et al., 2006; Sah et al., 2014) and major depression (Widerlöv et al., 1988; Sharma et al., 2022). Chang et al. (2016) showed that individuals with a genetic variant with high NPY expression had lower scores on anxiety and depression inventories during high stress than individuals with low NPY expression (Chang et al., 2016). Indeed, reduced NPY levels correlate with anxiety behaviors in patients with major depression (Widerlöv et al., 1988). Thus, NPY might reduce psychological distress and exerts anxiety-relieving effects. However, this effect could not be found within the correlation analysis. Instead, NPY slightly affected tension-anxiety scores in the non-finisher group at D2 by increasing them. However, at this measurement point, there was a chaos of feelings and transmitters in non-finishers, and no clear picture emerged.

Also, adiponectin affects mood by reducing depressive symptoms (Cao et al., 2018). But no negative correlation was found in the present study between the depressive mood score and adiponectin. The opposite effect emerged in non-finishers; adiponectin correlated positively with the depressive mood score. Roupas et al. (2013) evaluated the impact of an ultramarathon (180 km) on serum leptin and adiponectin. They could not find any changes in the adiponectin level but a significant decrease in leptin pre-vs. post-exercise (Roupas et al., 2013). Nevertheless, the exposition in the present study compared to that in Roupas' study differs in several aspects. In Roupas et al. (2013), the distance was much shorter, and the race staff provided the participants with food and drinks. But the most important difference compared to the present study was certainly the weather conditions. The ultramarathon took place during the summer and not during cold winter conditions. In another ultramarathon study, adiponectin levels changed. Arakawa et al. (2016) showed that adiponectin levels increase in response to an ultramarathon (180 km) held during summer. While leptin levels first decreased and then increased after the race (Arakawa et al., 2016). This suggests that leptin and adiponectin regulation also depends on other factors besides temperature. In the control and non-finisher groups, adiponectin was related to leptin. Thus, leptin might have increased adiponectin or the other way around. But not in the finisher group; there was even no negative correlation between adiponectin and leptin. Zaccaria et al. (2002) examined the effects of three different endurance events on serum leptin concentrations in athletes: a half marathon (21.097 km), a ski mountaineering race (45 km), and an ultramarathon (100 km). The results suggest that only long-duration endurance exercises with high energy expenditure, such as alpine skiing and ultramarathon, cause a significant reduction in circulating serum leptin levels (Zaccaria et al., 2002). It takes much longer stretches for a change to occur at the hormonal and transmitter levels. Perhaps this is also dependent on the previous training sessions. It seems that from a certain point in extreme situations, a reduction of the leptin level is needed to survive or to cope with strenuous exercise. In non-finishers, leptin was associated with higher depressive mood scores. Clinical studies found elevated leptin levels in individuals with major depressive disorder (Milaneschi et al., 2017). Increases in leptin are associated with starchy food-seeking (Licinio et al., 2014). In contrast, decreased leptin levels and increased NPY levels activate feeding behavior by stimulating the seeking and finding of food (Woods et al., 1998; Licinio et al., 2014), which might be an important evolutionary motivational effect for athletes in such extreme ultramarathons.

In conclusion, the study reveals an essential interplay of hormones and peptides affecting mood states during endurance exercise: Leptin appears to be connected with depressive mood and anger scores, and decreases vigor scores. NPY seems to reduce confusion scores and enhances the quality of recovery. It has also been shown that some mood states are conditional on others: depressive mood might cause confusion, anger, and tension-anxiety. Confusion might cause fatigue and tension-anxiety, while increasing perceived exertion. Vigor might improve the quality of recovery.

However, it should be remembered that this is a small and hard-to-reach population, and conducting a study under such extreme conditions is likely to remain a challenge.

6 Limitations

The race distance limits the present study results because the observed changes in physiological markers of exercise might vary in shorter or longer distances. The race under examination is one of the longest and coldest ultramarathons worldwide. In order to achieve a decent number of participants, the athletes from 2015, 2017, and 2019 were pooled. Thus, caution is needed to generalize the findings to other distances and climate conditions. Furthermore, a limitation of the present study was that it had a pre-experimental design. Participants would compete in cold and normal temperatures in a true experimental model, i.e., a randomized-groups model. However, such an optimal design was not feasible. The study is about changes in mood states and not about the diagnosis of depression in ultramarathon runners. The authors would like to point out that the POMS-SF is a questionnaire, not a clinical diagnostic tool.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics commitee, Charité—Universitaetsmedizin Berlin, Corporate member of Freie Universitaet Berlin, Humboldt-Universitaet zu Berlin, and Berlin Institute of Health, Berlin, Germany (EA4/109/12). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MS designed, planned, and implemented the study. AS, CK, LM-M, and MS performed measurements and collected data. MJ prepared data and provided feedback. NG, MG, CK, and MS, performed statistical analyses. KB performed laboratory analyses. RC contributed to the study design and provided logistical support, expertise, and feedback. CK wrote the manuscript. MAM prepared and planned the study, revised the manuscript and provided feedback. All authors read and approved the final manuscript.

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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.

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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.2022.970016/full#supplementary-material

SUPPLEMENTARY FIGURE S1
POMS-SF scores at the four different time points and the three groups: finisher (FIN, dark grey), non-finisher (NON, middle grey dotted line), and control (CON, light grey dotted line).

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2.5 Fifth paper: Glycocalyx, Stress, Body Composition, and Energy Expenditure

Steinach, M., Biere, K., Coker, R.H., Gaul, A.L., Hoerl, M., Jörres, M., Kienast, C., Mascarell-Maricic, L., Schalt, A., Gunga, H.C., Choukér, A., Rehm, M.: *Influences on glycocalyx shedding during the Yukon Arctic Ultra: the longest and the coldest ultramarathon.* J Appl Physiol., 2022

In this fifth paper we evaluated the changes in the endothelial lining along with stress, body composition and energy expenditure among the YAU study participants of 2015, 2017, and 2019. 13 participants were included in this evaluation of which ten reached the finish after 690 km. Seven volunteers served as control. It was the focus of this work to assess the influence the YAU on endothelial changes expressed through serum glycocalyx (GX) parameters in conjunction with changes in stress parameters, body composition, and energy expenditure.

The endothelial GX lines the lumen of every blood vessel maintaining its integrity and controlling vascular function (59). Stress and trauma may lead to degradation ("shedding") of the GX (60), so that its elements can be quantified in peripheral blood. High intensity exercise has been shown to induce shedding (61-63), however, no studies have investigated shedding of GX elements among healthy athletes in reaction to a marathon or ultramarathon, let alone in subarctic climate. Furthermore, previous studies regarding exercise induces GX shedding only investigated changes in male participants but not among women. It was therefore of interest to evaluate GX changes during the YAU and to evaluate any changes regarding influences of age, sex, body composition, energy expenditure, and to relate such changes to other stress parameters, such as creatine kinase, cortisol, CRP, and NT-Pro BNP. Figure 9 depicts the evaluated parameters of this study in the context of the integrated evaluation.

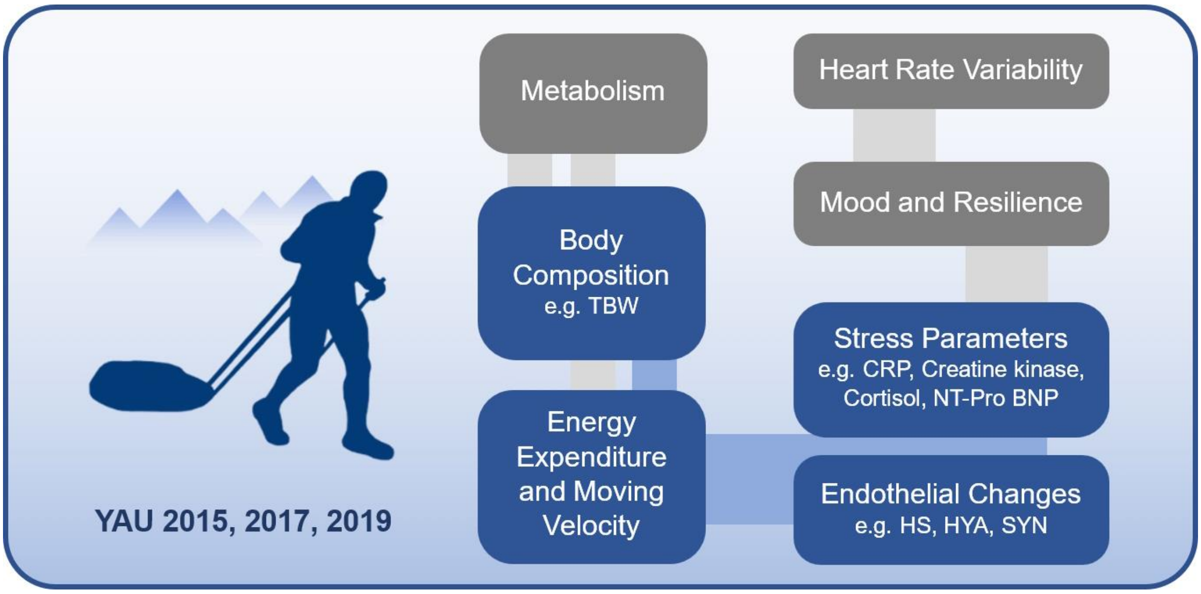

Figure 9: Evaluated parameters (dark blue boxes) in the fifth paper. (Graphic created by Mathias Steinach)

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RESEARCH ARTICLE

Influences on glycocalyx shedding during the Yukon Arctic Ultra: the longest and the coldest ultramarathon

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Abstract

The endothelial glycocalyx maintains vascular structure and may be subject to shedding during inflammation and also during high-intensive exercise. There are no studies on shedding during ultra-endurance exercise. The "Yukon Arctic Ultra" (YAU) is one of the longest and coldest ultramarathons and its impact on glycocalyx shedding was investigated. Thirteen adults (38.92 ± 8.67 yr, 6 females) of YAU editions 2015–2019 completed 657.03 ± 71.65 km at a moving velocity of 4.17 ± 0.62 km/h. Mean daily temperatures ranged from −12.6°C to −30.5°C. Glycocalyx elements heparan sulfate, hyaluronan, and syndecan CD-138 were quantified from serum at start, 277 km, 383 km, and 690 km. Cortisol, C-reactive protein, creatine kinase, and N-terminal-prohormone of brain natriuretic peptide were also quantified. Seven YAU volunteers (36.14 ± 11.04 yr, 5 females) served as control. There were no time-changes among the control. Among finishers, there was a significant increase for hyaluronan and a significant decrease for syndecan CD-138. Values were greater among female finishers for heparan sulfate at start, 383 km, and 690 km, and among male finishers for hyaluronan at 277 km. Values for syndecan CD-138 were greater among older finishers at all timepoints. There were weak significant correlations ($R^2 <$ 0.215) between hyaluronan and distance, creatine kinase, and NT-Pro BNP, respectively. Shedding of glycocalyx elements is shown among participants of the YAU. Greater shedding of heparan sulfate among female, greater increases of hyaluronan among male, and greater shedding of syndecan CD-138 among older athletes indicate complex glycocalyx shedding during ultra-endurance exercise.

NEW & NOTEWORTHY This is the first study to investigate changes in glycocalyx elements in an endurance footrace and first study to investigate exercise-induced shedding in both sexes. This study comprised of an athlete group who finished the ultra-long distance of up to 690 km during the Yukon Arctic Ultra as well as a control group. Results indicate relevant and different shedding of glycocalyx elements heparan sulfate, hyaluronan, and syndecan CD-138. Sex, age, BMI, and covered distance appear to have an influence on the shedding. Other serum parameters indicative of stress appear to be associated with shedding.

cold environment; endurance; heparan sulfate; hyaluronan; syndecan

INTRODUCTION

An increasing number of athletes engage in ultra-endurance events, such as ultramarathons, i.e., distances longer than 42.195 km (1). Furthermore, the number of athletes seeking additional challenges by partaking in ultramarathons in extreme environments, is increasing as well (2), which provide an excellent opportunity to investigate human resilience and adaptability (3).

The Yukon Arctic Ultra (YAU), taking place in the Yukon Territory in February each year, is considered the longest and the coldest ultramarathon in the world (4), as it combines the challenge to complete the distance of 690 km with the extreme climate conditions of subarctic winter. YAU athletes are faced with the challenges of long-term endurance exercise, prolonged exposure to cold climate, and diminished resting conditions.

Every healthy blood vessel is lined by an endothelial glycocalyx, comprising of proteoglycans, glycolipids, glycosaminoglycans, and glycoproteins (5). They form a mesh-like network into which other soluble molecules of plasmatic and endothelial origin can bind so that composition and

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thickness vary with values between 0.2 μm for smaller vessels to 5 μm in larger arteries (6). Its main components are membrane-bound proteoglycans, such as syndecan, to which other glycosaminoglycans, such as hyaluronan (HYA) and heparan sulfate (HS), are attached (7). The glycocalyx plays essential roles in maintaining vascular integrity (8), controlling vascular permeability (9), mechanotransduction (10), and shear stress-induced vasodilation, inflammation, and coagulation (5, 6). The glycocalyx may be subject to enzymatic and shear stress-induced shedding, so that quantification of glycocalyx elements as biomarkers in peripheral blood has been introduced to be used in the evaluation of various pathological states, such as sepsis, trauma, and ischemia-reperfusion injuries (6).

Only few studies have investigated the relationship between glycocalyx shedding and exercise, indicating that regular moderate exercise may support to maintain the integrity of glycocalyx elements among healthy young men (11). Chronically strained muscles in rodents may develop a disruption of their capillaries' glycocalyx, which was attributed to shear stress caused by increased exercise-induced blood flow (12). Further research showed that glycocalyx shedding takes place among untrained participants performing at high intensities of 70% of the individual maximal oxygen consumption ($\dot{V}O_{2max}$) (13), among moderately active participants during short-term moderate and high-intensity exercise testing (10), and among well-trained athletes performing at very high intensities of 85% peak power (14), whereas two other studies among untrained (15) and moderately active participants (16) showed no changes in glycocalyx shedding during high-intensity exercise cycling test.

To our knowledge, there exist no studies that have investigated shedding of glycocalyx elements among healthy human adults in reaction to the continuous endurance strain of a marathon or ultramarathon, let alone in subarctic climate. We had formulated four distinct hypotheses as basis of this investigation: *1*) that there would be time effects on serum levels of glycocalyx elements heparan sulfate (HS), hyaluronan (HYA), and syndecan CD-138 (also known as syndecan 1, SYN) for YAU athlete group; *2*) that there would be effects on serum levels of glycocalyx elements between the athlete group and the control group at each time point; *3*) that there would be influences of sex, age, and body mass index (BMI) when evaluating athlete subgroups regarding serum levels of glycocalyx elements at each time point and through comparison of each subgroup's time effect; and *4*) that there would be correlation among serum levels of glycocalyx elements, parameters known to indicate stress [cortisol (COR), C-reactive protein (CRP), creatine kinase (CK), N-terminal-prohormone of brain natriuretic peptide (NT-Pro BNP)] and covered distance by the athlete group.

METHODS

The Yukon Arctic Ultra: The Longest and the Coldest Ultramarathon

The Montane Yukon Arctic Ultra is an ultramarathon that takes place in the Yukon Territory of North-Western Canada (17). The race starts at the first Sunday of each February and the distances alternate biennially where the longest distance of 690 km (430 mi) is conducted during every odd-numbered year. Athletes can enroll in three categories: hiking footrace, cross-country skiing, and mountain biking. The race is limited to 14 days by which it must be completed. The trail of the YAU is prepared with snowmobiles making it relatively stable to hike and the trail is marked with poles every 20 to 30 m and athletes use maps and GPS-devices to navigate. The trail-surface is mostly flat; however, cumbersome elevations can occur by piled-up ice floes and there are general elevation gains of ∼500–700 m for the first and last sections of the race. Each athlete is equipped with a GPS-device (Spot, Spot LLC, VA) that allows being tracked and athletes can use the device to call for emergency assistance. Athletes must partake in a training course concerning the possible hazards of the YAU and demonstrate their ability to cope with the challenges. Medical staff is on duty at ten checkpoints ∼50 km apart to administer medical support, and to screen for injuries, especially frostbite, in which case the athlete would be disqualified. Except for these few resting facilities, athletes are required to conduct all activities, including preparation of meals, personal hygiene, resting, etc., on the trail. The weather conditions have been previously reported in detail for YAU editions 2015 to 2017 (18), with conditions not being different in 2019. Mean 24-h temperatures in Whitehorse at the start in 2015, 2017, and 2019 were: −26.6°C, −21.1°C, and −30.5°C, respectively; mean 24-h temperatures in Dawson City, each time at the finish were: −12.6°C, −24.0°C, and −22.9°C, respectively (19). More detailed information about the Montane YAU can be found on the official website (17).

Subjects and Study Implementation

A total number of 93 athletes enrolled to compete in the 690 km footrace of the YAU during the years 2015 (19), 2017 (35), and 2019 (39). From those, n = 32 (13 females and 19 males) athletes volunteered to partake in the study as participants (n = 9 in 2015, n = 10 in 2017, and n = 13 in 2019). Most participants (n = 31) were of Caucasian descent and one male athlete was of Asian origin.

From these 32 athletes participating in the study, n = 10 athletes were able to complete the YAU of 690 km (n = 1 in 2015, n = 5 in 2017, and n = 4 in 2019). In addition, a further three athletes in 2015 reached considerable distances of 483 km and 515 km (2 men), and 643 km (1 woman), respectively, with whom the collection of serum samples and measurements were possible under the same conditions as with the 690 km finishers stated earlier, so that these three participants were also included as finishers in our analyses. Thus, this report includes a total n = 13 athletes (6 females, 7 males) of the YAU editions 2015 to 2019 as finishers of the YAU footrace. Furthermore, in 2019, a control group of n = 7 (5 females, 2 males) was included in the measurements and serum sample collection, which were obtained at the respective checkpoints as with the athletes. This control group consisted of the volunteers, medical staff, and scientists during the YAU 2019. The groups will be referred to as "FIN" and "CON" henceforth.

Among FIN, 12 of 13 athletes (92.3%) had prior ultramarathon experience and 9 (69.2%) had engaged in the YAU before taking part in the study. In CON, 3 of 7 (42.9%) had ultramarathon experience and one person (14.3%) had previously partaken in the YAU.

The recruitment was conducted through the YAU organizers to the athletes enrolled in the 690 km footrace as well as to the volunteers for CON in 2019. Interested individuals then contacted the principal investigator through e-mail and received further detailed information. The potential study participants had several weeks to pose questions and to decide whether to partake in the study. All adult athletes enrolled in the YAU 690 km footrace were eligible for the study; there existed no further inclusion or exclusion criteria. All athletes had to present a health certificate issued by their physician to the race organizers to be enrolled in the race. Four to seven days before the race-start, the potential study participants met with the investigators in Whitehorse, Yukon, Canada, to finally give their informed written consent to take part in the study. The study was approved by the Charité Ethics Board (IRB-Number: EA4/109/12). All conducted measurements and procedures complied with the Declaration of Helsinki (7^{th} revised version, 64^{th} World Medical Association meeting, Fortaleza, Brazil) concerning the treatment of human subjects.

Experimental Protocol, Measurements, and Serum Collection

Baseline.

During three to seven days preceding the race (PRE), baseline anthropometric data were obtained. Body mass was measured using a calibrated scale (Seca GmbH, Hamburg, Germany, precision: 0 to 50 kg = ±50 g, 50 to 150 kg = ±100 g, above 150 kg = ±200 g) while height was taken from interview statements. Baseline body composition was measured using bioelectrical impedance analysis (BIA), a widely accepted method to determine body composition (20), with the BIA-101 device (Akern, Florence, Italy) on the participants who had remained in a supine position for at least 10 min. Fat mass (FM), lean body mass (LBM), skeletal muscle mass (SMM), as percentage from body mass as well as total body water (TBW, L) were calculated using appropriate equations (21–23). To evaluate the participants' endurance training status, resting heart rates (RHR) were obtained upon awakening while in supine position for 10 min using a validated heart rate monitor (RS800CX, Polar, Kempele, Finland) during the years 2015 and 2017 (24) and via a validated mobile ECG-system (Faros, Bittium, Oulu, Finland) in 2019 (25).

Fifteen milliliters of blood were collected under aseptic conditions by a trained physician from a cubital vein into tubes containing clot activator (Sarstedt S-Monovette, Sarstedt GmbH und Co. KG, Nümbrecht, Germany) and immediately stored at $+2$ to $+8°$C. The samples were centrifuged (10 min at 2,000 *g*) to separate the serum, which was pipetted into cryovials. Finally, the serum samples were stored in a liquid nitrogen dewar at below −80°C for transport.

All measurements and blood collections were conducted after the participants' overnight rest, in a quiet environment, with voided bladders and before breakfast. For body mass and BIA measurements, participants only wore light underwear.

In-race and post-race.

Further measurements and serum collections were conducted at two in-race checkpoints and at the finish. The checkpoints were chosen for accessibility, being indoors, availability of electrical power and running water, and reasonable quietness and ambient temperature to perform all procedures after the participants' overnight rest under comparable controlled conditions as at PRE. To this end, the first checkpoint during the race (During 1, D1) was located after 277 km in the city of Carmacks, the second checkpoint (During 2, D2) was located after 383 km in the city of Pelly Crossing, and finally the post measurements (POST) took place after 690 km in Dawson City. Body mass and BIA measurements were conducted the same manner as at PRE to track changes in body mass and TBW. To account for the participants' activity throughout the YAU, all were equipped with validated actimeter (Sensewear, Bodymedia, Pittsburgh, PA) that allowed the calculation of their mean daily energy expenditure (26).

Analysis of Serum Parameters

Without interrupting the cooling chain, the stored serum samples were transported to Berlin, Germany and Munich, Germany, to be analyzed. Due to limited volumes of serum samples, four measurements were missing at D1 among FIN and two were missing at D1 among CON.

Analysis of glycocalyx parameters.

All glycocalyx parameters were analyzed in the Laboratory of Translational Research "Stress and Immunity," Department of Anesthesiology, Ludwig-Maximilians-University Munich, Germany, by the same trained laboratory-technicians. Commercially available enzyme-linked immunosorbent assay (ELISA) kits for heparan sulfate (HS): Seikagaku Corporation, Tokyo, Japan; for hyaluronan (HYA): Echelon Biosciences Inc., Salt Lake City, UT; and for syndecan CD-138 (SYN): Diaclone SAS, Besançon, France, were used to quantify the three glycocalyx parameters in the serum samples, according to each manufacturer's instruction. The intra/inter-assay variability for the ELISA kits was as follows: HS: 6.4%/4.9%, HYA: 7.1%/5.0%, and SYN: 3.6%/5.7%. All samples were analyzed in duplicates.

Analysis of other serum stress parameters.

In a certified laboratory (Labor 28 GmbH, Berlin, Germany, accredited at the "DAkkS," Deutsche Akkreditierungsstelle GmbH), the serum samples were additionally analyzed for cortisol (COR), C-reactive protein (CRP), creatine kinase (CK), and N-terminal-prohormone of brain natriuretic peptide (NT-Pro BNP). COR and NT-Pro BNP were both analyzed using electrochemiluminescence immunoassays (ECLIA) with monoclonal antibodies specifically directed against COR and NT-Pro BNP, respectively, measured with a calibrated Cobas E 411 Analyzer (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). CRP was analyzed using particle enhanced immunoturbidimetric assays. CRP agglutinated with latex particles coated with monoclonal anti-CRP antibodies. The aggregates were determined turbidimetrically through a 2-point end assay type measured with a calibrated Cobas C 701/702 Analyzer (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). CK was analyzed following the recommendation of the International Federation of Clinical Chemistry (IFCC) based on enzyme-facilitated reactions leading to the formation of NADPH, which was analyzed with a calibrated Cobas C 701/702

Analyzer (Roche Diagnostics International Ltd., Rotkreuz, Switzerland), where the photometrically measured rate of formation of NADPH was directly proportional to the CK activity. The intra-/intertest variability was as follows: COR: 1.4%/2.7%, CRP: 0.9%/2.2%, CK: 0.5%/1.4%, NT-Pro BNP: 2.7/3.8%. All samples were analyzed in duplicates.

Data Analysis and Statistics

Definition of cohorts.

Regarding sex distribution among both groups a simple χ^2 test was applied.

Anthropometric data, resting heart rate, and race performance.

Regarding anthropometric data (age, body mass, height, BMI, FM, LBM, SMM, TBW, and RHR), normal distribution was tested with Shapiro–Wilk test and variance with Equal-Variance test. The data were tested with *t* tests for statistical difference for between group effects (Women vs. Men, and FIN vs. CON) and regarding data on race performance for FIN (distance covered, finishing time, average total velocity, and average moving velocity), which were evaluated from each athletes' GPS-device data and between-group effects were tested for statistical difference (Women vs. Men) using *t* tests. Average total velocity was defined to be the gross velocity for the entire race including resting and sleeping periods, while average moving velocity was defined to be the net velocity only while being in movement. Changes in body mass and TBW were tested through analysis of variance for repeated measurements (RM-ANOVA).

Determination of glycocalyx elements.

Within-group effects "FIN and CON over time". Regarding *hypothesis 1*, for within-group effects in FIN and CON over time (PRE, D1, D2, POST) for HS, HYA, and SYN, analysis of variance for repeated measurements (RM-ANOVA) were applied. For non-normally distributed data, RM-ANOVA on ranks was applied. In cases of significant effects over time, post hoc multiple comparison procedures (Holm–Sidak test), or rank-based post hoc multiple comparison procedures (Tukey test) were used to test between time points.

Between-group effects "FIN versus CON" and between- and within-group effects "Sex". Regarding *hypothesis 2* between-group effects FIN versus CON and regarding *hypothesis 3*, between-group effects Women versus Men among FIN at each time point for HS, HYA, and SYN, *t* tests were used. For non-normally distributed data, nonparametric rank-sum tests were applied (Mann–Whitney *U* test). In addition, within-group effects over time were tested for both sexes among FIN as described above for FIN and CON over time. In addition, an analysis of covariance (ANCOVA) was performed to evaluate the influence of sex (male or female) as covariate. The actimeter data between FIN and CON were compared through *t* test.

Between- and within-group effects "Age" and between- and within-group effects "BMI". Regarding *hypothesis 3* between-group effects age, the median age among FIN was calculated (38 yr) and chosen as cut-off value for an even distribution. For between-group effects among FIN at each time point for HS, HYA, and SYN, *t* tests were used. For non-normally distributed data, nonparametric rank-sum tests were applied (Mann–Whitney *U* test). In addition, within-group effects over time were tested for both age groups among FIN as described above for FIN and CON over time. In addition, an analysis of covariance (ANCOVA) was performed to evaluate the influence of age (distinct values) as covariate.

Regarding *hypothesis 3* between-group effects BMI, the same analysis was performed regarding BMI with the median BMI among FIN (23.5 kg/m^2) chosen as cut-off value for an even distribution. This analysis was included because there was no statistical difference in BMI between the sexes so that BMI could be tested as a separate entity (see RESULT). In addition, within-group effects over time were tested for both BMI groups among FIN as described above for FIN and CON over time. In addition, an analysis of covariance (ANCOVA) was performed to evaluate the influence of BMI (distinct values) as covariate.

Correlation with "Completed Distance," "Age," and "Other Serum Stress Parameters". Regarding *hypothesis 4*, simple linear correlation analyses were performed for HS, HYA, and SYN being the dependent variables and "Completed Distance" being the independent variable among FIN; for "Age" being the independent variable among FIN and CON, and for other serum stress parameters (COR, CRP, CK, and NT-Pro BNP) obtained at the same time points.

For graphic representation, boxplots were plotted for within-group effects "FIN and CON over time" including between-group effects "FIN vs. CON" as well as for within- and between-group effects among FIN separated for "Sex," "Age," and "BMI" for HS, HYA, and SYN, respectively. Boxes represent the 25th to 75th percentile range and median line within, whereas error bar whiskers represent the minimum and maximum ranges. Significant differences are marked with asterisks, one asterisk indicating a *P* value of $0.01 \leq P < 0.05$, two indicating $0.001 \leq P < 0.01$, and three indicating $P < 0.001$. All statistical analyses and plotting for graphic representation were performed using the statistical program-package "SigmaPlot" Version 14.5 (Systat Software Inc., San José, CA). Data are reported as means ± standard deviation (SD). Statistical significance was assumed at a *P* value of <0.05.

RESULTS

Definition of Cohorts, Anthropometric Data, and Resting Heart Rate

Among FIN, the sexes (6 females, 7 males) were equally distributed (χ^2 value = 0.143, P = 0.705). Also, among CON, with 5 female and 2 male participants, there was no significant difference in the distribution of both sexes (χ^2 value = 1.800, P = 0.179).

The anthropometric data are presented in Table 1. The female study participants exhibited a significantly lower body mass, were of smaller height, and showed greater percentage of FM, lower percentage of LBM, and lower TBW, respectively, than men, both among FIN and CON. There were no differences between the sexes for age (mean age for all participants: 37.95 ± 9.37 yr) nor for BMI (mean BMI for all participants: 23.45 ± 2.85 kg/m^2) nor for RHR (mean RHR for all participants: 62.53 ± 9.18 beats/min). Male CON exhibited greater SMM than female CON. There were no statistical

Table 1. *Anthropometric data and resting heart rate*

Parameter	FIN (*n* = 13; *w* = 6, *m* = 7)	CON (*n* = 7; *w* = 5, *m* = 2)	*P* value (FIN vs. CON)
Age, yr			
All (means ± SD)	38.92 ± 8.67	36.14 ± 11.04	0.541
Women (means ± SD)	36.67 ± 5.82	36.60 ± 12.54	0.991
Men (means ± SD)	40.86 ± 10.16	35.00 ± 9.90	0.509
P value (Women vs. Men)	0.408	0.880	
Body mass, kg			
All (means ± SD)	71.85 ± 11.60	67.43 ± 7.84	0.381
Women (means ± SD)	63.98 ± 9.31	63.52 ± 4.69	0.923
Men (means ± SD)	78.60 ± 9.03	77.20 ± 3.68	0.843
P value (Women vs. Men)	**0.015**	**0.015**	
Height, cm			
All (means ± SD)	172.3 ± 8.8	174.6 ± 11.4	0.625
Women (means ± SD)	166.5 ± 7.8	169.4 ± 8.9	0.578
Men (means ± SD)	177.3 ± 6.3	187.5 ± 0.7	0.064
P value (Women vs. Men)	**0.018**	**0.041**	
Body mass index, kg/m^2			
All (means ± SD)	24.13 ± 2.86	22.21 ± 2.57	0.156
Women (means ± SD)	23.07 ± 2.76	22.30 ± 3.08	0.673
Men (means ± SD)	25.03 ± 2.83	21.96 ± 1.21	0.193
P value (Women vs. Men)	0.234	0.891	
Fat mass, %			
All (means ± SD)	20.68 ± 5.02	21.70 ± 6.64	0.702
Women (means ± SD)	24.66 ± 3.72	25.25 ± 3.24	0.785
Men (means ± SD)	17.27 ± 3.08	12.83 ± 1.36	0.098
P value (Women vs. Men)	**0.002**	**0.004**	
Lean body mass, %			
All (means ± SD)	79.32 ± 5.02	78.30 ± 6.64	0.702
Women (means ± SD)	75.34 ± 3.72	74.75 ± 3.24	0.785
Men (means ± SD)	82.73 ± 3.08	87.17 ± 1.36	0.098
P value (Women vs. Men)	**0.002**	**0.004**	
Skeletal muscle mass, %			
All (means ± SD)	40.77 ± 5.84	38.61 ± 7.81	0.490
Women (means ± SD)	37.66 ± 5.06	34.45 ± 3.92	0.278
Men (means ± SD)	43.43 ± 5.37	48.98 ± 1.72	0.210
P value (Women vs. Men)	0.073	**0.005**	
Total body water, L			
All (means ± SD)	41.12 ± 7.00	37.48 ± 9.06	0.330
Women (means ± SD)	34.80 ± 3.78	32.34 ± 1.16	0.197
Men (means ± SD)	46.54 ± 3.46	50.33 ± 4.97	0.243
P value (Women vs. Men)	**<0.001**	**<0.001**	
Resting heart rate, beats/min			
All (means ± SD)	58.71 ± 6.77	69.63 ± 9.21	**0.007**
Women (means ± SD)	60.80 ± 8.13	67.56 ± 10.24	0.252
Men (means ± SD)	56.91 ± 5.33	74.80 ± 3.82	**0.003**
P value (Women vs. Men)	0.323	0.396	

Anthropometric data and resting heart rate are stated as means ± SD. *P* values are stated for differences between groups (FIN vs. CON) for all study participants and separately for women and men; and between sexes (women vs. men) for all study participants and separately for FIN and CON, with "FIN" being the athlete group and "CON" being the control group. Statistically significant *P* values printed in bold.

differences for any anthropometric data between groups (FIN vs. CON), neither among all participants nor among women nor men separately. RHR among FIN was significantly lower compared with CON and among male FIN compared with male CON.

Race Performance

Race performance results are presented in Table 2. Ten study participants completed the entire YAU and three athletes of 2015 reached considerable distances beyond D2, and were thus included among FIN. To this end, the finishers completed a mean distance of 657.03 ± 71.65 km, with no statistical differences between women and men. Men completed their distances in significantly shorter time than women (233.99 ± 33.48 h vs. 284.15 ± 21.88 h, $P = 0.01$) and exhibited a faster average moving velocity (4.55 ± 0.24 km/h vs. 3.73 ± 0.65 km/h, $P = 0.009$), whereas there was no difference in average total velocity between both sexes. Evaluation of actimeter data revealed that FIN exhibited a mean daily energy expenditure of 25,253.5 ± 4,441.8 kJ vs. 11,929.2 ± 2,388.9 kJ for CON ($P < 0.001$). Expressed as physical activity level in terms of metabolic equivalents (MET), where 1 MET resembles resting metabolic rate, the actimeter data revealed respective results with 3.5 ± 0.45 MET for FIN vs. 1.7 ± 0.31 MET for CON ($P < 0.001$). The evaluation of changes in TBW revealed a significant increase in total body water among FIN throughout the YAU from 41.12 ± 7.00 L at PRE to 44.81 ± 7.60 L at POST ($P < 0.001$), whereas body mass among FIN decreased from 71.85 ± 11.60 kg at PRE to 68.68 ± 11.22 kg at POST ($P < 0.001$). There were no significant changes for these parameters among CON (TBW: 37.48 ± 9.06 L at PRE to

Table 2. *Race performance*

Parameter	All Finisher (*n* = 13; *w* = 6, *m* = 7)	Women (*n* = 6)	Men (*n* = 7)	
Distance covered, km				*P* value (Women vs. Men)
Means ± SD	657.03 ± 71.65	682.29 ± 18.89	635.38 ± 93.74	0.256
Finishing time, h				
Means ± SD	257.14 ± 37.91	284.15 ± 21.88	233.99 ± 33.48	**0.010**
Average total velocity, km/h				
Means ± SD	2.51 ± 0.40	2.42 ± 0.23	2.59 ± 0.52	0.467
Average moving velocity, km/h				
Means ± SD	4.17 ± 0.62	3.73 ± 0.65	4.55 ± 0.24	**0.009**

Race performance data are stated as means ± SD. *P* values are stated for differences between sexes (Women vs. Men). Statistically significant *P* values printed in bold.

37.66 ± 9.01 L at POST, $P = 0.180$; body mass: 67.43 ± 7.84 kg at PRE to 67.56 ± 8.06 kg at POST, $P = 0.394$).

Determination of Glycocalyx Elements

Within-group effects "FIN and CON over time" and between-group effects "FIN versus CON".

The results for within-group effects "FIN and CON over time" and between group effects "FIN versus CON" for HS, HYA, and SYN are presented in Table 3.

The results indicate that there were no changes over time among CON for any of the glycocalyx elements. Among FIN, there were no changes over time for HS. There was a significant increase over time among FIN for HYA ($P = 0.001$) as indicated by the asterisks in Fig. 1*B* with subsequent multiple comparison analysis revealing significant differences between PRE versus D1 ($P = 0.002$), PRE versus D2 ($P < 0.001$), and PRE versus POST ($P = <0.001$). In addition, there was a significant decrease over time among FIN for SYN ($P = 0.007$) as indicated by the asterisks in Fig. 1*C* with subsequent multiple comparison analysis revealing significant difference between PRE versus D1 ($P = 0.008$), PRE versus D2 ($P = 0.001$), and PRE versus POST ($P < 0.001$). Detailed results of the respective multicomparison analyses can be found in the Supplemental Material.

Regarding between-group effects, there were significantly greater values among CON compared with FIN for HS at time points PRE ($P = 0.002$), D2 ($P = 0.013$), and POST ($P = 0.01$) as indicated by the asterisks in Fig. 1*A*. In addition, there were significantly greater values among FIN compared with CON for HYA at time point D2 ($P = 0.017$) as indicated by the asterisks in Fig. 1*B*. There were no differences between groups for SYN.

Within- and between-group effects and ANCOVA "Sex," "Age," and "BMI".

The results regarding between- and within-group effects and ANCOVA among FIN regarding sex, age, and BMI for HS, HYA, and SYN are presented in Table 4. As for all participants among FIN, there were comparable changes over time when separating for sex, for age, and for BMI. Specifically, there were no changes over time for HS separately for women and men, nor for finishers younger and older than 38 yr, nor for finishers with a BMI below and above 23.5 kg/m^2. Likewise, as for all participants among FIN, changes over time can be found for HYA and SYN, both among women and men separately (HYA increases: Women $P = 0.004$ and Men $P = 0.001$; SYN decreases: Women $P = 0.002$ and Men $P = 0.007$); for younger and older finishers (HYA increases: Age <38 yr $P = 0.002$ and Age >38 yr $P = 0.005$; SYN decreases: Age <38 yr $P = 0.005$ and Age >38 yr $P = 0.001$); and for finishers with a BMI below and above 23.5 kg/m^2 (HYA increases: BMI <23.5 kg/m^2 $P = 0.014$ and BMI >23.5 kg/m^2 $P < 0.001$; SYN decreases: BMI <23.5 kg/m^2 $P = 0.004$ and BMI >23.5 kg/m^2 $P = 0.003$) as indicated by the

Table 3. *Determination of glycocalyx elements: within-group effects "FIN and CON over time" and between-group effects "FIN vs. CON"*

Parameter	PRE	D1	D2	POST	*P* value within group effects "FIN and CON" over time
Heparan sulfate, ng/mL					
FIN (means ± SD)	1,705.0 ± 972.7	3,127.1 ± 2,629.2	2,932.8 ± 3,361.9	3,460.9 ± 5,301.1	0.175
CON (means ± SD)	3,792.9 ± 1,457.3	3,836.2 ± 1,294.9	5,091.9 ± 979.5	5,342.3 ± 2,007.1	0.222
P value between-group effects "FIN vs. CON"	**0.002**	0.171	**0.013**	**0.010**	
Hyaluronan, ng/mL					
FIN (means ± SD)	126.7 ± 50.8	286.9 ± 135.2	284.4 ± 165.4	248.9 ± 135.6	**0.001**
CON (means ± SD)	124.2 ± 28.9	167.2 ± 46.2	130.7 ± 19.3	153.0 ± 39.4	0.131
P value between-group effects "FIN vs. CON"	0.634	0.182	**0.017**	0.081	
Syndecan CD-138, ng/mL					
FIN (means ± SD)	43.7 ± 28.1	39.6 ± 22.9	33.4 ± 25.8	30.2 ± 23.3	**0.007**
CON (means ± SD)	49.0 ± 24.5	41.5 ± 18.1	38.9 ± 13.7	39.2 ± 14.2	0.320
P value between-group effects "FIN vs. CON"	0.681	0.877	0.303	0.369	

Data for heparan sulfate (HS), hyaluronan (HYA), and syndecan CD-138 (SYN) are stated as means ± SD. *P* values are stated for within group effects "FIN and CON" over time as well as for between group effects "FIN vs. CON" at each time point ("PRE" at baseline, "D1" at 277 km, "D2" at 383 km, and "POST" after the race at 690 km), with "FIN" being the athlete group and "CON" being the control group. Statistically significant *P* values printed in bold.

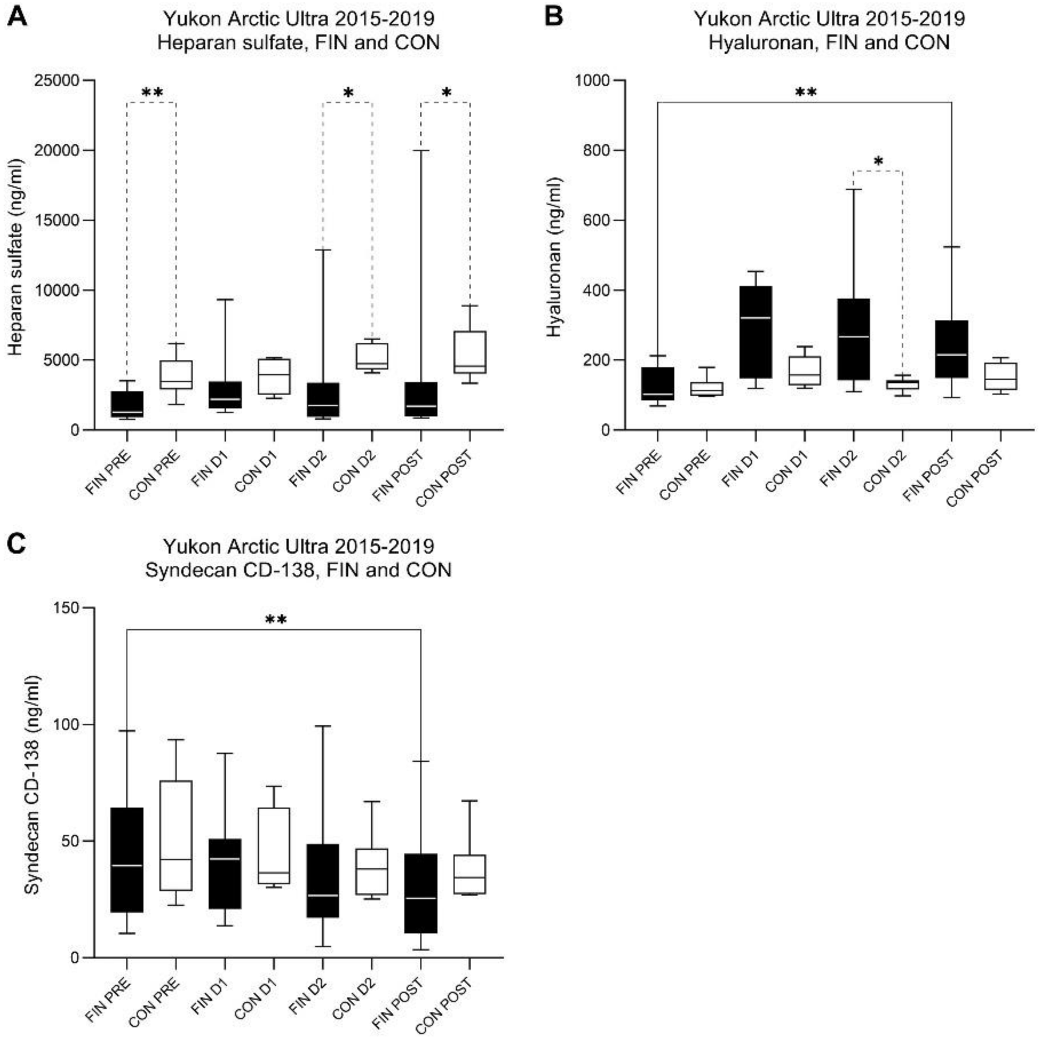

Figure 1. Boxplots for within-group effects "FIN and CON over time" and for between-group effects "FIN vs. CON" with black boxes representing "FIN" and white boxes representing "CON", with "FIN" being the athlete group and "CON" being the control group. Each plot represents the concentration of each glycocalyx element in the serum. Results for heparan sulfate (HS; *A*), for hyaluronan (HYA; *B*), and for syndecan CD-138 (SYN; *C*), respectively. The *x*-axis signifies the respective groups and measurement time points ("PRE" at baseline, "D1" at 277 km, "D2" at 383 km, and "POST" after the race at 690 km). Due to limited volumes of serum samples, four measurements were missing at D1 among "FIN" and two were missing at D1 among "CON". The dashed line signifies statistical difference regarding between-group effects while the solid line signifies within-group effects over time, with *P* value of *$0.01 \leq P < 0.05$ and **$0.001 \leq P < 0.01$.

asterisks in Fig. 2, *B* and *C*, Fig. 3, *B* and *C*, and Fig. 4, *B* and *C*, respectively. Detailed results of the respective multicomparison analyses can be found in the Supplemental Material.

In addition, results indicate the following between-group effects: for HS, women exhibited significantly greater values than men at PRE ($P = 0.007$), at D2 ($P = 0.003$), and at POST ($P = 0.018$), which was corroborated by ANCOVA ($P = 0.002$). For HYA, men showed significantly greater values than women at D1 ($P = 0.001$), which was corroborated by ANCOVA ($P < 0.001$). Finally, results indicate an age-related effect for SYN, with significantly greater values throughout all time points for the older finishers compared with the younger ones (PRE: $P = 0.015$, D1: $P = 0.016$, D2: $P = 0.025$, and POST: $P = 0.034$, respectively), which was corroborated by ANCOVA ($P < 0.001$). There were no sex-related differences for SYN and no age-related differences for HS and HYA. There were no BMI-related differences for neither of the glycocalyx elements when tested through *t* tests after categorization in above and below median BMI; however, ANCOVA revealed that athletes with higher BMI values showed significantly greater values of HYA ($P = 0.036$). Figures 2, 3, and 4 represent the within- and between-group effects for sex, age, and BMI, with asterisks indicating significant differences.

Correlation with "Completed Distance," "Age," and "Other Serum Stress Parameters".

Regarding correlation of glycocalyx elements with completed distance among FIN, only HYA showed a weak significant

Table 4. *Determination of glycocalyx elements: within-group effects over time, between-group effects, and ANCOVA for "Sex," "Age," and "BMI" among the athlete group (FIN)*

Parameter	PRE	D1	D2	POST	
					P value within group effects "Sex" over time
Heparan sulfate, ng/mL					
Women (means ± SD)	2,513.6 ± 1,047.1	4,530.5 ± 3,262.4	5,284.8 ± 4,351.6	6,304.9 ± 7,700.6	0.314
Men (means ± SD)	1,127.4 ± 263.6	1,723.7 ± 484.3	1,252.8 ± 439.9	1,429.6 ± 672.3	0.219
P value between-group effects "Sex"	**0.007**	0.140	**0.003**	**0.018**	
	ANCOVA for covariate "Sex" *P* value = **0.002**				
Hyaluronan, ng/mL					
Women (means ± SD)	109.5 ± 35.4	185.4 ± 80.2	194.6 ± 77.3	201.7 ± 58.5	**0.004**
Men (means ± SD)	141.5 ± 59.8	413.8 ± 38.9	361.3 ± 186.5	289.3 ± 172.6	**0.001**
P value between-group effects "Sex"	0.277	**0.001**	0.066	0.263	
	ANCOVA for covariate "Sex" *P* value **<0.001**				
Syndecan CD-138, ng/mL					
Women (means ± SD)	50.4 ± 30.7	44.2 ± 26.4	38.5 ± 32.9	37.3 ± 27.9	**0.002**
Men (means ± SD)	38.0 ± 26.7	33.8 ± 19.9	28.9 ± 19.4	24.2 ± 18.5	**0.007**
P value between-group effects "Sex"	0.452	0.536	0.527	0.332	
	ANCOVA for covariate "Sex" *P* value = 0.140				
					P value within-group effects "Age" over time
Heparan sulfate, ng/mL					
Age <38 yr (means ± SD)	1,607.9 ± 1,065.3	3,901.3 ± 3,772.3	3,588.1 ± 4,602.6	4,860.0 ± 7,443.0	0.342
Age >38 yr (means ± SD)	1,802.1 ± 961.2	2,352.9 ± 549.1	2,277.5 ± 1,628.0	2,061.9 ± 1,323.5	0.692
P value between-group effects "Age"	0.589	0.448	1.000	0.589	
	ANCOVA for covariate "Age" *P* value = 0.405				
Hyaluronan, ng/mL					
Age <38 years (means ± SD)	127.1 ± 50.6	298.1 ± 120.4	244.3 ± 117.5	242.0 ± 133.7	**0.002**
Age >38 years (means ± SD)	126.2 ± 55.9	272.9 ± 170.2	331.1 ± 210.4	256.9 ± 150.0	**0.005**
P value between-group effects "Age"	0.945	0.801	0.368	0.853	
	ANCOVA for covariate "Age" *P* value = 0.234				
Syndecan CD-138, ng/mL					
Age <38 yr (means ± SD)	27.3 ± 18.9	24.5 ± 10.7	19.2 ± 12.9	18.0 ± 17.1	**0.005**
Age >38 yr (means ± SD)	62.9 ± 25.5	58.6 ± 19.6	49.8 ± 28.2	44.5 ± 22.4	**0.001**
P value between-group effects "Age"	**0.015**	**0.016**	**0.025**	**0.034**	
	ANCOVA for covariate "Age" *P* value **<0.001**				
					P value within-group effects "BMI" over time
Heparan sulfate, ng/mL					
BMI <23.5 kg/m^2 (means ± SD)	2,028.3 ± 1,026.9	2,554.9 ± 983.7	2,607.8 ± 1,668.0	2,277.7 ± 1,147.9	0.668
BMI >23.5 kg/m^2 (means ± SD)	1,474.1 ± 939.2	3,699.3 ± 3,780.1	3,164.9 ± 4,326.1	4,306.1 ± 6,975.7	0.344
P value between-group effects "BMI"	0.355	0.579	0.876	0.530	
	ANCOVA for covariate "BMI" *P* value = 0.228				
Hyaluronan, ng/mL					
BMI <23.5 kg/m^2 (means ± SD)	120.2 ± 46.0	235.1 ± 140.2	206.6 ± 111.3	201.5 ± 81.7	**0.014**
BMI >23.5 kg/m^2 (means ± SD)	132.3 ± 57.6	351.7 ± 111.8	351.0 ± 182.2	289.5 ± 164.4	**<0.001**
P value between-group effects "BMI"	0.628	0.219	0.120	0.261	
	ANCOVA for covariate "BMI" *P* value = **0.036**				
Syndecan CD-138, ng/mL					
BMI <23.5 kg/m^2 (means ± SD)	47.0 ± 33.2	42.1 ± 28.7	37.2 ± 33.5	32.9 ± 31.2	**0.004**
BMI >23.5 kg/m^2 (means ± SD)	40.9 ± 25.3	36.5 ± 16.7	30.1 ± 19.2	28.0 ± 16.2	**0.003**
P value between-group effects "BMI"	0.718	0.740	0.640	0.725	
	ANCOVA for covariate "BMI" *P* value = 0.717				

Data for heparan sulfate (HS), hyaluronan (HYA), and syndecan CD-138 (SYN) are stated as means ± SD. *P* values are stated for within group effects regarding "Sex," "Age," and "BMI" over time as well as for between-group effects for these group-distinctions at each time point ("PRE" at baseline, "D1" at 277 km, "D2" at 383 km, and "POST" after the race at 690 km), and for results of performed ANCOVA regarding influence of "Sex," "Age," and "BMI" as covariates, with "BMI" representing the values of body mass index. Statistically significant *P* values printed in bold.

relationship, following the equation: HYA (ng/mL) = 0.24 × Distance (km) + 139.1 (R^2 = 0.211, $P < 0.001$), indicating an increase of HYA as covered distance increases.

Regarding correlation of glycocalyx elements with age among FIN, only SYN showed a moderate significant relationship, following the equation: SYN (ng/mL) = 1.64 × Age (years) − 27.9 (R^2 = 0.318, $P < 0.001$), indicating an increase of SYN as age increases among FIN. Regarding correlation of glycocalyx elements with age among CON, only HYA showed a weak significant relationship, following the equation: HYA (ng/mL) = 1.52 × Age (years) + 86.7 (R^2 = 0.202, P = 0.021), indicating an increase of HYA as age increases among CON.

Correlation results of glycocalyx elements with other stress parameters among FIN showed a weak significant relationship for HYA and CK, following the equation: HYA (ng/mL) = 0.0484 × CK (U/L) + 171.0 (R^2 = 0.150, $P < 0.001$), indicating an increase of HYA as CK increased among FIN. Furthermore, results showed a weak significant relationship

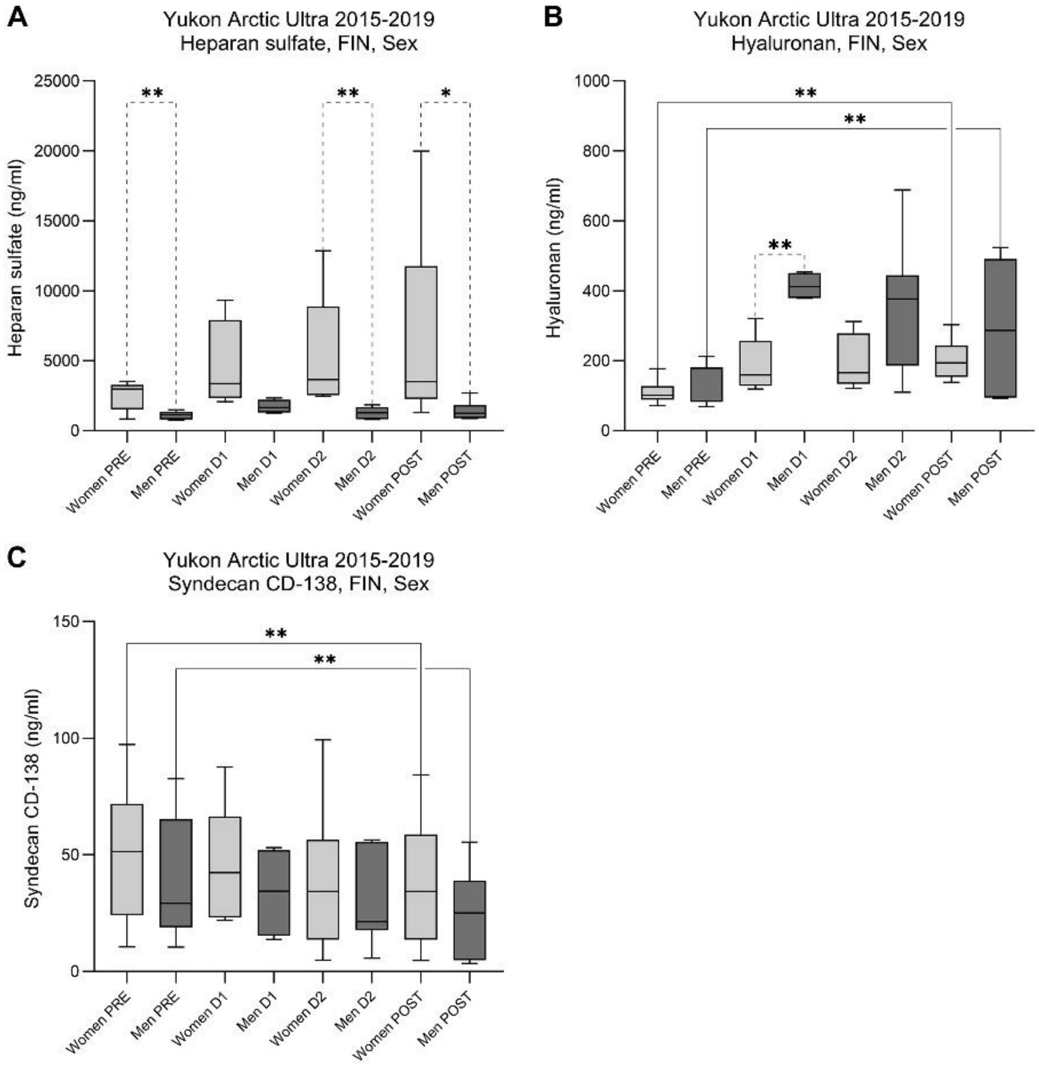

Figure 2. Boxplots for within- and between-group effects among "FIN", with "FIN" being the athlete group, separated for "Sex" with light gray boxes representing "Women" and dark gray boxes representing "Men". Each plot represents the concentration of each glycocalyx element in the serum. Results for heparan sulfate (HS; *A*), for hyaluronan (HYA; *B*), and for syndecan CD-138 (SYN; *C*), respectively. The *x*-axis signifies the respective groups and measurement time points ("PRE" at baseline, "D1" at 277 km, "D2" at 383 km, and "POST" after the race at 690 km). Due to limited volumes of serum samples, one measurement was missing at D1 among "Women" and three were missing at D1 among "Men." The dashed line signifies statistical difference regarding between-group effects while the solid line signifies within-group effects over time, with a P value of $*0.01 \leq P < 0.05$ and two indicating $**0.001 \leq P < 0.01$.

for HYA and NT-Pro BNP, following the equation: HYA (ng/mL) = 0.116 × NT-Pro BNP (pg/mL) + 175.1 (R^2 = 0.089, P = 0.009), indicating an increase of HYA as NT-Pro BNP increased among FIN. Among CON, results indicated a weak significant relationship for HS and COR, following the equation: HS (ng/mL) = 103.6 × COR (μg/dL) + 3313.2 (R^2 = 0.182, P = 0.042). Detailed results of the correlation analyses can be found in the Supplemental Material.

DISCUSSION

With regards to the formulated hypotheses, we found that: *1*) there were effects over time (within-group effects) with an increase for HYA, and a decrease for SYN among the athlete group FIN of the Yukon Arctic Ultra; *2*) there were effects regarding the glycocalyx elements between the athlete group and the control group (between-group effects) with greater values for CON at PRE, D2, and POST, as well as for HYA with greater values for FIN at D2; *3*) there were influences of sex, age, and BMI when evaluating athlete subgroups (between-group effects: between male vs. female participants, between younger vs. older participants, and between participants of lower vs. higher BMI) with greater values among women for HS at PRE, D2, and POST, greater values among men for HYA at D1, and greater values among older athletes for SYN at all time points, and an influence of BMI

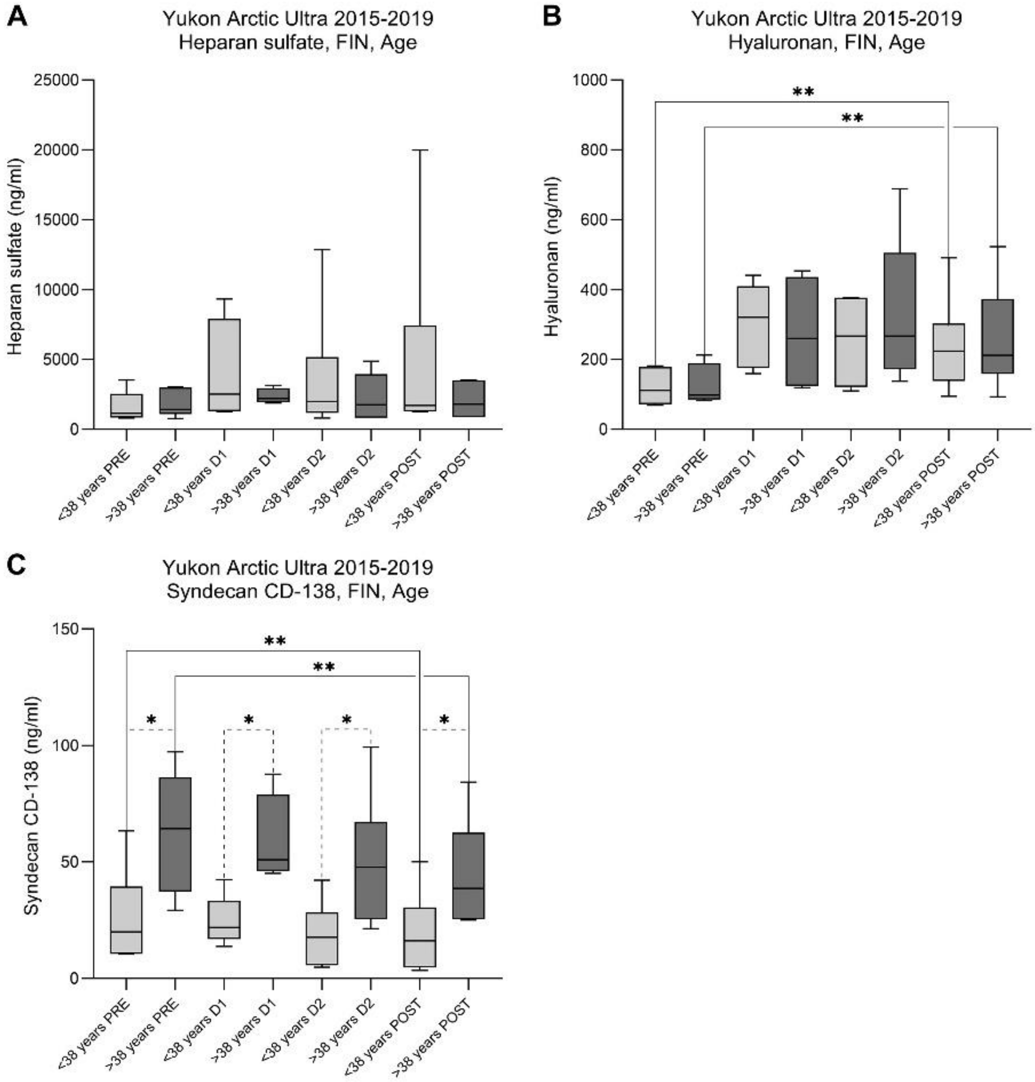

Figure 3. Boxplots for within- and between-group effects among "FIN", with "FIN" being the athlete group, separated for "Age" with light gray boxes representing "Age < 38 yr" and dark gray boxes representing "Age > 38 yr". Each plot represents the concentration of each glycocalyx element in the serum. Results for heparan sulfate (HS; *A*), for hyaluronan (HYA; *B*), and for syndecan CD-138 (SYN; *C*), respectively. The *x*-axis signifies the respective groups and measurement time points ("PRE" at baseline, "D1" at 277 km, "D2" at 383 km, and "POST" after the race at 690 km). Due to limited volumes of serum samples, two measurements were missing at D1 among "Age < 38 yr" and two were missing at D1 among "Age > 38 yr." The dashed line signifies statistical difference regarding between-group effects while the solid line signifies within-group effects over time, with a *P* value of $*0.01 \leq P < 0.05$ and $**0.001 \leq P < 0.01$.

with greater values for athletes with a higher BMI; and *4*) there were positive correlations between the results of HYA and creatine kinase, NT-Pro BNP as well as the amount of covered distance among the athlete group.

Definition of Cohorts, Anthropometric Data, and Race Performance

The participants were of comparable age and BMI with no differences between men and women, whereas women were smaller, lighter, had higher percentage of fat mass than men and lower total body water. Except for resting heart rates obtained at PRE, with lower values among the athlete group reflecting their better endurance training status (27), there were no differences between athletes and the control for any of these parameters, which can thus be construed as comparable homogeneous groups.

The finishers completed an ultra-long distance of ~657 km during the Yukon Arctic Ultra footrace 2015 to 2019, with a more than twofold greater mean daily energy expenditure compared with CON. The completion times were different between sexes and correspond to a mean completion time of 9 days, 17 h, and 59 min for men and 11 days, 20 h, and 9 min for women, indicating that on average, men covered 65 km per day and women of 57 km per day, respectively. Men

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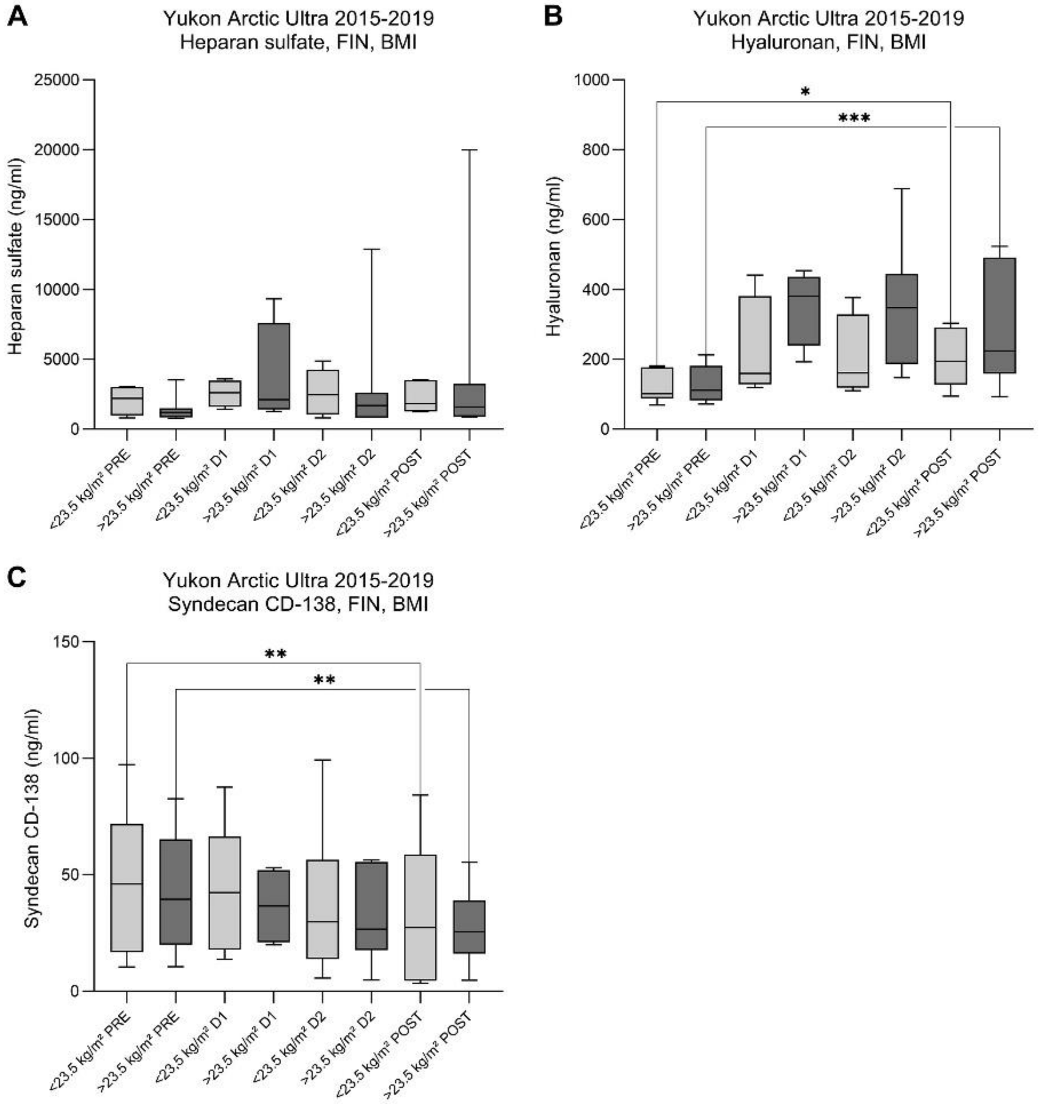

Figure 4. Boxplots for within- and between-group effects among "FIN", with "FIN" being the athlete group, separated for "BMI" (body mass index) with light gray boxes representing "BMI $<$23.5 kg/m^2" and dark gray boxes representing "BMI $>$ 23.5 kg/m^2." Each plot represents the concentration of each glycocalyx element in the serum. Results for heparan sulfate (HS; *A*), for hyaluronan (HYA; *B*), and for syndecan CD-138 (SYN; *C*), respectively. The *x*-axis signifies the respective groups and measurement time points ("PRE" at baseline, "D1" at 277 km, "D2" at 383 km, and "POST" after the race at 690 km). Due to limited volumes of serum samples, one measurement was missing at D1 among "BMI $<$ 23.5 kg/m^2" and three were missing at D1 among "BMI $>$ 23.5 kg/m^2." The solid line signifies within-group effects over time, with a *P* value of $*0.01 \leq P < 0.05$, $**0.001 \leq P < 0.01$, and $***P < 0.001$.

completed their distances with significantly greater moving velocity than women (4.55 km/h vs. 3.73 km/h), which is in line with other investigations (28). However, the athletes' overall moving velocities were low (4.17 km/h among all finishers) compared with other ultra-endurance events (29). It was therefore of interest to investigate changes in glycocalyx shedding among YAU athletes since the few previous studies regarding glycocalyx shedding and exercise had only investigated the impact of all-out exercise testing (11, 15, 16), of short-term moderate- and high-intensity testing (10), of strenuous bouts of exercise at 70% $\dot{V}O_{2max}$ (13) and at 85% peak power (14), and of continuous strain on muscles over 2 to 4 days in a rodent model (12). Even though some had evaluated the impact of moderate endurance training (11, 13), they had only reported on glycocalyx shedding occurring during high-intensity exercise testing, but not during low-intensity endurance exercise itself, which makes the results of this study more relevant, since the adaptational mechanisms of exercise training associated with glycocalyx shedding, such as increased NO synthesis (30) and angiogenesis (31) apply more specifically to adaptation to endurance exercise (32). Furthermore, all previous studies regarding glycocalyx shedding and exercise in humans had only investigated men (10, 11, 13–16). Finally, none of the previous studies had taken into account influences of renal function or body water. Although it was beyond the scope of this study to account for renal function (e.g., through creatine clearance), we kept track of changes in total body water.

Determination of Glycocalyx Elements

Within-group effects "FIN and CON over time" and between-group effects "FIN vs. CON".

With regards to within-group effects, i.e., changes over time during the YAU, the results indicate no significant changes for any of the three glycocalyx parameters among the control group, whereas there was a significant increase for hyaluronan among the finishers as well as a significant decrease for syndecan CD-138 for this group, and no changes over time for heparan sulfate among the finishers. This indicates that shedding of glycocalyx elements does take place during long-duration endurance exercise of low intensity and that the glycocalyx elements appear to be differently susceptible to this shedding, possibly because of their different structure within the glycocalyx network (7). Causes for this shedding could be attributed to the increased formation of reactive oxygen species known to occur during prolonged endurance exercise (33), of which the glycocalyx is highly sensitive (34). In addition, abnormal shear stress has been discussed as an additional cause both among humans (13) and in a rat model with exercise-induced shedding model characterized by continuously stimulated muscle over 2–4 days (12). Although this rat model might be nonapplicable because of the extremely prolonged stimulation, it could be argued that also the YAU athletes engage in endurance exercise that leads to "supra-physiological" muscular stimulation.

The impact of shear forces (shear stress) on endothelial cells, glycocalyx production, and maintenance has been investigated in the context of atherogenesis (35). Although normal shear stress is considered endothelium-protective, atherosclerosis develops when the flow is perturbed, associated to pro-proliferative and proinflammatory gene expression, more leucocyte adhesion, and other proinflammatory sequences (36). Although laminar shear stress does protect the function in health, inadequate shear stress as seen in severe inflammatory states contributes to multiple pathologies (37).

The glycocalyx shedding observed in this study among healthy ultramarathon athletes requires comparison to shedding in other settings. It has been summarized that clinical conditions like sepsis and trauma usually lead to a three- to fourfold increase of glycocalyx elements in peripheral blood (6). However, the description in that recent review shows a complex picture with increases for certain clinical conditions such as heart failure, while other studies found no changes or even decreases (6). Nevertheless, a majority of studies indicate approximately threefold increases during sepsis and fourfold increases during abdominal surgery for heparan sulfate and four- to eightfold increases for syndecan CD-138 under these conditions (38–48). Increases can be much higher with 10- and 42-fold increases for syndecan CD-138 and heparan sulfate, respectively, during ischemia-reperfusion injuries (49) and 159-fold increases for syndecan CD-138 among women with HELLP syndrome (50). With regards to exercise-induced shedding, previous studies reported increases in a range of 21% for heparan sulfate, 44% for hyaluronan, and 5%–22% for syndecan CD-138 during high-intensity exercise (10, 11, 13–15), whereas there were also decreases for heparan sulfate by 4% and for hyaluronan by 45% following intensive exercise (15). The results found by this study seem to be in line as they both resemble comparable changes (~2.3-fold increase for hyaluronan from PRE to D1 among finishers) but also underscore the complex and diverse reactions of glycocalyx shedding as there were no changes over time for heparan sulfate and a decrease over time for syndecan CD-138 among the athletes of this study. It is important to note that the amount of glycocalyx elements detectable in peripheral blood is influenced by the rate of glycocalyx synthesis, the amount of shedding as well as their clearance rates (6, 51, 52). The latter have been shown to be rapid through renal elimination (53) and that fivefold differences in syndecan CD-138 may ensue because of variations in kidney function (52). However, it could be argued that in response to ultra-endurance exercise during the YAU, glycocalyx shedding would appear to be a prerequisite for increased amounts of glycocalyx elements to be detectable in peripheral blood and that any altered kidney function would then modify this amount (51).

Rates of glycocalyx synthesis, shedding, and elimination of shed elements might also explain differences between the finishers and control group for heparan sulfate. Although not significant through χ^2 test, there were more women than men among the control, which might have led to sex-specific differences.

Evaluation of total body water showed an increase among FIN by 8.9% and body mass decreased by 4.4%, whereas there were no changes for these parameters among the control, which indicates hyperhydration among the athletes, as has been observed during ultramarathons before due to fluid overload (54). It is important to note that the observed changes of the evaluated glycocalyx elements (e.g., increase in HYA, decrease in SYN) each changed by a greater amount than could be explained by the observed changes in total body water. Since TBW changes indicate hyperhydration among FIN, the more than twofold increase in HYA (by 126.4% PRE to D1) becomes more noteworthy while the decrease in SYN (by 30.8% PRE to POST) might in part be explained by a dilution effect, where hyperhydration may lead to increased plasma volume and to a decrease in plasma electrolytes, hematocrit, and osmolarity (55).

Furthermore, it was shown in vitro that glycocalyx elements react differently to shear stress where heparan sulfate and glypican-1 were mobilized and reorganized within the glycocalyx network clustering in the cell junctions, while in contrast chondroitin sulfate, bound albumin, and syndecan CD-138 did not move indicating that the mobility of transmembrane bound syndecan CD-138 was constrained during exposure to shear stress (56), which might be an indication that syndecan CD-138 does not move and is shed. Yet, another recent study indicated that shear stress induces an ongoing synthesis of heparan sulfate and chondroitin sulfate, which may form numerous long "stress fibers" that stabilize syndecan CD-138 (57) that might result as one speculated reason why syndecan-1 shedding lowered over a prolonged time-period of ultra-endurance exercise as observed in our study.

Within- and between-group effects and ANCOVA "Sex," "Age," and "BMI".

Since all previous studies regarding glycocalyx shedding and exercise in humans had only included men (10, 11, 13–16), it was of great interest to investigate sex-specific responses. The female finishers exhibited greater values of heparan sulfate compared with men at time points PRE, D2, and POST, the influence of sex as covariate was corroborated by ANCOVA. Conversely, men showed greater values of hyaluronan compared with women at D1 (with a 2.9-fold increase at D1 compared with PRE), which was corroborated by ANCOVA, whereas there was no influence of sex for syndecan CD-138. These results indicate that sex seems to exert an influence on glycocalyx shedding among ultra-endurance athletes. Although the observed changes in glycocalyx elements measured in peripheral blood cannot be linked directly to changes in glycocalyx function, the observed results appear to be in line with a previous study where women appeared to be more susceptible to develop reduced glycocalyx barrier function among patients with coronary artery disease (58); however, no glycocalyx elements were measured in that study. In addition, it has been noted that glycocalyx shedding among women may be influenced by the timing of the menstrual cycle (59), and that controlled ovarian stimulation could increase glycocalyx shedding affecting vascular barrier function among women (60), which may have led to greater fragility of the glycocalyx and susceptibility to shedding in women in our study. Since the male athletes of our study were significantly faster than the women, it is conceivable that their greater moving velocity might have contributed to the observed results, especially the greater increase of shed hyaluronan among men at the first stage of the YAU, whereas the female athletes were significantly slower but were on the trail for a significantly longer time, which might have led to the continuous increase in hyaluronan. However, although the female athletes had a significantly longer race duration and thus longer exposition to the race conditions, the changes among female athletes for hyaluronan and syndecan CD-138 became apparent already at the first part of the race between PRE and D1.

Little is known regarding the influence of age on glycocalyx shedding. It has been shown that higher age might be associated with increased shedding of syndecan CD-138 among burn patients (61). However, that study included only patients with severe burns with a burn injury of more than 20% total surface area among elderly patients, the oldest being 78 yr old. In addition, an age effect was shown for syndecan CD-138 in another study, but the investigated cohort were patients treated for hypertension, diabetes, and other diseases (62). Therefore, it was surprising to find approximately twofold greater values for syndecan CD-138 among the older finishers (>38 yr) throughout all time points, which was corroborated by ANCOVA for age as distinct values as covariate, which indicates an influence of age on shedding of syndecan CD-138 among healthy adults.

Although the results regarding the influence of BMI after categorization in above and below median BMI values, were not significant, athletes with higher BMI (>23.5 kg/m^2) showed greater values as a trend for heparan sulfate and hyaluronan. ANCOVA revealed that BMI analyzed as distinct values had significant influence as covariate on hyaluronan with greater BMI leading to higher values in hyaluronan. Since there were no statistical differences between women and men regarding BMI, it is conceivable that BMI might have an influence on glycocalyx shedding that is not confounded by sex.

Correlation with "Completed Distance," "Age," and "Other Serum Stress Parameters".

Hyaluronan positively correlated with completed distance indicating that this glycocalyx element is being increasingly shed as the distance increases. Hyaluronan also showed positive correlation with CK and NT-Pro BNP, being indicative of muscular damage (63) and cardiac volume stress (64), respectively. The increase along CK might reflect simple collinearity based on the muscular breakdown of the continuous strain on the athletes by the YAU (63). NT-Pro BNP has been shown to increase during ultramarathons of low intensity (65). In addition, it has been shown that A-, B-, and C natriuretic peptides might induce glycocalyx shedding of their own (66). The increase of total body water among FIN while their body mass decreased throughout the YAU suggests that hyperhydration may had taken place among the athletes, which in turn might have contributed to the increased amounts of NT-Pro BNP among FIN (67). Thus, greater excreted amounts of NT-Pro BNP, as a possible reaction to the observed hyperhydration (68), among the YAU athletes might be related to augmented glycocalyx shedding.

The discussed age effect for syndecan CD-138 was also shown by the correlation of syndecan CD-138 and age, with greater values for increasingly older athletes. This relationship with age was also found between hyaluronan and age among the control group, which is another indicator that age appears to be an influence on glycocalyx shedding among healthy adults.

Despite no significant increase during the YAU over time, there appears to be an association between shedding of heparan sulfate and cortisol among the control, as expressed by the correlation analysis. Although it was shown that administered cortisol might alleviate glycocalyx shedding (69) that investigation had studied sole administration of hydrocortisone in a clinical setting while in our study an interplay of stress-related reactions might have contributed to a stress reaction leading to shedding of heparan sulfate among the control. This stress could be attributed to the volunteers demands of taking care of athletes' safety and making sure the race goes as planned.

Glycocalyx shedding during endurance sports: destruction or adaptation?

The shedding of the glycocalyx coating of the vascular epithelium was quantified by the respective categories of glycocalyx compounds shed into the blood of humans undergoing extreme physical and environmental stress. These pathophysiological effects of extreme challenges to the vascular bed might have contributed to endothelial lesions, fluid shift toward the interstitial space, and edema formation (70, 71)—symptoms, which have been previously reported in association with exercise-induced endothelial dysfunction among ultramarathon runners (72). Since the YAU took place in subarctic climate, the cold climate conditions might also have contributed to the results. Although there exist no studies regarding glycocalyx shedding due to cold injuries, some studies indicate endothelial lesions in association with frostbite and cold injuries (73). Even though none of the study participants showed any signs of frostbite or hypothermia throughout the YAU, for which they were examined regularly, it is conceivable that cold exposure might have had an additional augmentative effect on the glycocalyx shedding among YAU finishers.

In addition, shedding of the pulmonary epithelial glycocalyx might have contributed to the observed results among the athletes through the influence of breathing very cold air. There exist no studies that have investigated the impact of breathing very cold air on pulmonal glycocalyx shedding. However, as has been shown that inhalation of altered air composition with higher oxygen concentrations may lead to glycocalyx injury (74), it is conceivable that inhalation of very cold air might also have detrimental effects on the pulmonary epithelial glycocalyx.

The results of this study pose the question if ultra-endurance exercise, even at low intensities at hiking speed but over a very prolonged time, may be "too much," as has been discussed with regards to marathon and ultramarathon running (75), but not with the focus on glycocalyx shedding. On the other hand, it has been shown that endurance athletes possess a more resilient glycocalyx, where regular moderate endurance training can help maintain the glycocalyx and certain shear stress appears to be required to maintain glycocalyx structure and function (11). Furthermore, a correlation between glycocalyx thickness measured by sublingual dark field imaging and maximal exercise capacity has been shown (76), indicating enhanced glycocalyx integrity among well-trained athletes.

The results of our study, in conjunction with the findings of the few previous studies regarding glycocalyx shedding and exercise (10, 11, 13–16), suggest that there might exist an ideal range of exercise duration and intensity that is beneficial to sustain the endothelial glycocalyx. In such a region of adequate exercise, the beneficial effects of endurance exercise [e.g., increase of NO synthase function (30) through glycocalyx-mediated mechanotransduction (10)] would prevail over the detrimental effects that may have led to glycocalyx shedding among YAU finishers, such as mechanically caused shedding (13) and the influence of NT-Pro BNP (66) as a possible response to hyperhydration (68).

As a final remark it should be mentioned that although glycocalyx shedding has been associated with negative health effects (6), so have several other aspects of exercise training, such as exceeding the lactate threshold (77) and increased formation of reactive oxygen species (78), but which have been shown to be necessary training stimuli to provoke training adaptation and long-term increase in exercise capacity (77, 79). Furthermore, it should be noted that the changes might not necessarily be construed as abrasive shedding but as a reorganization of glycocalyx elements within the network (56) and physiological release into the blood stream, where the elements serve further functions, such as anticoagulative effects of heparan sulfate (5). Based on the results of this study, we propose that this reorganization and physiological release of glycocalyx elements could be understood as a relevant step of an adaptational process in response to endurance exercise, taking place on the endothelial level since it was shown that glycocalyx reorganization leads to accessibility of E-selectin by leucocytes with a subsequent expression of vascular endothelial growth factor (VEGF) (80), where VEGF function can even be augmented by released glycocalyx elements (81), which in turn might increase the build-up of new capillaries and lead to enhanced oxygen uptake by the tissue (31).

Limitations and Outlook

As a first limitation it should be noted that it would be extraordinarily difficult to replicate the conditions and physical exertion of the YAU in another setting. Furthermore, this study, as many in the field of physiological adaptations of humans in extreme environments, is confronted with only a low number of participants. However, this investigation comprised of 13 participants in the athlete group FIN with an equal representation of men and women, whereas many other studies have much lower numbers and often only comprise of men. In addition, this study included a control group, which allowed for further comparison. Although the distribution of men and women among the control seemed unequal, the χ^2 test revealed that the difference in representation of both sexes among the control was not statistically significant. Nevertheless, further studies should aim to include an even greater number of participants and to include men and women in equal distribution within the studied groups.

Since the results of our study indicate diverging shedding of the three investigated glycocalyx elements, further studies are necessary to better understand the underlying causes and mechanisms of glycocalyx shedding with regards to the structure of the glycocalyx network. Although no athletes of this study had shown signs of frostbite, they were still being exposed to the deeply cold climate, which might have had an influence on glycocalyx shedding. Further studies should address the effects of cold exposure on glycocalyx shedding as a separate entity.

In addition, it is conceivable that the alveolar epithelial glycocalyx in the lung may have been subject to shedding as well and thus contributed to the detected glycocalyx elements, especially under the deeply cold temperatures, which should be focused on in future studies to elucidate and differentiate between the different possible sources of glycocalyx shedding.

As the results of our study have indicated several associations and dependencies between the shedding of glycocalyx elements and other independent variables like sex and age among the athletes, as well as with other serum stress parameters, further studies seem warranted to investigate whether and to which extent such relations can be found outside the field of an ultramarathon in extreme cold, and if dependencies from sex, age, and BMI can be confirmed. Furthermore, timing and relation of glycocalyx synthesis, shedding, and elimination, with focus on kidney function, as well as hyperhydration and increased excretion of NT-Pro BNP, should receive more attention in future investigations as well as imaging measurements to determine glycocalyx function.

Finally, future studies might aim to investigate possible protective as well as detrimental effects of exercise on the endothelial glycocalyx and to determine possible ideal ranges of exercise intensity and duration to maintain glycocalyx structure and function as well as to investigate the involvement of glycocalyx reorganization as a conceivable necessary step in endurance training adaptation.

Conclusions

For the first time, shedding of glycocalyx elements is shown among participants of an ultramarathon of very long duration and low intensity in very cold climate. There was no shedding among athletes over time for heparan sulfate but an increase for hyaluronan and a decrease for syndecan CD-138, and no changes over time for neither of the investigated glycocalyx parameters among the control. Greater overall shedding of heparan sulfate among the female and greater increases of shedding of hyaluronan among the male athletes as well as greater overall shedding of syndecan CD-138 among the older athletes along with greater BMI leading to higher values in hyaluronan show that sex, age, and BMI appear to be of influence on glycocalyx shedding among healthy human adults and that the glycocalyx elements are differently susceptible to shedding under the investigated conditions. Greater covered distance as well as higher values of NT-Pro BNP, as a possible reaction to hyperhydration, seems to be associated with increased shedding of hyaluronan. The observed changes in glycocalyx elements during this ultramarathon indicate that there might exist an ideal range of exercise intensity and duration that might be most beneficial to glycocalyx stability. Considering the complex interactions taking place during adaptation to endurance training, we furthermore propose that glycocalyx reorganization and shedding might be a conceivable necessary step in endurance training adaptation.

SUPPLEMENTAL DATA

Supplemental Material: https://doi.org/10.6084/m9.figshare.19420238.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.S., R.H.C., H.-C.G., A.C., and M.R. conceived and designed research; M.S., K.B., M.H., C.K., L.M.-M., A.S., A.C., and M.R. performed experiments; M.S., K.B., A.L.G., M.H., M.J., C.K., L.M.-M., A.S., A.C., and M.R. analyzed data; M.S. interpreted results of experiments; M.S. prepared figures; M.S. drafted manuscript; M.S., R.H.C., H.-C.G., A.C., and M.R. edited and revised manuscript; M.S., K.B., R.H.C., A.L.G., M.H., M.J., C.K., L.M.-M., A.S., H.-C.G., A.C., and M.R. approved final version of manuscript.

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3. Discussion

3.1 Energy Expenditure, Body Composition, and Metabolism

The results from actigraphy revealed considerable mean daily energy expenditures of about 26,000 kJ among the participants – with daily peak values of up to 38,000 kJ –, which borders on the assumed upper limits of daily human endurance exercise expenditure (64). Stated in metabolic equivalents (MET), where 1 MET resembles resting metabolic rate (65), this surmounts to a an average 3.25-fold increase – and even 4.75-fold increase at peak – compared to resting metabolic rate, which again is close to the assumed maximal human physical activity level per day of about 5 MET that can be sustained to several days (66). It should be noted that this enormous energy expenditure increase among the YAU participants was not being achieved through running at high speeds or other peak power activities, but contrarily through continuous long-term exercise at mere walking speed. The participants' GPS data allowed the precise analysis of their moving velocity (i.e., excluding sleeping and resting states), which revealed that at average the participants moved at about 4.1 km/h. Interestingly, the fastest participants were overall not much "faster" than the slow ones but simply spent more time moving on the YAU track – and thusly more time above a certain activity level expressed in MET – compared to the slower participants, as Figure 10 illustrates.

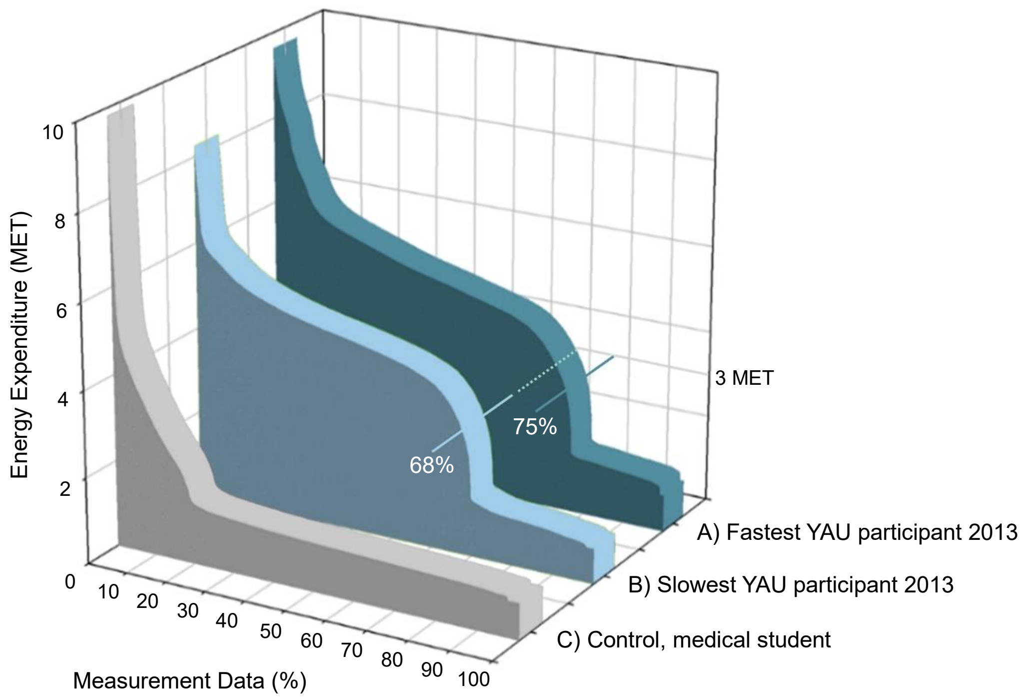

Figure 10: Histogram of the cumulative energy expenditure in MET of the A) fastest and the B) slowest YAU participant in 2013 and of a control (C, medical student who engaged in 120 minutes of general physical exercise per week). The slower participant had spent 68% of the time at ≥ 3 MET while the faster participant had spent 75% of the time at ≥ 3 MET. Previously unpublished data. (Graphic created by Mathias Steinach)

At the same time, the YAU participants exhibited to a remarkable energy deficit of about 10,000 kJ per day, as described for ultramarathons (67) and during military training (68). It was shown that a prolonged energy deficit negatively affects protein turnover rate and maintenance of FFM (69), leading to muscle atrophy and a reduction in physical performance (70, 71). The effects appear even greater when the energy deficit is combined with extended physical exertion (72). Contrarily, participants of ultramarathons, also in cold environments, have shown to retain FFM (43, 73) and recent research showed that strenuous military training in a winter environment with negative energy balance does not lead to reduction in physical performance (74).

The retention of FFM among the YAU participants, despite the marked energy deficit and prolonged endurance exercise, might be attributed to the observed increase of follistatin shown to preserve muscle mass (45), and a possible interorgan transfer of amino acids thereby conserving musculature (75). In addition, exercise intensity has been shown to greatly influence protein breakdown during exercise with a liner relationship between intensity and amino acid oxidation (76), while fat oxidation has been shown to be highest at low to moderate intensities at around 60% VO_2max (77). Thusly, the preservation of FFM among the YAU participants might also be attributed to the low intensity of this particular ultramarathon conducted at walking speed. In addition, the observed energy deficit, while significant, was less severe than during other ultramarathons with a deficit of up to 28,000 kJ per day (67).

The utilization of fat as an important fuel during the YAU is supported by the eightfold rise of plasma acetoacetate during the race indicating an increased reliance on ketones as energy source (78), which can be understood as a fuel source from fat during endurance exercise (79, 80). This is supported by the observation that endurance training augments the utilization of fatty acids by musculature (81) with an increased activity of fat-metabolizing enzymes and whole-body fat oxidation at a given intensity (82). Consequentially, we observed a significant decrease in FM among the YAU participants, which has been shown among ultramarathon athletes in various settings (51, 83, 84). The reductions of FM during the YAU could also be attributed to the changes in cytokines linked to the BAT. Irisin showed an increase in 2013, while there were no changes for meteorin-like and only modest increases for FGF-21. On the other hand, there was an increase for follistatin during the YAU 2017. Both irisin and follistatin have been associated with activation of BAT (27, 46), so that the observed rises of these cytokines could have led to activation of BAT among the YAU participants and thereby increasing fat oxidation and energy expenditure (85). Furthermore, not only cold exposure but also exercise has been associated with transformation of white into beige adipose tissue (86), showing similarities with BAT (87), which might have further contributed to the increased fat utilization during the YAU.

Although not evaluated regarding change in body composition, the adipokines adiponectin and leptin were analyzed in the fourth publication. There, we found a marked decrease in leptin by about 68% among the YAU athletes compared to no changes among the control group. This corresponds to several reports of leptin reduction during prolonged endurance exercise (88, 89), especially when combined with negative energy balance (90), as well as reduction of circulating leptin through cold exposure (91), thusly signaling insufficient energy stores (92).

For adiponectin, one previous investigation found no change (89), while another found an increase towards the end of an ultramarathon (12). We found a marked increase for adiponectin during the YAU, especially among the finishers, with a 2.5-fold increase, while there were no changes over time among the control. This corresponds to previous studies where serum concentrations of adiponectin were negatively correlated with fat mass (93, 94) and that exercise (95) and reduction of fat mass led to an increased secretion of adiponectin (96), with the function of a starvation hormone, inducing food-seeking behavior and insulin sensitization (97). In addition, adiponectin secretion was shown to be augmented through cold exposure (98).

NPY, as an orexigenic peptide, influences appetite and thusly energy metabolism (99). During the YAU, there was an increase in NPY towards the middle of the race that returned to baseline among the athletes, while there was an initial rise form already heightened values that decreased at the end among the control, so the differences between the groups were less obvious for NPY. Studies about the effects of endurance exercise on NPY are scarce and one previous study revealed no changes of 4 week training in highly trained rowers (100), while another study found an increase in NPY after a 25 km swim competition (57). Cold exposure has been shown to increase NPY expression in rodents (101), where NPY may act as a limiting factor to reduce heat loss through attenuated BAT activity (102), thusly conserving energy stores.

The participation in the YAU elicits comparable changes in leptin, adiponectin, and NPY as in previous analyses, although both the decrease in leptin and increase in adiponectin during the YAU were greater than in many previous reports (89, 90, 98) and the reduction in leptin was greater than in a fasting study where 52 hours of zero-calorie fasting led to a leptin reduction of 61-63% in both obese and normal-weighted individuals (103), so the observed changes during the YAU were remarkable since the YAU participants were endurance trained individuals with relatively low fat mass.

It is conceivable that the challenges of the YAU, like long-term endurance exercise and prolonged cold exposure, multiply and have led to the observed changes with high energy expenditure, reduction in FM and altered adipokine profile.

3.2 Stress Parameters

The fourth and fifth publication evaluated stress parameters in different contexts, although the stress parameters only functioned as secondary outcomes. Stress parameters cortisol, CK, CRP, and NT-Pro BNP correlated positively with a shed glycocalyx parameter (hyaluronan), which also correlated with covered distance during the YAU. This is in line with many other ultramarathon studies that have shown an increase in cortisol (104, 105), CK (84, 106), CRP (58, 107), and NT-Pro BNP (84, 108).

However, the changes in cortisol observed among the YAU athletes were modest and already started at elevated values, which may in part be attributed to the stress of race preparation (109) and the stress of traveling to the Yukon, as has been described for long-distance flights (110). The comparably modest changes could be explained by the relatively low intensity at which the YAU is conducted, i.e., at walking speed. It has been shown before that, in general, exercise intensity is associated with cortisol release with higher intensities resulting in cortisol elevation while low intensities might even lower circulating cortisol (111) and that shorter ultramarathon completion times (i.e., races conducted at higher race speeds) led to greater rises in cortisol (112). Cold exposure has been shown to lead to increased stress responses with heightened cortisol levels (113), although also a blunting of cortisol response during long-term exposure to cold environments has been found, indicating a habituation effect of the pituitary-adrenal cortex axis to cold exposure (114).

CK, has extensively been used as a serum marker for muscle use and breakdown (115), both for endurance exercise (116) as well as short-term resistive exercise (117). The parameter was shown to be related to muscle fatigue (118) and it was recommended to monitor CK in order to avoid rhabdomyolysis-related complications after endurance competition events (119). Since the rise of CK among the YAU participants with an about 10-fold increase was relatively modest compared to other studies, where more than 200-fold increases were found among marathon (120) and ultramarathon runners (84), it could be derived that the impact of the YAU on muscle breakdown, while not negligible, has been low in comparison to other endurance events. Again, exercise intensity has to be taken into consideration, where greater endurance intensity was associated with greater increases in CK (121), while – as discussed before – the intensity during the YAU was relatively low. Furthermore, training status was shown to impact CK responses after exercise with greater values among less trained individuals (122), while the YAU athletes were relatively well trained. In addition, cold exposure has also been described as influence on CK increases, although with a low increase (123) so that the impact of exercise appears to be of greater importance than that of cold exposure.

CRP as an inflammation marker has been used to assess the impact of exercise on human physiology with notable increases during exercise (124). However, it has been found that long-term regular moderate exercise leads to decreased release of pro-inflammatory markers, such as tumor necrosis factor-α, interleukin 1-α (125), which in turn reduces the release of CRP from the liver among individuals who exercise regularly (126). For this parameter, the analysis revealed an about 20-fold increase during the YAU, which is comparable to other reports with about 5- to 25-fold increases during ultramarathons (58, 127-129), but considerably less compared to clinical acute-phase-reactions, where CRP concentrations can increase 1000-fold (130). Cold injuries such as frostbite have shown to increase CRP concentrations (131) and although the YAU athletes were screened regularly for cold-related injuries, it may still be conceivable that the cold exposure may have contributed to the elevated CRP concentrations.

NT-Pro BNP is released in response cardiac volume load through stretching of cardiomyocytes and thusly used to assess cardiac stress (132) and specifically in the diagnosis of congestive heart failure (133), but also with increases during marathon and half-marathon runs (134), and during ultramarathons (9, 135). The analysis during the YAU revealed an about 8-fold increase, which is in line with previous studies during ultramarathons (9, 135), although shorter ultramarathons also reported smaller increases around a 2-fold rise (136), which was also shown for marathon runners (137). It has been reported that less the intensity, but exercise duration is of relevance to the NT-Pro BNP elevation (84), so it is comprehensible that despite the low intensity there is a marked increase during the long-duration YAU. Indeed, there are no studies so far to have evaluated the possible impact of cold exposure on this marker, it thusly remains unclear to what extend the cold climate might have contributed to its changes during the YAU.

3.3 Heart Rate Variability

Acute exercise is often associated with a reduction in parasympathetic drive and HRV (39), while parasympathetic dominance is usually restored post-exercise leading to an increase in HRV, which has also been described for ultramarathons (9, 138, 139). Due to the considerable length of the YAU, this vagal tone recovery could already be seen among the successful YAU athletes around the last third of the race, where resting heart rates began to decrease again with respect to an initial rise at the first part of the race. This demonstrated that successful competitors were able to adapt to the race stress by recovering to baseline conditions, which has been described before regarding functional overreaching training interventions (36). Among the non-finisher group, the parasympathetic drive reduction during the YAU was greater compared to the finisher. Since stress has been associated with reduced HRV and reduced parasympathetic drive (140) as well as its negative influence to fall asleep (141), this indicates that the unsuccessful athletes might have been less able to cope with the race demands, while the successful athletes were able to sufficiently relax and rest under the conditions of the YAU. This supports the hypothesis that vagal tone flexibility may reflect one's capacity for adaptation to environmental stressors (142). On the other hand, the analysis of the long-term self-similarity coefficient α2, which has been associated with increased alertness and vigilance (143), revealed that only among the finisher this parameter was increased throughout the entire YAU, which could indicate their ability to induce heightened alertness and need for vigilance, e.g., when sleeping in the wilderness, making the correct decisions, et cetera. It was furthermore shown that cold exposure influences autonomous control as expressed through HRV, with increased sympathetic drive before cold adaptation and a heightened parasympathetic drive after such cold adaptation had taken place (144), so that the successful athletes appear to have been better adapted to the climate influence during the YAU than the less successful athletes. The HRV analysis thusly revealed relevant differences between the finisher and non-finisher groups in reaction to long-duration endurance exercise and cold exposure.

3.4 Mood and Resilience

Stress as an environmental factor appears to be most influential on the development of mood-related disorders like depression (145), while the term "resilience" refers to the ability to withstand and cope with stressful events without developing a pathology (146). Various aspects have been shown to be associated with the development of mood-disorders as well as the resilience against it, such as oxidative stress (147), inflammatory reactions (148), and changes in the gut microbiome (149) – all of which may be challenged during an ultramarathon (51, 150, 151). In addition, it has been shown that personality traits are inherited (152) and affected by childhood experiences (153), thereby influencing stress-coping mechanisms and resilience (154), so that some individuals would be better equipped to cope with stress while other may be more susceptible to it (155).

Several studies exist that have investigated mood and resilience with regards to ultramarathons. It was shown that during a 80.5 km treadmill run, participants with greater emotional intelligence, defined as the ability to regulate one's emotions (156), showed lower mood disturbance and less perceived effort than those with lower emotional intelligence (157). Mental toughness, defined as the ability to produce consistently high levels of subjective or objective performance despite present stressors (158), was found to be higher among ultramarathon runners of a Hawaiian 100 mile ultramarathon than athletes from other sports (159), and mental toughness was found to be associated with reduced depression and anger and increased vigor during a 120 mile ultramarathon in cold climate (160). These previous findings are in line with the results from the YAU, taken from POMS-SF questionnaires (161). YAU athletes exhibited less depression, anger, fatigue, and confusion before the race compared to normative data, while after the race there were no differences to normative data except lower vigor among the YAU athletes. In addition, vigor reduced throughout the race to rise again after completion, and fatigue increased complementary and decreased after the race but remained above the baseline, while tension continually decreased throughout all time points. These results reveal that mental toughness may be higher in YAU athletes compared to the general population, while the exceptional demands of the race take their toll and eventually diminish these differences towards the end of the race. It is further of interest to note that before the YAU start, there were no differences in tension, anger, and depression between the later finisher and non-finisher groups, however, at D1, non-finisher scored higher in these categories than finisher, indicating that their own perception at early stages of the run may influence and predict the eventual outcome.

Compared to the classic marathon run, or shorter ultramarathons in temperate climates, YAU athletes are faced with an up to 14 day long challenge not only of their athleticism and endurance, but overall ability to cope with numerous stressors, to make correct decisions, and to apply their skills to field conditions, e.g., clothing and food management, setting up shelter, being able to make fire in a certain time, et cetera. Mental toughness, resilience against stressors, and the confidence in one's own abilities may play an important role in this regard as it has been suggested that "perceived self-efficacy is concerned with judgements of how well one can execute courses of action required to deal with prospective situations" (162). Comparable results were found during an Antarctic expedition consisting of 26-day ship travel followed by 24-day stay in the Antarctic field, where negative dimensions like depression increased while vigor decreased over time (163). Furthermore, it was shown that cold stress led to increased negative mood among soldiers (34) and that cold-related negative mood could be reduced through exercise where continuous exercise was more effective than intermittent bouts (164), so it is conceivable that the more active YAU athletes, i.e., the ones who spent more time moving, were able to generate more heat and thusly also regulated their mood towards a more positive state. In general, it has been shown that exercise leads to a more positive mood with greater vigor and that regular exercisers show a greater effect than those who do not regularly exercise.

3.5 Endothelial Changes

The endothelial glycocalyx fulfills numerous important functions maintaining and controlling vascular stability (165), permeability (166), mechanotransduction (62), vasodilation, and coagulation (167). Previous investigations have shown that the GX may be disrupted and shed in response to high-intensity exercise (61-63). However, no previous study had investigated GX changes in response to long-duration endurance exercise, let alone in cold climate. Furthermore, all previous studies had only included men, so that any influence of sex were of interest.

During the YAU, no changes for the three investigated GX parameters HS, HYA, and SYN were found among the control group, however, there was an increase among the athletes for HYA and a decrease for SYN, which indicates that shedding of GX elements takes place during long-duration endurance exercise of the YAU. Furthermore, it demonstrates that the glycocalyx elements appear to be differently susceptible to shedding, possibly because of their different structure within the GX network (168) and their different reactions to outside influences on that network (169). The causes might lie in the increased formation of reactive oxygen species known to occur during prolonged endurance exercise (170), of which the GX network was shown to be highly sensitive (171). In addition, high shear stress has been discussed as a cause for GX shedding both among humans (61) and in a rodent model with an exercise-induced shedding model characterized by continuously stimulated muscle up to four days (172). Shedding of GX elements during the YAU is therefore conceivable since continuously activated and strained musculature is a key characteristic of this long-duration ultramarathon. Clinical conditions like trauma and sepsis often lead to three- to fourfold increases of GX elements in peripheral blood (60), so the results found during the YAU seem to be in line as they resemble comparable changes but also underscore the complex reactions of the different GX elements.

It should be noted that shed GX elements are subject to renal elimination (173), so that any GX elements detectable in serum would depend on rates of synthesis, shedding, and elimination. Sex appeared to be an influence as men showed greater values at D1 for HYA, with one possible reason being the faster moving speed of men and thusly shear stress (174), while ovarian and menstrual cycles may had an influence on GX shedding (175, 176). In addition, age seemed to have an influence with about twofold greater values of SYN during the race for older athletes.

The observed GX changes during the YAU may have contributed to endothelial lesions, fluid shift toward the interstitial space, and edema formation, because of the disruption of the GX barrier (177) – symptoms that have previously been described in association with exercise-induced endothelial dysfunction during ultramarathons (178). Although no studies exist regarding the influence of cold stress on GX shedding, it is conceivable that the harsh conditions of the YAU may have contributed to the observed reactions, since some studies have indicated cold injuries, e.g., frostbite, to be associated with endothelial lesions (179).

Finally, it should be noted that the observed changes should not necessarily be construed as a destructive effect but as a reorganization of GX elements within the network (169). In addition, it was shown that shed GX elements released into the blood stream may serve physiological functions there, such as HS with an anticoagulative effect (167). GX shedding might even be a necessary step in endurance training adaptation on the endothelial level since it was shown that shedding may enable leucocytes to access endothelial E-selectin with subsequent expression of vascular endothelial growth factor (180), which then may lead to the build-up of new capillaries and enhances tissue oxygen uptake (181). Since the endothelium and GX network seems to be involved in adaptation to endurance exercise in this manner, it becomes comprehensible that moderate endurance training has a positive influence on glycocalyx layer integrity itself (182).

3.6 Integrated Aspects

As discussed for energy expenditure, body composition, and metabolism, changes in either area will affect the other (183). Aside from the discussed possible interactions, stress hormones may also have contributed to the observed effects, since cortisol and CRP may contribute to muscle catabolism and CK to indicate muscle breakdown (184). Except for CRP, which was in comparable ranges of serum concentration as during other ultramarathons (58, 127-129), the increases in cortisol and CK were modest, indicating that the exercise intensity during the YAU conducted at walking speed was low enough to limit muscle breakdown and thusly to preserve FFM. In addition, cold exposure might not only have influenced metabolism and increased energy expenditure as discussed, but cold-induced changes in metabolites might have also contributed to preservation of FFM, as was shown for irisin, follistatin, and FGF-21 (185).

The possible influence of sex on energy expenditure and metabolism should also be considered. The female study participants during the YAU were quite successful: 46% were able to complete the entire distance of 690 km, compared to 48% among the male participants. However, when accounting for the overall completed distance, i.e., including covered distance beyond the D2 checkpoint before having to quit the race, women indeed covered a greater distance than men (682 km vs. 635 km). This is in line with other investigations that have shown that women may outperform men in ultra-long endurance competition (186). Furthermore, women during the YAU exhibited a lower moving speed (3.7 km/h vs. 4.6 km/h), thereby likely conserving energy stores (187) and being less prone to injuries (188). With regards to energy use, it was shown that the female metabolism favors fat metabolism (189), which appears to allow female endurance athletes to access a more prolonged fuel source (190). It was furthermore shown that women may have a higher pain tolerance than men (191), which, considering the continuous musculoskeletal strain and cold exposure during the YAU, would be advantageous for women and could explain why women might be more successful in ultra-endurance competition (192), especially under extreme circumstances as the YAU.

The analyses of HRV in conjunction with psychometric measurements revealed that the finisher group, i.e., the athletes who would eventually complete the YAU, were able to restore their positive mood and wellbeing after D1, with a decrease in tension and increase in vigor traits along with an observed recovery in vagal tone, which is in line with previous studies that have shown associations of an enhanced parasympathetic drive and POMS vigor among well trained athletes (193), as well as patients suffering from chronic fatigue (194). Furthermore, lower POMS total scores, indicating reduced mood disturbance, were associated with higher scores in Borg total quality of recovery (TQR) scores. This is in line with previous research that showed that intensive training leads to disturbed mood and a decreased quality of recovery (195). Reduced resting capability and sleep disturbances during ultramarathons and its impact on exercise performance have been shown before, leading to reduced endurance capacity, like the time to exhaustion (196). Further research has shown less qualitative sleep among patients suffering from post-traumatic stress disorder associated with impaired vegetative control compared to resilient individuals (23). As discussed, higher values of positive mood restoration along with a greater recovery in vagal tone were found among the successful finisher group and thusly might have led to a higher quality of recovery and overall better sleep, which is underscored by the observation that the less successful non-finisher group scored higher in sleepiness before the start. Overall, resilience has been found to be positively associated with better sleep (197). Lower sleepiness and higher alertness would be essential to cope with the YAU, so it seems comprehensible that more resilient and better rested athletes would experience less cognitive and physical impairment and would thusly yield better performance (198).

Regarding resilience, NPY has been proposed as a possible transmitter (199). As discussed, NPY was shown to increase during endurance events as well as cold exposure and to act as an orexigenic peptide, influencing energy metabolism. NPY, however, also appears to be involved with strengthening mental and physical performance (56). During the YAU, NPY was correlated positively with TQR among finishers as well as negatively correlated with the confusion trait. This is in line with previous works where NPY was found to counteract negative impact on mood, emotional processing, and stress resilience (200). Another study revealed that the highly resilient U.S. Special Forces soldiers produced greater concentrations of NPY during stressful training along with enhanced performance compared to regular soldiers (201). It was suggested that elevated concentrations in NPY might improve self-confidence (202), which was underscored by a study that showed genetic variations for NPY (NPY haplotypes) to be associated with increased vulnerability to anxiety symptoms (203).

The analysis on GX shedding showed GX shedding takes place in reaction to an ultramarathon in cold climate. The evaluation in conjunction with other stress parameters revealed a positive correlation of HYA with CK and NT-Pro BNP, indicating an increase of HYA as CK and NT-Pro BNP increased, respectively, among the YAU finishers. HYA also positively correlated with completed distance indicating that this GX element was being increasingly shed as the distance increased. The increase along with CK might reflect simple collinearity with muscular breakdown in reaction to the muscular strain of the YAU (115, 116). On the other hand, NT-Pro BNP has been shown to increase during ultramarathons of low intensity (84) and it was furthermore shown that A-, B-, and C-natriuretic peptides may induce glycocalyx shedding (204). Since bioimpedance measurements revealed hyperhydration among the finisher group – with an increase of TBW from 41.1 liter at PRE to 44.8 liter at POST, while the body weight decreased from 71.8 kg to 68.7 kg – it is conceivable that this hyperhydration may have led to the observed increase in NT-Pro BNP (205) and that the increased concentrations in NT-Pro BNP may have induced shedding in HYA among the YAU athletes (206).

All in all, the analyses indicate a network of interactions taking place during the YAU, where changes in various physiological systems influence and complement each other.

3.7 Application

Application of the findings of this investigation would be areas of occupational health regarding physical labor in a cold environment, e.g., military personnel, emergency workers, bush pilots, workers on oil rigs, et cetera. They may have clinical implications when considering that vast proportions of Western populations suffer from a sedentary lifestyle (1, 2) and overfeeding (207), while it shows that an increase – not of intensity – but in exercise duration each day can lead to a significantly heightened energy expenditure and that a significant reduction in serum fatty acids as well as body fat may occur while lean body mass is being retained. Although some of this knowledge has already been incorporated in health guidelines, e.g., to increase general daily activity (208-210), still the prevalence of inactivity and obesity are high and to some part even increasing (211-213), so that results from events like the YAU may help in the efforts to reduce inactivity and the prevalence of associated diseases. Of course, events like the YAU are extreme and cannot be understood as a general recommendation to the public or even patients, however, it demonstrates human's capability for adaptation and does so in a time-lapse manner, i.e., in a very short time, whereupon it has been proposed that ultramarathons are an outstanding model to study human adaptability under unique and stressful circumstances (214). It shares this characteristic with studies in other extreme environments, e.g., regarding real or artificial weightlessness or in confined and isolated places such as Antarctica (215-217), where changes under such conditions occur very rapidly to be studied and applied to clinical fields (218).

3.8 Limitations and Outlook

Like all studies, the presented investigation has its limitations. The Yukon Arctic Ultra poses a specific set of circumstances (distance, climate, altitude, circadian setting) that cannot readily be generalized or applied to other settings. Furthermore, although the investigation included a considerable number of participants (about 34% of all competitors during the investigated years in the longest distance took part as study participants) and included both sexes (one third of all participants were women and eventually about half of all finishers were female) the number is still small compared to studies in marathons, where far greater numbers can be achieved. In addition, the setting of a field study during a competition race in an extreme environment demands compromises, so that only a limited number of measurements can be taken at a given time in order not to exceed the participants' tolerance and compliance.

The five studies introduced here represent the work of the past ten years and several results of high interest and significance could be discovered. Nevertheless, the investigation continues. Future studies are planned to evaluate energy balance along with macronutrient intake. In addition, stress parameters shall be evaluated as primary outcomes. Furthermore, sleep measurements will be analyzed in conjunction with HRV data. In a broader perspective, it is also planned to employ the study at ultramarathons in other extreme environments such as hot climates and in high altitudes, which might allow an even more comprehensive evaluation.

All in all, this research, although difficult and demanding to conduct, has been very productive and not only exemplifies the adaptability of the human body to extreme environments and helps to increase our knowledge of physiological processes, but has also proven invaluable to bundle the expertise of scientists of different research topics and to interest students and doctoral candidates to this field and to encourage them to pursue a career in the science of physiology.

4. Summary

The Yukon Arctic Ultra is a unique ultramarathon that poses manyfold challenges to its participants: ultra-long endurance exercise of up to 690 km distance, exposure to subarctic climate, and diminished resting conditions. The presented investigation revealed considerable increases in energy expenditure among the athletes, of up to more than four times resting metabolic rate, along with a marked energy deficit, changes in body composition with reductions in fat mass while fat free mass could mostly be retained, possibly to the exercise- and cold-induced release of myokines like irisin and follistatin.

Furthermore, analysis of vegetative control expressed through heart rate variability, as well as of mood through psychometric measurements, revealed that the more successful athletes, who would eventually be able to finish the race, showed better adaptation to the race demands, with less depression, anxiety and anger, but greater vigor and higher alertness. They also exhibited a faster restoration of vagal predominance during the race with a better ability to relax and restore, leading to less sleepiness and greater vigilance compared to the non-finishing athletes.

Resilience, as the ability to cope with stressful events, appears to be a key element during such a race and neuropeptide Y has been discussed as a mediator in resilience reactions. The analyses revealed that during this race, neuropeptide Y was associated with less confusion and better quality of recovery among the finisher group and that overall neuropeptide Y was increasingly released among the athletes compared to the less challenged control group.

Another influence was the factor sex, indicating that the female athletes were not only as successful as the male participants to complete the whole race, but that women completed an overall greater distance, when accounting for all covered distances. Women, with a moving speed of 3.7 km/h, were considerably slower than men at 4.6 km/h, which may have saved energy stores and allowed them to predominantly use fat as a long-term energy source.

In addition, it was shown that ultra-long endurance exercise may lead to shedding of endothelial glycocalyx elements and that these elements appear to be differently susceptible to that shedding. Sex, age, and covered distance all appeared to have an influence and hyperhydration with subsequent release of NT-Pro BNP may have contributed to the observed changes.

The analyses of physiological changes during the Yukon Arctic Ultra have revealed a multitude of endurance- and cold-exposure-related alterations. This ultramarathon has thusly proven to be an outstanding model to study human adaptation capabilities to extreme environments under real-life field conditions that could otherwise not be replicated in a laboratory setting.

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7. Eidesstattliche Erklärung

Hiermit erkläre ich an Eides Statt, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,

- die vorliegende Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Erkenntnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern bzw. Wissenschaftlerinnen und mit technischen Hilfskräften und die verwendete Literatur vollumfänglich in der Habilitationsschrift angegeben wurden,

- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.