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

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Abstract

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.

Keywords

COLD EXPOSURE; EXERCISE; METABOLISM; EXERTION

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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 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 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 enzymelinked immunosorbent assay kit. The intra-assay %CV was <10% for irisin, <10% for meteorin-like and a sensitivity of <10 pg·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$_2$O, 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$_2$O) 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 (μmol·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·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·mL^{-1} at pre-event and rose ($P = 0.06$) up to 1289 ± 24 ng·mL^{-1} by the end of the event (Fig. 2). Serum meteorin-like remained stable 7.7 ± 0.1 ng·mL^{-1} (pre-event) to 7.4 ± 0.3 ng·mL^{-1} (post-event) (Fig. 3). Despite an absolute increase in FGF-21 due to a marked elevation in one participant (i.e., $+ 110$ pg·mL^{-1}), there was no change from pre-event (46 ± 4 pg·mL^{-1}) to post-event (79 ± 36 pg·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 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.

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, 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.

Acknowledgments

References

1. Baxmann AC, Ahmed MS, Marques NC, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clin J Am Soc Nephrol. 2008; 3(3):348–54. [PubMed: 18235143]

2. Bogren LK, Murphy CJ, Johnston EL, Sinha N, Serkova NJ, Drew KL. 1H-NMR metabolomic biomarkers of poor outcome after hemorrhagic shock are absent in hibernators. PLoS One. 2014; 9(9):e107493. [PubMed: 25211248]

3. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-a-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature. 2012; 481(7382):463–8. [PubMed: 22237023]

4. Coker RH, Williams RH, Yeo SE, et al. The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity. J Clin Endocrinol Metab. 2009; 94(11):4258–66. [PubMed: 19808853]

5. Cooper JA, Nguyen DD, Ruby BC, Schoeller DA. Maximal sustained levels of energy expenditure in humans during exercise. Med Sci Sports Exerc. 2011; 43(12):2359–67. [PubMed: 21606870]

6. Cypess AM, Lehman S, Williams G, et al. Identification and importance of brown adipose tissue in adult humans. N Engl J Med. 2009; 360:1509–17. [PubMed: 19357406]

7. Davis TR. Chamber cold acclimatization in man. J Appl Physiol. 1961; 16:1011–5. [PubMed: 13883973]

8. Department of the Army. Technical Bulletin. Prevention and Management of Cold-Weather Injuries. Washington, DC: Headquarters, Dept. of the Army; 2005. TB Med 508.

9. Dressendorfer RH, Wade CE. Effects on a 15-d race on plasma steroid levels and leg muscle fitness in runners. Med Sci Sports Exerc. 1991; 23(8):954–8. [PubMed: 1956271]

10. Fenzi A, Kiefer FW. Brown adipose tissue and thermogenesis. Horm Mol Biol Clin Investig. 2014; 19(1):25–7.

11. Goldstein DL, Pinshow B. Taking physiology to the field: an introduction to the symposium. Integr Comp Biol. 2002; 42(1):1–2. [PubMed: 21708688]

12. Gunga, HC. Human Physiology in Extreme Environments. 1. Oxford: England, Academic Press; 2015. p. 336

13. Hao Q, Yadav R, Basse AL, et al. Transcriptome profiling of brown adipose tissue during cold exposure reveals extensive regulation of glucose metabolism. Am J Physiol Endocrinol Metab. 2015; 309(5):E380–92. [PubMed: 26081284]

14. Knecthtle B, Salas OF, Andonie JL, Kohler G. Effect of a multistate ultra-endurance triathlon on body composition; World Challenge Deca Iron Triathlon 2006. Br J Sports Med. 2008; 42:121–5. [PubMed: 17601765]

15. Lee P, Linderman JD, Smith S, et al. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab. 2014; 19:302–9. [PubMed: 24506871]

16. Lieberman HR, Castellani JW, Young AJ. Cognitive function and mood during acute cold stress after extended military training and recovery. Aviat Space Environ Med. 2009; 80:629–36. [PubMed: 19601505]

17. Marsh RL. How muscles deal with real-world loads: the influence of length trajectory on muscle performance. J Exp Biol. 1999; 202(Pt 23):33377–85.

18. Nicholson JK, Foxall PJ, Spraul M, Farrant RD, Lindon JC. 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. Anal Chem. 1995; 67:793–811. [PubMed: 7762816]

19. Nicholson JK, Lindon JC, Holmes E. 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. Xenobiotica. 1999; 29:1181–9. [PubMed: 10598751]

20. Norheim F, Langleite TM, Hjorth M, et al. The effects of acute and chronic exercise on PGC-1a, irisin and browning of subcutaneous adipose tissue in humans. FEBS J. 2014; 281(3):739–49. [PubMed: 24237962]

21. Palinkas LA. Mental and cognitive performance in the cold. Int J Circumpolar Health. 2001; 60:430–9. [PubMed: 11590885]

22. Petrovic N, Walden TB, Shabalina EG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-receptor gamma (PPARgamma) activation of epididymally derived white adipocyte culture reveals a population of thermogenically competent, UCP-1 containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem. 2010; 285:7153–64. [PubMed: 20028987]

23. Rao RR, Long JZ, White JP, et al. Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. Cell. 2014; 157(6):1279–91. [PubMed: 24906147]

24. Saito M, Okamatsu-Oguma Y, Matsushita M, et al. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes. 2009; 58:1526–31. [PubMed: 19401428]

25. Saugy J, Place N, Millet GY, Degache F, Schena F, Millet GP. Alterations of neuromuscular function after the world's most challenging mountain ultra-marathon. PLoS One. 2013; 8(6):e6556.

26. Schnyder S, Handschin C. Skeletal muscle as an endocrine organ: PGC-1a, myokines and exercise. Bone. 2015; 80:115–25. [PubMed: 26453501]

27. Segal KR, Van LM, Fitzgerald PI, Hodgdon JA, Van Itallie TB. Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. Am J Clin Nutr. 1988; 47:7–14. [PubMed: 3337041]

28. Serkova N, Fuller TF, Klawitter J, Freise CE, Niemann CU. H-NMR-based metabolic signatures of mild and severe ischemia/reperfusion injury in rat kidney transplants. Kidney Int. 2005; 67:1142–51. [PubMed: 15698456]

29. Steinach M, Kohlberg E, Maggioni MA, Mendt S, Opatz O, Stahn A. Changes of 25-OH-vitamin D during overwintering at the German Antarctic stations Neumayer II and III. PLoS One. 2015; 10(12):e0144130. [PubMed: 26641669]

30. Väänänen II, Vihko V. Physiological and psychological responses to 100 km crosscountry skiing during 2 days. J Sports Med Phys Fitness. 2005; 45(3):301–5. [PubMed: 16230981]

31. Virtanen KA, Lidell ME, Orava J, et al. Functional brown adipose tissue in healthy adults. N Engl J Med. 2009; 360(15):1518–25. [PubMed: 19357407]

32. Wu, HB. Tietz Clinical Guide to Laboratory Tests. 4. St. Louis, MO: Saunders/Elsevier; 2006. p. 1856

33. Yoneshiro T, Aita S, Matsushita M, et al. Recruited brown adipose tissue as an antiobesity agent in humans. J Clin Invest. 2013; 123(8):3404–8. [PubMed: 23867622]

| 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.

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 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.

Serum FGF-21

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.

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 $(76 \pm 5^{*})$	69 ± 6 $(76 \pm 5^{*})$	74 ± 3	$73 \pm 4^{**}$
BMI (kg·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	$12 \pm 3^{***}$

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.

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 ($P = 0.02$).