Title

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Journal

Journal of wilderness medicine, 34(3)

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Publication Date

2023-09-01

DOI

10.1016/j.wem.2023.05.003

Peer reviewed

HHS Public Access

Author manuscript

Wilderness Environ Med. Author manuscript; available in PMC 2024 September 01.

Published in final edited form as:

Wilderness Environ Med. 2023 September ; 34(3): 341–345. doi:10.1016/j.wem.2023.05.003.

Alaska Backcountry Expeditionary Hunting Promotes Sustained Muscle Protein Synthesis Short Title: Alaska Hunting Promotes Muscle Retention

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Abstract

Introduction.—We have previously described negative energy balance (ie, −9.7±3.4 MJ/d) and weight loss (Δ−1.5 ± 0.7 kg) influenced by high levels of energy expenditure (ie, 17.4±2.6 MJ/d) during remote expeditionary hunting in Alaska. Despite negative energy balance, participants retained skeletal muscle. The purpose of this pilot study was to measure skeletal muscle protein synthesis and examine molecular markers of skeletal muscle protein metabolism under similar conditions of physical and nutrient stress.

Methods: The “virtual biopsy method” was used to evaluate integrated fractional synthetic rates (FSR) of muscle protein from blood samples in four participants. Muscle biopsies were taken to measure molecular markers of muscle protein kinetics (ie, FSTL1, MEF2, MYOD1, B2M and miR-1-3p, −206, −208b, 23a, 499a) using real-time polymerase chain reaction.

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AUTHOR CONTRIBUTIONS

Study concept and design (RC, BR, LB, MS, MH, WE), data acquisition (RC, MC, BK, AG, TB, MS), data analysis (RC, MC, BK, AG, TB, MS), drafting and critical revision of manuscript (RC, BR, MC, AG, MS, MH, WE), approval of final manuscript (RC, BR, MC, BK, AG, LB, TB, MS, MH, WE).

DISCLOSURE STATEMENT

Disclosures: None. We declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Results: Our findings in 4 participants (2 females [28 and 62 y of age; 66.2 and 71.8, body weight (kg); 25.5 and 26.7 body mass index (kg/m^2)]) and (2 males [47 and 56 y of age; 87.5 and 91.4 body weight (kg); 26.1 and 28.3 body mass index (kg/m^2)]) describe mean muscle FSR of serum carbonic anhydrase (2.4%) and creatine kinase M-Type (4.0%) and positive increments in molecular regulation.

Conclusions: Preservation of skeletal muscle under conditions of physical and nutrient stress seems to be supported by positive inflection of skeletal muscle FSR and molecular activation.

INTRODUCTION

Previous work has confirmed the preservation of skeletal muscle during sustained physical activity under conditions of negative energy balance (1,2). The levels of energy expenditure and energy deficit during backcountry hunting in Alaska may provide a surrogate model for military training and/or operational scenarios where physiological resilience can be diminished (3)

With respect to mission-centric objectives in a tactical environment, maintenance of skeletal muscle ensures adequate functional performance and reduces the risk of musculoskeletal injury (3). Without the undue influence of confounding variables such as extreme heat, sleep deprivation and/or failure to meet minimal recommendations for protein intake (4), it seems that muscle remodeling is sufficient even during moderate energy deficit, especially under conditions of movement constancy.

Whereas resistance training and positive caloric balance will likely result in increased muscle mass and/or absolute strength, essential amino acids and/or endurance training positively influence skeletal muscle synthesis (5). Muscle microRNAs (myomiRs) such as miR-1, 23, 208 and 499 have been implicated in the “fine tuning” of muscle gene expression (ie, FSTL1, MEF2C, MYOD1, B2M) (6). Laboratory-based studies in this area of investigation have evaluated acute responses to dietary intake, physical activity, or pre- and post- frame lifestyle interventions, but these approaches have important drawbacks that include assessments that fail to capture all aspects of the operational scenario. Therefore, the primary purpose of this pilot study was to measure integrated fractional synthetic rates (FSR) of muscle protein using the virtual biopsy technique uring the unscripted, demanding conditions previously described (7).

METHODS

Study Design and Participants

Healthy adults were recruited to participate in two separate excursions in the Brooks Range of northeastern Alaska (Table 1). All described methods and materials were approved by the institutional review board of the University of Alaska Fairbanks (1102840–12). Each individual had independently secured the consulting services of Pristine Ventures, Inc (Fairbanks, AK). Each participant had backcountry hunting experience and without any current history of metabolic, pulmonary or heart diseases, cancer or other chronic inflammatory condition).

Alaska Backcountry Expeditionary Hunting—Experienced backcountry clients received basic “hunt planner” information on preparation, harvest preservation, and load carriage. These instructions were delivered by Pristine Ventures (Fairbanks, Alaska, USA), but the clients were not guided, in accordance with Alaska state law. Clients were then provided with an opportunity to become participants in our research study focused on an evaluation of muscle protein kinetics. Upon obtaining informed consent from these individuals, they were asked to complete a health history questionnaire as research participants. None of the participants were taking medications that would influence metabolism and all were nonsmokers. None of the participants had been diagnosed with nor exhibited symptoms of neurological, cardiovascular, respiratory, or metabolic disease.

Remote air travel services in 2019 and 2020 were provided by Shadow Aviation (Fairbanks, Alaska, USA). Participants were already committed to the expeditions, independent from their study participation. No attempts were made to provide shelter, control sleep, or manipulate dietary intake. Our previous work in this cohort described total energy expenditure of ~17 MJ/d, energy intake of ~8 MJ/d and negative energy balance of ~10 MJ/d. These data indicate a sustained range of mild to intense physical activity (1,2). Such conditions are consistent with a threshold of negative energy balance that may result in reduced physical performance (3). The durations of the 2019 (ie, 13 d) and 2020 (ie., 8 d) excursions differed only due to the inextricable connection between bush plane travel and unpredictable weather in the Alaskan wilderness.

PROCEDURES

Body Mass and Body Composition

A Lunar iDXA scanner (General Electric Healthcare, Chicago, Illinois, United States) was utilized to measure body composition <24 h pre- and post-expedition. For all measurements taken, participants were provided identical surgical scrubs to wear during both exams.

Virtual Biopsy: Protocol and Analysis

To measure the synthesis of skeletal muscle proteins, we employed the isotopic labeling of newly synthesized proteins via oral dosage of deuterated water (^2H_2O) (Fig. 1b). Participants were given 50 mL of 70% enriched ^2H_2O (Cambridge Isotope Laboratories, Andover, Massachusetts, USA) 3× per day on day 1, 50 mL 2× per day on days 2 and 3, and 30mL 2× per day for the remainder of the expedition. To measure background enrichment levels of total body water, participants were asked to collect saliva samples throughout the expedition. Blood samples were obtained within 72 h of return to determine the FSR of skeletal muscle protein. After collection and centrifugation, samples were immediately stored at −80°C until isotopic analysis.

Creatine kinase M-type (CK-M) and Carbonic Anhydrase - 3 (CA-3) were immunoprecipitated from 1.0–1.5 mL of human serum, followed by in-solution digestion with trypsin for LC/MS analysis, as described previously (8). Fifty-three CK-M and 26 CA-3 peptides were identified by LC/MS/MS analysis. Kinetic data only include peptides

that passed all analytical criteria, and muscle FSR was calculated as described previously (8).

Muscle Biopsy and Analysis

For the purpose of examining molecular regulation, muscle biopsies were from the vastus lateralis using sterile procedures and a local anesthetic (1% lidocaine) <24 h pre- and post-expedition. Muscle tissues were immediately flash-frozen in liquid nitrogen and stored at −80°C until subsequent analysis.

Total RNA including small RNA was isolated from muscle tissue with QIAGEN miRNeasy Tissue/Cells Advanced Mini Kit. Total RNA concentration was measured, and the quality of RNA was evaluated by Qubit fluorometer, NanoDrop spectrophotometer and a Bioanalyzer automated electrophoresis system. Real-time PCR was conducted to obtain expression values of FSTL1, MEF2C, MYOD1 and B2M. After validation of potential reference genes, YWHAS demonstrated stable expression. The reverse transcription was carried out with SuperScript IV VILO (SSIV VILO) Master Mix (ThermoFisher Scientific) in 20 μL following the protocol. Real-time PCR was conducted in triplicates on an ABI-7900 HT using the TaqMan Fast Advanced Master Mix and Applied Biosystems TaqMan Gene Expression assays (ThermoFisher Scientific). Human skeletal muscle RNA was used as a positive control (ThermoFisher Scientific).

For quantification of mature miRNAs, cDNA was generated with a TaqMan Advanced miRNA cDNA Synthesis Kit (ThermoFisher Scientific). Real time PCR was conducted to obtain expression values of miR-1-3p, miR-206, miR-208b-3p, miR-23a-3p, and miR499a-5p in skeletal muscle using the TaqMan Fast Advanced Master Mix and Applied Biosystems TaqMan Advanced miRNA Assays (ThermoFisher Scientific). We chose miR-16-5p as a reference according to recommendations of ThermoFisher Scientific (Waltham, MA). Two preparation steps were performed before reverse transcription and the miR-Amp reaction: the poly(A) tailing reaction and the adaptor ligation. Synthesized cDNA from pooled RNA was 10-fold diluted 4 times and used for obtaining standard curves and calculating reaction efficiency. Gene and miRNA expression between pre- and post-expedition was calculated with Pfaffl method which considers PCR efficiencies of target and reference genes. A fold-change in level of expression of a target gene or miRNA relative to a reference gene or miRNA was calculated for each sample.

STATISTICS

Data were analyzed using Microsoft Excel and General Electric iDXA and Prism software. Data are reported as mean±SD.

RESULTS

Clinical Characteristics and Body Composition

Four adults (2 females, 2 males; age = 48±11 y; BMI = 25.1±0.69 kg/m^2) were recruited for this study. Data are reported in Tables 1 and 2. There were absolute reductions in

body weight, total fat, trunk fat, and android fat mass (Table 2). There were also absolute reductions in percent total, arm, trunk, and android fat masses (Table 2).

Integrated Fractional Synthetic Rate of Protein Synthesis

CA-3 - derived FSR ranged from 1.3 % to 5.1 % in participants 4 and 1, respectively (Figure 1). CK-M – derived FSR ranged from 1.4 % to 8.2 % in participants 3 and 1, respectively (Figure 1). Overall, CA-3 - derived FSR and CK-M - derived FSR was 2.4 ± 1.8 % per day and 4.0 ± 2.9 % per day, respectively.

Muscle Gene Expression and MyomiRNA

We evaluated changes in miR-1-3p, miR-206, miR-208b-3p, miR-23a-3p and mIR-499a-5p. Of these, there was a ~fourfold increment in miR-206 (Table 3). Potential alterations in muscle gene expression (ie., FSTL1, MEF2C, MYOD1 and B2M) were also examined. There was a ~three-fold increment in FSTL1 (Table 4).

DISCUSSION

The primary objective of this pilot study was focused on the measurement of muscle protein FSR in humans using the virtual biopsy method during backcountry hunting in Alaska. The elevations in integrated muscle protein FSR exceed data reported in response to sprint interval training in young athletes (9). Our data represent the first to employ the “virtual biopsy method” in a remote unsupported field setting offering insight into the regulation of muscle metabolism under conditions of physical and nutrient stress.

The level of physical activity and negative energy balance previously reported under similar circumstances is similar to short-term military operations and/or training scenarios (1,2,3). Hunters are focused on operational maneuverability and time constraints for insertion/extraction points that are ~160 km apart, and in the context of a remote environment. These factors limit nutritional provisions and demand movement constancy. It is impossible to replicate these unpredictable conditions in a well-controlled laboratory setting. Despite the discrepancy between energy intake and energy expenditure under similar circumstances, lean tissue mass was maintained (1,2). The unique constructs of this model then provides an opportunity to study alterations in muscle protein synthesis elicited by physical and nutrient stress.

Countless investigations have evaluated exercise-induced and/or dietary-induced alterations in muscle protein synthesis (4). While informative, stable isotope strategies have primarily focused on acute responses to dietary intake in conjunction with functional overload, usually in the form of resistance exercise (10). These studies have paralleled intense interest in strategies to promote muscle hypertrophy and/or sports performance. On the other hand, the metabolic intricacies of longer training and/or operational scenarios require a more comprehensive assessment of muscle protein synthesis to advance understanding of protein metabolism in the context of operational conditions. We observed higher integrated (muscle protein synthesis in our middle aged to older research participants than reported in younger adults following 9 sessions of sprint interval training over a 3-wk period (9). However, to inform strategies for future countermeasures we recognize that CK-M FSR and CA-3 FSR

primarily represent structural and cytosolic proteins and assert the need for an examination of specific alterations in muscle proteome dynamics.

We also conducted preliminary observations of myomiRs (ie, miR-1-3p, miR-206, miR-208b-3p, miR-23a-3p, miR499a-5p) and FSTL1, MEF2C, MYOD1 and B2M in skeletal muscle which have been implicated in the regulation of skeletal muscle plasticity (6). While underpowered to evaluate significance, potential alterations in miR-206 and FSTL1 inform the need for larger investigations that connect molecular mechanisms to skeletal muscle resilience under the combined circumstances of chronic physical and nutrient stress.

LIMITATIONS

We recognize that the small number of participants negatively affects the statistical power of this study and therefore limits the extrapolation of the data presented herein.

CONCLUSION

Utilizing the virtual biopsy technique for the first time in a remote field setting, we report levels of skeletal muscle FSR during Alaska backcountry expeditionary hunting that exceeds that reported following sprint interval training during positive energy balance (9). We also provided skeletal muscle myomiRNA and anabolic gene expression data that support our data describing skeletal muscle FSR. Collectively, these data demonstrate the mechanisms that support muscle remodeling despite chronic physical stress and negative caloric balance.

ACKNOWLEDGEMENTS

This research was primarily supported by Pristine Ventures, Inc. (Fairbanks, AK). This research was also supported in part by the National Institute of General Medical Sciences of the National Institutes of Health under awards P20GM130443 (Center for Transformative Research in Metabolism) and P20GM103395 (Alaska INBRE). We would like to express our sincere gratitude to our research participants and Andy Greenblatt of Shadow Aviation.

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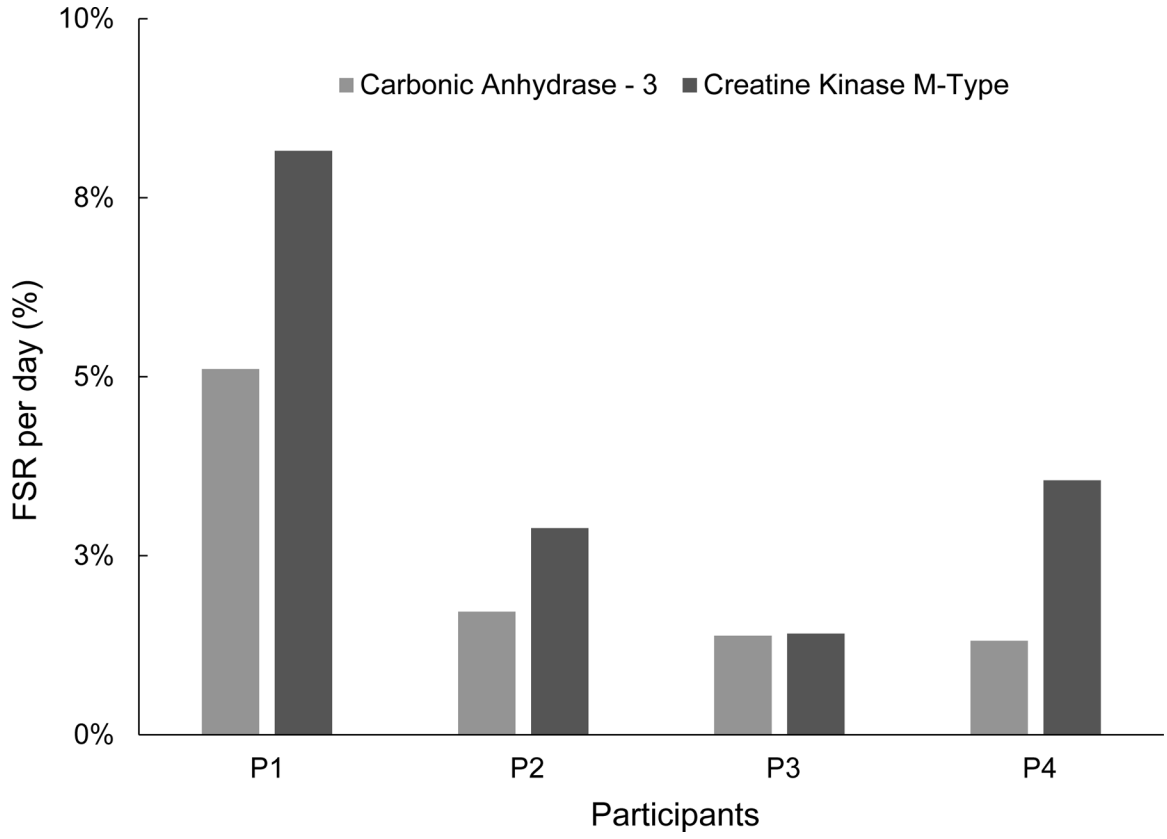

Figure 1.

Integrated measurement of muscle protein synthesis represented by CA-3 and CK-M FSR in 4 research participants (ie, P1-P4). Twenty-six CA-3 peptides and 53 CK-M peptides were identified by LC/MS/MS analysis and kinetic data only include peptides that passed analytic criteria.

Table 1.

Participant Information and Body Composition

	Age	Sex	Height (cm)	Body Weight (kg)	Body Mass Index (kg/m^2)	Δ Body Weight	Δ Body Mass Index (kg/m^2)
Participant 1	47	M	182.9	87.5	26.1	−0.5	+0.0
Participant 2	28	F	167.6	71.8	25.5	−2.5	−0.8
Participant 3	56	M	180.3	91.4	28.1	−3.1	−1.0
Participant 4	62	F	146.1	66.2	26.7	−2.6	−1.1
Mean ± SD	48±15	-	170±17	79.2±12.1	26.6±1.1	−2.1±1.2	−0.8±0.5

Table 2.

Changes in DXA-derived measurements of lean tissue, fat mass, and percentage of fat mass.

	Total (kg)	Arms (kg)	Legs (kg)	Trunk (kg)	Android (kg)	Gynoid (kg)
			Lean Tissue Mass			
Δ	+0.3 ± 1.0	+0.2 ± 0.1	−0.4 ± 0.7	1.5 ± 3.1	0.1 ± 0.2	0.3 ± 0.4
			Fat Mass			
Δ	−1.6 ± 0.2	−0.1 ± 0.1	−0.4 ± 0.3	−1.1 ± 0.3	−0.2 ± 0.1	−0.2 ± 0.1

Data are presented as mean±SD.

Table 3.

Change in myomiRNA

	Fold Change
miR-1-3p	8.865
miR-206	4.793
miR-208b-3p	9.867
miR-23a-3p	24.357
miR499a-5p	4.242

Table 4.

Change in muscle gene expression

	Fold Change
FSLT1	3.255
MEF2C	1.014
MYOD1	1.290
B2M	−1.303