PROTOCOL TITLE: Effects of Pioglitazone on Exogenous Carbohydrate Oxidation during Steady-State Exercise at High Altitude

SECTION A: RESEARCH TEAM AND LOCATIONS

A1. RESEARCH TEAM

Study Role	Institution/Company and Contact Information
Sponsor	*N/A*
Principal Investigator	*Name, Rank, and Degree:* Lee M. Margolis, PhD *Title:* Nutritional Physiologist *Institution:* MND, USARIEM *Address:* 10 General Greene Ave Bldg. 42, Natick, MA 01760 *Phone Number:* 508-206-2335 *Email:* lee.m.margolis.civ@health.mil
Associate Investigator(s)	*Name and Degree:* Stefan M. Pasiakos, PhD *Title:* Division Chief *Institution:* MPD, USARIEM *Address:* 10 General Greene Ave Bldg. 42, Natick, MA 01760 *Phone Number:* 508-206-2250 *Email:* stefan.m.pasiakos.civ@health.mil *Name and Degree:* Roy M. Salgado, PhD *Title:* Research Physiologist *Institution:* TMMD, USARIEM *Address:* 10 General Greene Ave Bldg. 42, Natick, MA 01760 *Phone Number:* 508-206-2375 *Email:* roy.m.salgado.civ@health.mil *Name and Degree:* Benjamin J. Ryan. PhD *Title:* Research Physiologist *Institution:* TMMD, USARIEM *Address:* 10 General Greene Ave Bldg. 42, Natick, MA 01760 *Phone Number:* 508-206-2408 *Email:* benjamin.j.ryan.14civ@health.mil *Name and Degree:* Emily E. Howard, PhD *Title:* Nutritional Physiologist *Institution:* MND, USARIEM *Address:* 10 General Greene Ave Bldg. 42, Natick, MA 01760 *Phone Number:* 508-206-2309 *Email:* emily.e.howard14.civ@health.mil *Name and Degree:* Jessica A. Gwin, PhD *Title:* Research Physiologist *Institution:* MND, USARIEM *Address:* 10 General Greene Ave Bldg. 42, Natick, MA 01760 *Phone Number:* 508-206-2300 *Email:* jessica.a.gwin.civ@health.mil

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Study Consultant

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Study Coordinator

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Research Monitor

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Ombudsman

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A2. ROLES AND RESPONSIBILITIES

A2.1 Principal Investigator

Name: Lee M. Margolis
Study Responsibilities: Dr. Margolis is responsible for the safe and scientifically sound conduct of the study. He will oversee all aspects of the study, ensure safety and ethical treatment of volunteers; maintain required documentation for the study and obtain required approvals; and will have primary responsibility for data analysis, interpretation, and publication. Dr. Margolis will also be actively involved in the volunteer brief, obtaining consent, and data collection. Dr. Margolis will be responsible for the conduct of the study in accordance with the protocol. Maintenance of a list of appropriately qualified persons to whom significant study-related responsibilities have been delegated.

A2.2 Associate Investigator

Name(s): Stefan M. Pasiakos, Roy M. Salgado, Benjamin J. Ryan, Emily E. Howard, and Jessica A. Gwin, Devin Drummer, Michael Dawson
Study Responsibilities: Protocol development, formulation of protocol questions, hypotheses, experimental approach, and design. Assist PI with volunteer briefing, obtaining informed consent, data collection, and sample processing. Associate investigators will also ensure safety and ethical treatment of participants and will contribute to the analysis, interpretation, and publication of the data.

A2.3 Study Coordinator

Name: Marques Wilson
Study Responsibilities: Primary responsibilities include assisting with volunteer briefing, obtaining informed consent, data collection, preparing and administering study diets, preparing and maintaining data collection forms, and biological sample processing. Will contribute to the analysis, interpretation, and publication of the data. She may conduct procedures for which she is qualified and credentialed as outlined in the DOA log.

A2.4 Ombudsperson

Name(s): Adam Luippold and Andrew Greenfield
Study Responsibilities: The ombudsman shall protect the interests of the research volunteers. The ombudsman will do the following: read the consent form, observe the recruitment briefing and the signing of consent documents, make sure supervisory and command staff are not present, make sure the description of the study and all risks and discomforts are correct, complete, and understandable to the audience, and make sure that the volunteers are not threatened or pressured to participate. They will observe briefings for military participants in the Human Research Volunteer (HRV) program.

A2.5 Study Consultant

Name(s): Andrew J. Young
Study Responsibilities: Study consultant will provide insight and feedback on study design and data interpretation. Activities may include protocol development, formulation of protocol questions, hypotheses, experimental approach, and design. They will not engage with participants or assist with data collection tasks.

A2.6 Research Monitor

Name(s): Sarah L. Hanson
Study Responsibilities: The research monitor will review reports of SAEs/UPIRTSOs and provide an unbiased written report of the events. The research monitor should periodically observe the conduct of the study with subjects. The research monitor should provide concurrence that subjects met all protocol eligibility criteria for the study.

A3. RESEARCH LOCATIONS

USARIEM, Natick MA: The U.S. Army Research Institute of Environmental Medicine (USARIEM) is a DoD research facility within the U.S. Army Medical Research and Development Command. It is the Institute responsible for conducting basic and applied research to determine the effects of exposure to environmental extremes, occupational tasks, physical training, deployment, operational stress and nutritional factors on the health and performance of military personnel. The facility contains environmental chambers for controlling temperature and humidity, an environmentally controlled hypobaric chamber, a water immersion laboratory, as well as several dry and wet laboratories for animal and human experimentation. The dry laboratories are capable of a broad range of experiments, including biomechanical analysis, body composition, energy expenditure, muscle strength and endurance. The wet laboratories include general clinical chemistry analyzers, as well as equipment for ELISA, RIA, histology, and molecular biology assays. Each investigator at the facility has a personal computer with software for data management, analysis, presentation and report generation. Their computers are interfaced with a network server for easy, secure data handling and transfer. All testing procedures will take place at USARIEM.

Metabolic Solutions, Nashua, NH: Metabolic Solutions is a state-of-the art stable isotope analytical laboratory that has the basic laboratory facilities and equipment to analyze stable isotope in breath and blood. Equipment onsite includes an Agilent 6110 LC-Tandem Mass Spectrometer (LC-MS/MS) Triple Quad with Agilent 1100 LC, Thermo Finnigan Delta XP Isotope Ratio Mass Spectrometer (IRMS) with GC Isolink for ^{13}C, ^{15}N and ^{2}H GC-IRMS and Conflow IV interface, and a Thermo Finnigan Delta V Advantage IRMS with trace GC-Combustion III unit for ^{13}C, ^{15}N and D GC-C-IRMS and Conflow IV interface and elemental Analyzer EA112HT ^{13}C, ^{15}N and D sample combustion unit. Metabolic Solutions has been an industry leader in developing new stable isotope tracer applications. Metabolic Solutions will analyze samples on a fee for service basis.

Pennington Biomedical Research Center (PBRC), Baton Rouge, LA: The laboratory is accredited by the College of American Pathologists (CAP) and participates in proficiency testing programs administered by the CAP. Biochemical analyses will be conducted at PBRC on a fee for service basis.

A4. MULTISITE RESEARCH

***Lead Site**:* N/A
***Performance Site**:* N/A

SECTION B: RESEARCH METHODOLOGY

B1. ABSTRACT

Two recent investigations from our laboratory have consistently observed a reduction (30-50%) in the rate of exogenous glucose oxidation in native lowlanders performing metabolically-matched, steady-state exercise at high altitude (HA) compared to sea level (SL). Accompanying lower rates of exogenous glucose oxidation at HA were increases in circulating glucose and insulin concentrations, and reduced glucose rate of disappearance and metabolic clearance rate. Apparent hypoxia-induced insulin insensitivity along with alterations in glucose kinetics suggests reduction in glucose uptake by the peripheral tissue is a primary factor contributing to reductions in exogenous glucose oxidation at HA. As such, the primary objective of this study is to determine the ability of an insulin sensitizer (Pioglitazone, PIO) to enhance exogenous glucose oxidation and metabolic clearance rate during metabolically-matched, steady-state exercise during acute HA exposure compared to placebo (PLA) in native lowlanders. Secondary objective of this study will be to assess the impact of PIO on markers of inflammation and iron status compared to PLA. This randomized crossover placebo control double blinded study will examine substrate oxidation and glucose kinetic responses to ingesting supplemental carbohydrate (glucose) during metabolically-matched, steady-state exercise with acute (~5 h)

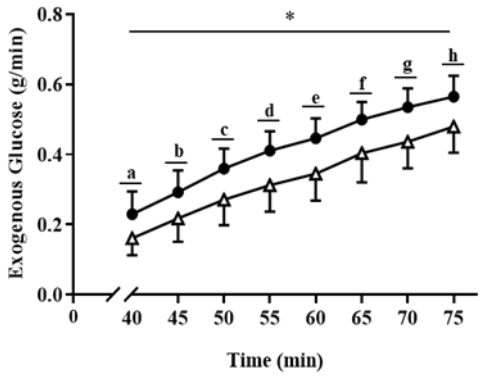
exposure to HA (460 mmHg, or 4300m, barometric pressure similar to Pike's Peak) after receiving PIO (HA+PIO), or after receiving a matched placebo (HA+PLA). Eight healthy, recreationally active males between the ages of 18-39 yrs will be required to complete this study. Following a 4 day glycogen normalization period receiving PIO or PLA daily, volunteers will complete two 80-min trials, performing metabolically-matched, steady-state aerobic (same absolute workload corresponding to ~55 ±5% of $\dot{V}O_2$peak at HA) exercise on a treadmill, and consuming 145 g of glucose (1.8 g/min); one trial with HA+PIO and the other with HA+PLA. A dual glucose tracer (^{13}C-glucose oral ingestion and [6,6-$^{2}H_2$]-glucose primed, continuous infusion) technique and indirect calorimetry will be used to selectively analyze endogenous and exogenous glucose oxidation, as well as glucose rate of appearance (Ra), disappearance (Rd) and metabolic clearance rate (MCR). Serial blood samples will be collected during each trial to assess endocrine and circulating substrate responses to exercise, carbohydrate, and hypoxia with or without PIO. All trials will occur at the same time of day in the USARIEM hypobaric/hypoxic chamber and be separated by a minimum 10-d washout period. The primary risks associated with this study include those associated with acute hypobaric hypoxia, exercise, and blood sampling.

B2. BACKGROUND AND SIGNIFICANCE

Acute High Altitude Exposure and Glucose Metabolism

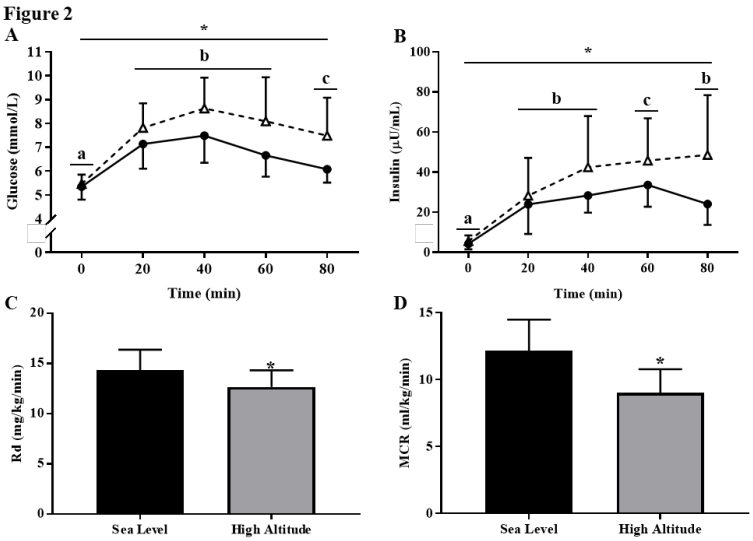
Deployments to austere and remote mountainous regions will occur during Multi-Domain Operations (MDO). Rapid ascent to high altitude (i.e., hypobaric hypoxia; HA) elicits physiologic and metabolic adjustments that collectively degrade physical performance and augment dietary requirements. In particular, HA exposure increases reliance on carbohydrate for fuel to sustain physical activity, resulting in rapid declines in muscle glycogen content which, in part, contributes to decreased performance (1-3). To combat declines in physical performance, the Modular Ration Enhancement Operations HA (MORE-HA) was created to provide additional dietary carbohydrate to fuel sustained physical activity. However, recent studies from our laboratory have consistently demonstrated a 30-50% reduction in oxidation of exogenous glucose consumed by unacclimatized lowlanders performing steady-state exercise during acute (< 8 hrs) high altitude (HA) exposure compared to metabolically-matched exercise performed at sea level (SL; **Figure 1**) (3, 4). Reduced exogenous glucose oxidation during acute (< 8 hrs) HA exposure has also been demonstrated by others (5-7). Across studies, reductions in exogenous glucose oxidation occurred whether exercise at SL and HA were matched for absolute (3-5) or relative (6, 7) intensity. Consistent results between investigations from multiple laboratories, matching for different exercise intensities, indicates a true physiological dysregulation in use of exogenous carbohydrate for fuel use during aerboic exercise after initial arrival to HA from SL.

Figure 1



Margolis et al. *Metabolism* 2019. Values mean ± SD. Values with different letters are different from each other. *HA different than SL. ● Sea Level △ High Altitude

Accompanying lower rates of exogenous glucose oxidation at HA are increases in circulating glucose and insulin concentrations (**Figure 2A,B)**, and reductions in glucose rate of disappearance and metabolic clearance rate (MCR; **Figure 2C,D**) during steady-state exercise compared to SL (3). These data are characteristic of insulin resistance (8) and suggest that reductions in peripheral glucose uptake may be a primary factor contributing to lower rates of exogenous glucose oxidation at HA. The inability to effectively use dietary carbohydrate as a metabolic fuel source and the likelihood that peripheral insulin resistance modulates those effects, suggests that a more aggressive solution than carbohydrate supplementation alone is required to optimize fuel utilization during hypoxia and enhance physical performance during future HA MDO.



Margolis et al. *Metabolism* 2019. Values mean ± SD. Values with different letters are different from each other. *HA different than SL. ● Sea Level △ High Altitude

One possible solution is to employ short-term use of insulin sensitizing drugs, such as biguanides (Metformin) and thiazolidinediones (Pioglitazone; PIO), which are commonly prescribed to maintain glucose homeostasis in type II diabetics. In the context of acute HA exposure, there is evidence that Metformin may improve hypoxia-induced insulin sensitivity and increase muscle glycogen synthesis at rest (9). Though Metformin enhances insulin sensitivity and peripheral glucose uptake, it also reduces intestinal glucose absorption and hepatic gluconeogenesis. The latter effects may limit the amount of exogenous and endogenous glucose available to fuel performance. Conversely, PIO's primary mechanism of action is to target peripheral tissue (e.g., muscle and lipid), as well as the liver to improve insulin sensitivity and glucose uptake without affecting intestinal glucose absorption. The mechanism of action for PIO suggests it is more appropriate than Metformin as an insulin sensitizer to enhance glucose uptake and oxidation to sustain physical performance during unacclimatized HA exposure. To date, no study has assessed the effectiveness of PIO on improving insulin sensitivity at HA to enhance exogenous glucose oxidation or glucose turnover.

Acute High Altitude Exposure, Inflammation and Iron Status

Recently, Hill et al.(10, 11) reported that following prolonged exercise (60 minutes at 65% sea level $\dot{V}O_2$peak) in normobaric hypoxia (FIO2 = 0.135 or ~3,500 m) there is an increase in gastrointestinal permeability, decrease inflammatory response of peripheral blood mononuclear cells (PBMC), and reduction in the ratio of pro- to anti-inflammatory circulatory biomarkers when compared to sea level. From their findings, the authors concluded that a short bout of exercise in normobaric hypoxia caused acute immune-disruption. An intriguing finding from their study(10) was a decrease in phosphorylated AMP-activated protein kinase (p-AMPK) one hour post exercise and a decrease in sirtuin 1 (SIRT1) one and four hours post exercise while simultaneously having greater reduction in nuclear factor kappaB (NF-κB) one hour post exercise in hypoxic conditions. Both p-AMPK and SIRT1 inhibit NF-κB inflammatory pathway (12, 13). Thus, the finding of reduced PBMC inflammatory response post exercise in normobaric hypoxia seems contradictory.

In addition to its insulin sensitizing effects, PIO has been shown to have anti-inflammatory and antioxidative effects. Zhang et al.(14) reported that PIO lowered circulating inflammatory markers in individuals with impaired glucose tolerance. PIO has been shown to increase p-AMPK(15) and SIRT1(16) and thus reduce inflammation. However, no studies have investigated the effects of PIO on inflammatory responses during acute exercise in hypobaric hypoxia particularly when normal health individuals have higher blood glucose levels.

An accumulating body of evidence indicates key interactions between hypoxia, insulin resistance, and systemic iron homeostasis (17, 18). A secondary effect of improving hypoxia-induced insulin resistance may also be an improvement in iron status. Indeed, several cellular mechanisms regulating iron homeostasis are influenced by

PIO-targeted signaling pathways (19, 20). These data may suggest that alleviation of hypoxia-induced insulin resistance may also improve iron status. As iron is vital to the oxygen carrying capacity of the blood, improved iron status under hypoxic conditions may facilitate improve performance during unacclimatized military operations. However, the influence of PIO on systemic iron regulation during acute HA exposure has not been determined.

B3. MILITARY RELEVANCE

Rapid deployment to remote mountainous regions will occur during MDO. Unacclimatized Service members experience altered carbohydrate metabolism, causing an increased reliance on glycogen for fuel, concomitant with a blunted ability to take up and oxidize exogenous carbohydrate. These negative physiological effects likely exacerbate declines in physical performance at HA. This proposal directly supports the Military Operational Medicine Research Program's Physiological Health and Performance Program Area under the Biomedical Performance Enhancement (BPE) Work Unit. Specifically, the proposed work aligns to BPE Strategic Plan's *Technical Objective 1.1.3: Material solutions (pharmacological) that enhance Soldier resistance to environmental stressors.*

This proposal will provide guidance for use of PIO to enhance exogenous carbohydrate oxidation at HA operations, and will provide the opportunity to determine if carbohydrate supplementation can indeed enhance performance if substrate metabolic dysregulation is avoided during HA exposure. Information from this proposal would be valuable to operational partners such as USSOCOM and the 10th Mountain Division who engage in HA MDO.

B4. OBJECTIVES/SPECIFIC AIMS/RESEARCH QUESTIONS

Objective:

1. Determine the effects of PIO on exogenous glucose oxidation and glucose turnover during exercise under acute HA exposure compared to PLA.
2. Determine the effects of PIO on inflammation during acute HA exposure compared to PLA.
3. Determine the effects of PIO on markers of iron status during acute HA exposure compared to PLA.

Hypotheses:

1. Exogenous glucose oxidation, Rd, and MCR during metabolically-matched, steady-state exercise at HA will be greater after PIO administration compared to PLA.
2. Inflammation will be lower during acute HA exposure after PIO administration compared to PLA.
3. Markers of iron status will be altered during acute HA exposure after PIO administration compared to PLA.

B5. RESEARCH PLAN

B5.1 Research Design

This study will be a randomized crossover double blind placebo control trial.

Table 1: Study Timeline

	Baseline					Trial 1					Washout	Trial 2				
Study Day	**SV**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10-19**	**20**	**21**	**22**	**23**	**24**
Medical Screening	X															
Body Composition		X														
Height		X														
Weight		X								X						X
VO_2peak		X	X													
High Altitude Exposure			X							X						X
Practice Exercise				X	X											
Glycogen Normalization						X						X				
PIO/PLA						X	X	X	X	X		X	X	X	X	X
Study Diet						X	X	X	X			X	X	X	X	
Steady-State Exercise										X						X
Carbohydrate Tracer Study										X						X
Blood Sampling										X						X
Breath Sampling										X						X

Timeline may shift based on participant availability, weekend, and holiday schedules.

B5.2 Research Subjects/Population(s)

B5.2.1 Subject Population(s)

Subject population will be representative of active duty male service members, being in good health and recreationally active. Previous studies at USARIEM (Protocol# 16-02HC and 18-09H) which have evaluated metabolically-matched exercise at HA compared to SL have only been performed in male volunteers. It is apparent that sex-based differences exist during low-to-moderate intensity endurance exercise at sea level. Sex-based differences in substrate oxidation during exercise at SL may result in differences in response to change in substrate oxidation at HA between men and women. Therefore, this study will recruit male volunteers only as sex differences in glucose metabolism at HA are not known. Based on findings from this and our previous studies, a similar series of investigations will be conducted in the future to assess the impact that unacclimatized HA exposure on females.

B5.2.2 Number of Subjects, Records, and/or Specimens

To complete testing on the 8 volunteers necessary to reach statistical power, we estimate we will need to enroll 24 individuals. All screening will stop once complete data has been collected on 8 volunteers. Records and specimen collection are described in the Research Procedures and Data Collection sections. During briefings and consenting potential participants will be informed that even though they may be eligible and want to participate, if we are able to obtain enough data from preceding subjects, they may not ultimately be tested. These individuals may be recruited from the HRV population, civilians, and active duty military personnel (on and off the installation).

B5.2.3 Inclusion Criteria

- Men aged 18 – 39 years
- Born at altitudes less than 2,100 m
- Physically active (exercise minimum 2 days per week)
- Have supervisor approval (permanent party military and civilians)
- Willing to refrain from alcohol, smokeless nicotine products and dietary supplement use during study periods

B5.2.4 Exclusion Criteria

- Born at altitudes greater than 2,100 m
- Musculoskeletal injuries that compromise exercise capability
- Metabolic or cardiovascular abnormalities, or gastrointestinal disorders
- Taking medication that affects macronutrient metabolism and/or the ability to participate in strenuous exercise
- Living in areas that are more than 1,200 m, or traveled to areas that are more than 1,200 m for five days or more within 2 months of data collection
- Evidence of apnea or other sleeping disorders
- Prior diagnosis of high altitude pulmonary edema or high altitude cerebral edema
- Presence of asthma or respiratory tract infections
- Smoking or vaping
- Taking medications that interfere with oxygen delivery and transport
- Anemia (HCT <38% and HBG <12.5 g/dL) and Sickle Cell Anemia/Trait
- Blood donation within 8 weeks of beginning the study
- Unwilling or unable to consume study diets or foods provided due to personal preference, dietary restrictions, and/or food allergies
- Unwilling or unable to adhere to study physical restrictions

B5.3 Research Procedures

Research procedures conducted by investigators and support personnel will be in accordance with the Delegation of Authority (DOA) Log for Key Research Personnel as described below.

COVID-19 risk mitigation: Study staff and participants will comply with all COVID-19 risk mitigation procedures in place at USARIEM during the time of data collection. As such, participants may be asked to wear face masks and use hand sanitizer during data collection activities (in accord with prevailing recommendations at the time of data collection) and may be asked to wear gloves (i.e., nitrile gloves) during data collection activities. Study staff and participants also may be asked to undergo COVID-19 testing (via nose swab performed at USARIEM) before conducting data collection activities and study participation in the HA chamber. Participants will be asked if they are experiencing symptoms of COVID-19 each day. If a volunteer is experiencing or reports symptoms, they will be restricted from study activity, including entering the altitude chamber and sent to Office of Medical Support and Oversight (OMSO) for further evaluation. Depending on the timing of symptom onset/positive COVID-19 results, the participant may be allowed to repeat study activities when symptoms/illness subside.

This randomized crossover placebo controlled double blinded study will examine substrate oxidation and glucose kinetic responses to ingesting supplemental carbohydrate (glucose) during metabolically-matched, steady-state exercise with acute (~5 h) exposure to HA (460 mmHg) after short-term (5 days) use of Pioglitazone (HA+PIO), or matched placebo (HA+PLA). Eight healthy, recreationally active males between the ages of 18-39 yrs will be enrolled. Following a muscle glycogen normalization period, volunteers will complete 80-min of metabolically-matched, steady-state (same absolute workload corresponding to ~55 ± 5% of $\dot{V}O_2$peak at HA) exercise on a treadmill, and consume 145 g of glucose (1.8 g/min) with HA+PIO and HA+PLA. A dual glucose tracer (^{13}C-glucose oral ingestion and [6,6-$^{2}H_2$]-glucose primed, continuous infusion) technique and indirect calorimetry will be used to analyze endogenous and exogenous glucose oxidation, as well as glucose rate of appearance (Ra), disappearance (Rd), and MCR. Serial blood samples will be collected during each trial to assess endocrine and circulating substrate responses to exercise, carbohydrate, and hypoxia, with or without PIO. Isotope methodology and aerobic exercise protocols will be identical to our previous work (3, 4), allowing comparison of outcomes across studies. All

trials will be conducted in the USARIEM hypobaric/hypoxic chamber and be separated by a minimum 10-d washout period.

A member of the research team will generate a randomization scheme using a random number generator (http://randomization.com or similar). Treatment type (PIO/PLA) will be coded to de-identify treatment (Example: treatment A and treatment B or similar) to blinded staff. Assigned unblinded staff, responsible for administering the treatment to volunteers, will be responsible for creating treatment codes to match treatment. They will maintain record of randomization scheme and treatment.

Body Composition*:* Body composition will be determined using dual energy x-ray absorptiometry (DEXA, DPX-IQ, GE Lunar Corporation, Madison, WI). The DEXA technique allows for the non-invasive assessment of soft tissue composition by region with a precision of 1-3% (21). The volunteer will lay face-up on the DEXA densitometer table in shorts, t-shirts, and stocking feet. Volunteers will be asked to remain motionless for the 8-10 min scan. These data will be used to calculate total body mass, fat-free mass, and fat mass. Calibration to external standards will be performed before actual data collection. Measurements of body composition will be used for participant characterization and to assess if fat mass and fat-free mass are confounding variables for substrate oxidation.

Height and Weights**:** Anthropometrics, performed using standardized techniques and equipment, will be used to determine volunteer eligibility and characterize study volunteers. Height will be measured to the nearest 0.1 cm using a stadiometer at screening. Body mass will be measured after an overnight fast (10 hr), using a calibrated digital scale to the nearest 0.1 kg at screening. Body mass will be measured at baseline and the morning of experimental trial days.

Determination of Peak Oxygen Uptake**:** Following a minimum of a 10 hour fast, volunteers will complete two separate maximal aerobic exercise sessions to determine peak oxygen uptake ($\dot{V}O_2$peak) on a treadmill. Two treadmill $\dot{V}O_2$peak assessments will be conducted because HA exposure of 4,300 m produces a decrease in $\dot{V}O_2$peak of ~27% compared to SL (22). As such, volunteers will complete one treadmill $\dot{V}O_2$peak at SL and one at HA so $\dot{V}O_2$peak is known under both conditions. $\dot{V}O_2$peak will be determined using an indirect, open circuit respirator system (True Max 2400, Parvomedics, Sandy, Utah, USA). Volunteers will be clothed in appropriate athletic attire and perform this assessment at standard ambient indoor temperature (20-22°C) and humidity conditions (20-30%). Volunteers will be given adequate time to become familiar with the testing procedures and allowed a 3-min self-paced warm-up on the treadmill. At the start of testing, the volunteer will put on a mask connected to a 2-way respiratory valve. The volunteers will begin by running for 4 min at a pace predetermined as comfortable at a 0% grade. At 4-min, the grade will be increased to 4% followed by an additional 2% every 2 min thereafter until volitional exhaustion.

Heart rate will be monitored using a heart-rate monitor (Polar Electro Inc, Oulu, Finland) the last 30 seconds of each workload during all testing. The test will be stopped immediately if the subject reports angina-like symptoms, exertional syncope, shows signs of poor perfusion (i.e., light-headedness, confusion, ataxia, pallor, cyanosis, nausea, or cold and clammy skin), or if testing equipment fails. Staff conducting this test are trained and certified in basic life support.

Glycogen Normalization**:** Following an overnight (10 hour) fast, volunteers will complete a glycogen normalization protocol on a cycle ergometer. The intensity will be based on $\dot{V}O_2$peak. Volunteers will begin with a 5-min warm-up before beginning the protocol. After a warm-up period, the cycle ergometer protocol is comprised of repeated periods of 2 min of work at 80 ±5% $\dot{V}O_2$peak followed by 2 min of recovery at 50 ±5% $\dot{V}O_2$peak. The protocol will last approximately 50-min (12 work:rest cycles). To ensure familiarity with the testing procedures, volunteers will perform one practice session during the baseline pre-study period. If volunteers are unable to maintain rotations per minutes (rpm) above 50 for 15 seconds during the work stage, watts will be lowered to ensure that the volunteers can complete all 12 work:rest cycles. Volunteers will be permitted to consume water *ad libitum* during the protocol.

Study Diet**:** After completing the glycogen normalization protocol, volunteers will be fed a controlled diet

prescribed to maintain energy balance for 4 days. The diet will provide 6.0 g carbohydrate/kg/d, 1.2 g protein/kg/d, and 1.0 g fat/kg/d. All food and beverages (except water) will be prepared and provided by study dietitians and consist largely of military combat ration and supplemental food items (ex. Frozen prepared dinners, breakfast sandwiches, commercial granola bars, etc.). The same diet will be consumed for both arms of the study. Volunteers will thus consume the study diet for 8 days total. Corn-derived sugars will be avoid in the study diets to reduce high natural abundance of ^{13}C. Caffeine will be restricted to no more than one calorie-free caffeinated beverage per day (if the research volunteer chooses to consume caffeine). The caffeinated beverage will be supplied by study staff and must not be consumed during fasting periods or prior to exercise. Volunteers will be asked to return all wrappers to study dietitians to ensure consumption of prescribed study foods.

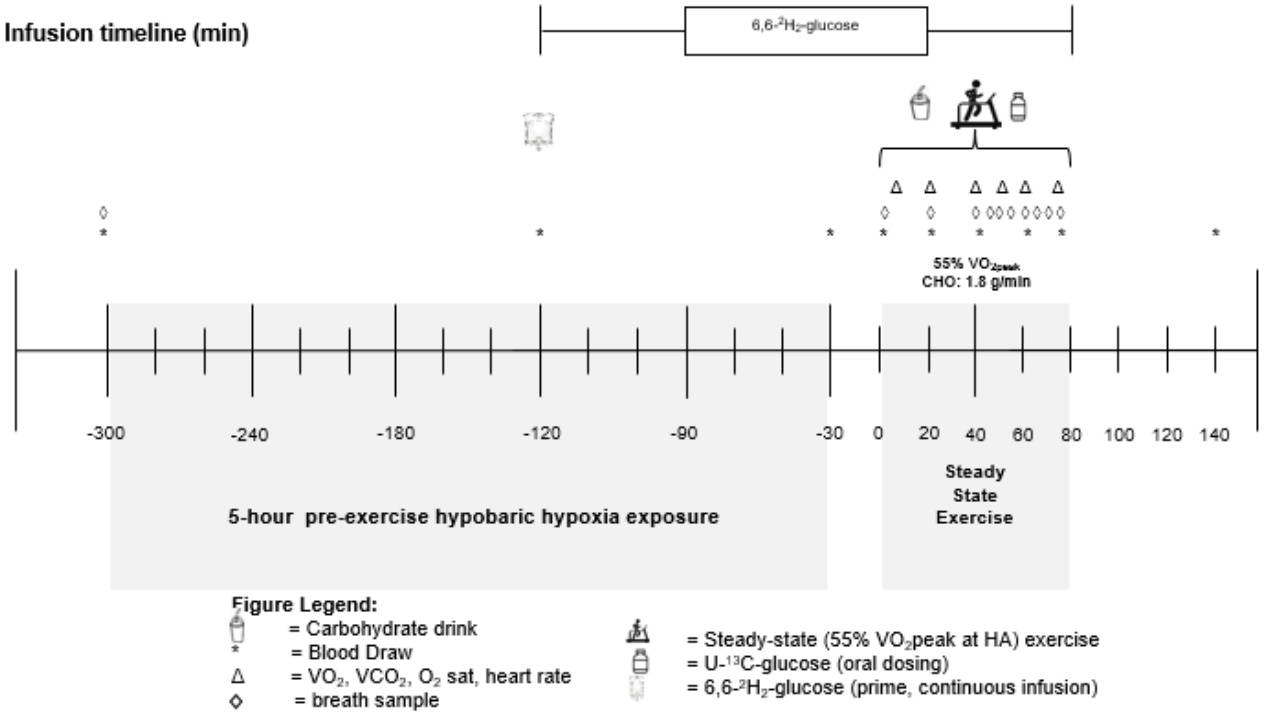
Insulin Sensitizer: Pioglitazone (PIO) will be administered as a 15 mg oral dose per day for 5 days during the HA+PIO arm of the study. A 15 mg dose was chosen because this is the minimal dose required to induce a clinically significant improvement in insulin sensitivity (23). Volunteers will take the oral dose in the morning while they are consuming the glycogen normalization diet (6.0 g carbohydrate/kg/d) for 4 days and once more on the carbohydrate tracer study day. Volunteers will also table a placebo (PLA) during one arm of the study. It will be administered in the same sequence as PIO to assure blindness to study participants and blinded study staff. The PLA used will be a closely matched tablet, Zeebo®, composed of 100% microcrystalline cellulose. PIO/PLA will be held and administered by OMSO staff only.

High Altitude Exposure: To simulate ascension to high altitude, experimental trial testing will occur in the hypobaric/hypoxic (altitude) chamber at USARIEM under temperate (20°C, 20-30% rh) ambient conditions. The chamber will reduce barometric pressure, and thus ambient oxygen pressure, to a value similar to that at the summit of Pike Peak, CO (4,300 m). Volunteers will be exposed to hypobaric/hypoxic conditions for 5-hrs before beginning steady-state exercise to mimic our past investigations. Total time in the chamber on the experimental trial day will be ~7-hrs. No more than two volunteers will be tested in the chamber at one time. Study personnel who will conduct data collection in the altitude chamber will be medically cleared by OMSO. Medical staff (OMSO) will be onsite while volunteers and staff are in the simulated altitude chamber. Additionally there will be at least three chamber operators in communication with the PI who will be in the chamber with volunteers at all times.

***Steady-State Treadmill Exercise*:** Results from a $\dot{V}O_2$peak assessment under HA conditions will be used to prescribe intensities for treadmill exercise on experimental trial days utilizing ACSM equations to estimate the speed and grade of the treadmill. Volunteers will complete 80-min of metabolically-matched, steady-state exercise at 55 ±5% of their $\dot{V}O_2$peak determined at HA. A practice session will be conducted during the baseline period to confirm prescribed speed and grade are appropriate to induce target $\dot{V}O_2$ and allow the research team to make modifications to prescribed speed and grade if needed to induce target VO_2. The practice session will range ~40-80 minutes in length. The speed and grade determined during the practice session will be used to induce the same absolute intensity between study arms. The mode, duration, and intensity of steady-state exercise were chosen to mimic our past investigations (3, 4).

Carbohydrate Tracer Studies: After a 10 hr overnight fast, two catheters will be placed into the lower arm (one in each arm). One arm will be used for infusion of 6,6-[$^2H_2$] glucose tracer and the other will be used for blood sampling. Following an initial blood and breath sample collection to determine background enrichments, a primed, continuous infusion of 6,6-[$^2H_2$] glucose (Cambridge Isotope Laboratory, Andover, MA, USA) will begin (prime, 82.2 μmol·kg^{-1}; continuous rate, 0.78 μmol·kg^{-1}·min^{-1}) (24, 25) for 2 hrs before and during exercise. Volunteers will complete 80-min of steady-state exercise on a treadmill, with $\dot{V}O_2$, $\dot{V}CO_2$, HR, and SaO_2 measured at approximately 5, 20, 40, 50, 60, and 75 min. During exercise volunteers will consume a glucose beverage at 1.8 g/min. Drinks will contain 145 g glucose, with a total volume of 1450 mL. The drink will be consumed in four boluses throughout the 80-min of steady-state exercise (0, 20, 40, and 60 min). The first bolus will be 550 mL, with the remaining three being 300 mL. The glucose beverage consumed during exercise will be enriched with 200 mg ^{13}C-glucose (Cambridge Isotope Laboratory) to increase the isotopic enrichment well above natural levels and optimize the measurement of exogenous glucose oxidation.

Figure 3: Carbohydrate Tracer Studies



Blood Sampling *(credentialed procedure)***:** Blood samples will be collected using an indwelling catheter kept patent using IV saline at approximately at approximately -300, -120, -30, 0, 20, 40, 60, 80, and 140 min during exercise by a USARIEM credentialed phlebotomist. Baseline blood sampling will occur after a 10-hr overnight fast on experimental trial days. A total of 18 blood samples will be completed during this study taking ~340 mL of blood sampled from each volunteer over the experimental trial days (**Table 3**). Blood samples will be used for isotope analysis assessment, substrate and hormone responses, and iron markers (**Table 3**).

For glucose kinetic analysis, the tracer/tracee ratio (6,6-[$^{2}H_{2}$] glucose/glucose) using gas-chromatography-mass spectrometry (GCMS; Metabolic Solutions, Inc., Nashua, NH) (24), while $^{13}C/^{12}C$ in plasma glucose will be measured using isotope-ratio mass spectroscopy (IRMS; Metabolic Solutions).

Breath Sampling *(non-credentialed procedure*):Breath samples will be collected at approximately -300, 0, 20, 40, 45, 50, 55, 60, 65, 70, 75, and 80 min during exercise to determine exogenous glucose oxidation using single-patient breath collection bags (Quin-Tron Instrument Company, Milwaukee, WI, USA). Baseline breath sample will also be collected prior to correct for background of naturally occurring ^{13}C. The natural enrichment of the glucose ingested and the $^{13}C/^{12}C$ in expired gas samples will be analyzed using isotope-ratio mass spectroscopy (Metabolic Solutions, Inc., Nashua, NH).

B5.4 Data Collection

Table 2.Outcome Variables

Data Element/Variable	Source	Operational Specification
Anthropometric data	Direct measurement	Height, weight, body composition
Substrate oxidation	Blood and breath	Glucose turnover, carbohydrate, fat, and protein oxidation
Metabolic response	Blood	Substrate analytes, hormones, inflammation, iron status

Table 3. Blood Analytes

	Time points (min)								
Analyte	**-300**	**-120**	**-30**	**0**	**20**	**40**	**60**	**80**	**140[4]**
Glucose[1]	x	x	x	X	x	x	x	X	
Insulin[2]	x			X	x	x	x	X	
Free fatty acids[2]	x			X	x	x	x	X	
Lactate[2]	x			X	x	x	x	X	
Glycerol[2]	x			X	x	x	x	X	
Norepinephrine[2]	x			X	x	x	x	X	
Epinephrine[2]	x			X	x	x	x	X	
Hemoglobin[1]	x			X				x	
Hematocrit[1]	x			X				x	
^{13}C-glucose[3]				X	x	x	x	x	
6,6-[$^{2}H_2$] glucose[3]		x	x	X	x	x	x	x	
PBMC[1]	x			X				x	x
TNFα[1]	x			X				x	x
IL-6[1]	x			X				x	x
IL-10[1]	x			X				x	x
Hemooxygenase 1[1]	x			X				x	x
Adiponectin[1]	x			X				x	x
Erythropoietin[1]	x			X					
Hepcidin[1]	x			X					
Soluble transferrin receptor[1]	x			X					
Erythroferrone[1]	x			X					
Platelet derived growth factor-BB[1]	x			X					
Ferritin[1]	x			X					
Serum Iron[1]	x			X					
Transferrin Saturation[1]	x			X					
C-reactive protein[1]	x			X					
Archive	x	x	x	X	x	x	x	x	x

Samples will be analyzed at [1]USARIEM, [2]Pennington Biomedical Research Center (Baton Rouge, LA), and [3]Metabolic Solutions (Nashua, NH). [4]Final blood draw at ~140 min will be taken under normoxic conditions in both trials. All blood samples will be separated into plasma and serum through centrifugation, aliquoted, and stored at –80˚C until analysis or shipment. Study samples will be collected and aliquoted at USARIEM and specific samples will be shipped on dry ice to Pennington Biomedical Research Center, and Metabolic Solutions. Serum iron and transferrin saturation will be measured using an automated clinical chemistry

and immunoassay analyzer (Dimension: Siemens Healthcare Diagnostic, Deerfield, IL, USA). Serum soluble transferrin receptor, interleukin-6, hepcidin, platelet-derived growth factor-BB, erythropoietin, erythroferrone, and C-reactive protein will be determined using enzyme-linked immunosorbent assays. Blood sent out to other laboratories for analysis will not have any remaining samples after analysis is completed. USARIEM will retain all archive samples.

Plasma Glucose Turnover Calculations

For calculation of plasma glucose turnover the Steele equation with modifications for non-steady state will be used (26). Enrichment (E) will be expressed as mole percent excess (MPE); calculated as (TTR)/(1 + TTR), where TTR is the tracer to tracee ratio. Appropriate corrections for skewed abundance distribution and overlapping spectra will be made for the TTR of the glucose tracers, 6,6-[$^2H_2$] glucose and U-^{13}C-glucose (26). From these calculations, total glucose Ra will be comprised of rates of appearance of exogenous (i.e., ingested) glucose and of endogenous (i.e., hepatic glucose production and negligible renal glucose production or splanchnic glucose) glucose (26):

$$\text{Total glucose } R_a \text{ (Total } R_a) = (F - ((pV \times ((C_2 + C_1)) / 2) \times ((E_2 - E_1) / (t_2 - t_1)))) / ((E_2 + E_1) / 2)$$

$$\text{Glucose } R_d = \text{Total } R_a - (pV (C_2 - C_1) / (t_2 - t_1))$$

$$\text{Exogenous glucose } R_a \text{ (Exo } R_a) = ((\text{Total Ra} + F) \times ((G2 + GE1) / 2) + ((pV \times ((C2 + C1) / 2)) \times ((G2 - G1) / (t2 - t1)))$$

$$\text{Endogenous glucose } R_a = \text{Total } R_a - \text{Exo } R_a$$

$$\text{Metabolic Clearance Rate (MCR)} = \text{Glucose } R_d / ((C_2 + C_1) / 2)$$

Where F represents the infusion rate of 6,6-[$^2H_2$] glucose; *p*V is the effective volume of distribution for glucose, C_1 and C_2 are plasma glucose concentrations at t_1 and t_2, respectively, E_1 and E_2 are plasma enrichments of 6,6-[$^2H_2$] glucose at t_1 and t_2, respectively, and E_d and E_p are tracer enrichments of U-^{13}C-glucose from the test drink and plasma, respectively.

Calculations of Carbohydrate and Fat Oxidation

Carbohydrate and fat oxidation rates will be calculated from $\dot{V}O_2$ (L/min) and $\dot{V}CO_2$ (L/min) during the 80-min exercise bout as described by Jeukendrup and Wallis (27):

$$\text{Fat oxidation (g/min)} = (1.695 \times \dot{V}O_2) - (1.701 \times \dot{V}CO_2)$$

$$\text{Carbohydrate oxidation (g glucose/min)} = (4.585 \times \dot{V}CO_2) - (3.226 \times \dot{V}O_2)$$

Calculations of Exogenous and Endogenous Glucose Oxidation

Exogenous and plasma glucose oxidation will be calculated as (7):

$$\text{Exogenous glucose (g/min)} = \dot{V}CO_2\ [(Rexp - Rref)/(Rexo - Rref)]/k$$

$$\text{Plasma glucose (g/min)} = \dot{V}CO_2\ [(Rexp - Rref)/(Rglu - Rref)]/k$$

where $\dot{V}CO_2$ is in L/min, Rexp is the observed isotopic composition of expired CO_2, Rref is the isotopic composition of expired CO_2 at rest before ingestion of the first dose of ^{13}C-glucose, Rexo is the isotopic composition of the exogenous glucose ingested, Rglu is the isotopic composition of plasma glucose, and k (0.747 L/g) is the volume of CO_2 provided by the complete oxidation of glucose. Total endogenous glucose oxidation can be calculated by subtracting exogenous glucose oxidation from total CHO oxidation. Endogenous glucose oxidation derived from muscle and liver can be determined by subtracting plasma glucose oxidation from total carbohydrate oxidation (muscle), and subtracting exogenous carbohydrate oxidation (liver) from plasma glucose oxidation (7). The first 40 min of steady-state exercise will allow for equilibration between the $^{13}C/^{12}C$ in expired CO_2 and the $^{13}C/^{12}C$ in CO_2 produced in tissues (28). Thus, endogenous glucose oxidation will only be calculated from samples obtained in the last 40 min of steady-state exercise (40 to 80 min).

Blood Analytes

Serum glucose, free-fatty acids, glycerol, and plasma lactate concentrations will be determined using enzymatic and colorimetric assays (Beckman Coulter DXC 600 Pro, Beckman Coulter, Brea, CA, USA).

Serum insulin concentrations will be determined using an advanced automated immunoassay instrument (ImmuliteR 2000: Siemens Healthcare Diagnostic, Deerfield, IL, USA). Serum epinephrine and norepinephrine will be determined using a BI-CAT® Adrenaline and Noradrenaline ELISA assay kit (Eagle Biosciences, Nashua, NH, USA). Blood analytes will be analyzed at Pennington Biomedical Research Center (PBRC) under an existing fee for service contract.

Circulatory and Intracellular Inflammatory Biomarkers
Following ~20 minutes of seated postural control, venous blood (8-10 mls) will be collected at four time points (-300, 0, 80, and 140 min) into EDTA treated collection tube. PBMC will be isolated using density gradation separation. Briefly, whole blood will be carefully layered on-top of a separating media (Histopaque 1077, Sigma-aldrich) at a 1:1 ratio then centrifuged at 450 x g for 30 minutes at 22°C. Blood plasma will be aliquoted into cyrovials and stored at -80°C freezer for batch analysis at a later time. The buffy coat containing the PBMC will be collected and transferred into a sterile conical tube and washed thrice with 10 ml phosphate buffer saline. The PBMC pellet will then be stored at -80°C for analysis of intracellular proteins using immunoblotting. Circulating (in blood plasma) and intracellular (PBMC) biomarkers will be assayed via molecular assay techniques (e.g., Western blot, enzymatic/colorimetric, multiplex, etc.) at the USARIEM laboratories. Intracellular inflammatory biomarkers will include, but not be limited to AMPK, SIRT1, NF-KB, IKK-B, and IL1RA.

B5.5 Managing Data and/or Human Biological Specimens for this Research

All data and medical information obtained will be considered privileged and held in confidence. Study volunteers will be assigned unique subject identification (ID) numbers that will not contain any personal identifiers such as name, social security number, address, date of birth, zip code, etc. This study subject ID number will be used on all data collection instruments, to include questionnaires, data collection forms, computer records, etc. A number will be assigned as each volunteer is medically cleared for participation. A master list linking the volunteers' names and ID numbers will be kept in a separate locked file in the principal investigator's office, or kept in a computer file with password-protected access restricted to the principal investigator and study coordinator. When the results of the research are published or discussed in conferences, no information will be included that would reveal identity. All samples will be stored in -80ºC freezer at USARIEM in room 322 or 304 using the subject identification number. De-identified samples for isotopic analysis will be shipped on dry ice to (blood) and room temperature (breath) to Metabolic Solutions. De-identified blood will be shipped on dry ice to Pennington Biomedical Research Center. All samples will be shipped via FedEx and stored in these laboratories until analyzed. Once samples have been analyzed, there will be no remaining sample for storage other than the archived sample stored at USARIEM. Coded data will be transmitted between the above mentioned laboratories via an encrypted email, a secure file transfer site, or using an approved removable media. Only USARIEM will maintain digital copies of these data. Body composition and $\dot{V}O_2$peak data will be shared with volunteers. No other data will be shared with volunteers.

Only the principal investigator and study coordinator will have access to personal identifiable data. No outside laboratory will have access to identifiable data. Hard copy data records will be stored for a minimum of three years from the time the study is completed. Electronic data records will be maintained for a period of at least ten years after the study has been completed. The master list will be destroyed upon the protocol's closure.

B5.6 Managing Data and/or Human Biological Specimens for Future Research

De-identified study samples will be stored in -80ºC freezer at USARIEM in room 322 or 304 for potential future use and maintained indefinitely. Only personnel assigned to the research study by the principal investigator will have access to samples. The de-identified data and samples will remain under the control of the PI and may be shared with outside collaborators for future research. Any use of the samples outside of this defined protocol will be submitted as a protocol amendment or a new protocol.

B5.7 Devices, Drugs, Dietary Supplements, Nutritional Supplements, And Biologics

B5.7.1 Devices

5.7.1.1 FDA-approved device being used in this research according to the approved labeling
DEXA, DPX-IQ, Lunar Corporation, True Max 2400, Parvomedics, Sandy, Utah, USA, Polar Electro Inc, Oulu, Finland

5.7.1.2 FDA-approved device being used in this research in a manner other than its approved labeling
N/A

5.7.1.3 Any device not approved by the FDA
N/A

B5.7.2 Drugs

B5.7.2.1 FDA-approved and used in accordance with the approved labeling

B5.7.2.2 FDA-approved and used in a manner not in accordance with its approved labeling
Pioglitazone, Thiazolidinedione, Takeda Pharmaceutical Company

B5.7.2.3 Any drug not approved by the FDA
N/A

B8 Statistical Analysis

B5.8.1 Sample Size Estimation

Based on results of exogenous carbohydrate oxidation and MCR rate between SL and HA in our previous investigation (3, 4), a sample size of 8 is required to achieve 80% power (**Table 4**). We will request to enroll up to 24 individuals to account for withdrawals from the study.

Table 4: Previous study effect sizes

Study	Outcome	Delta	Effect Size	Sample Size
Young et al. (4)	Exogenous Glucose Oxidation	0.35 ± 0.21	1.11	7
Margolis et al. (3)	Exogenous Glucose Oxidation	0.09 ± 0.06	0.95	8
Margolis et al. (3)	MCR	3.1 ± 2.0	0.98	7

B5.8.2 Data analysis

Statistical analyses will be conducted using either SPSS (IBM Corp. Armonk, NY), SAS 9.3 (SAS Institute Inc., Carey, NC), or equivalent. Shapiro Wilks will be used to assess all data for normality. Data that are not normally distributed will be log transformed for statistical analysis. Paired T tests will be used to assess effects of study arms (HA+PIO vs. HA+PLA) on chamber conditions (barometric pressure, temperature, and humidity), oxygen saturation, heart rate, $\dot{V}O_2$, $\dot{V}CO_2$, respiratory exchange ratio, ventilation exchange, total glucose and fat oxidation, plasma glucose, muscle, and liver oxidation. Mixed Model repeated measures ANOVA will be used to assess the effects of condition, time, and their interactions for breath ^{13}C-enrichments, exogenous glucose oxidation, and blood analytes. Bonferroni adjustments for multiple comparisons will be performed if significant interactions are observed.

SECTION C: HUMAN RESEARCH PROTECTIONS

C1. RECRUITMENT AND CONSENT

C1.1 Identification and Selection of Subjects

Volunteers will be recruited from the federally and non-federally employed civilian population, the Natick Human Research Volunteer (HRV) Pool, NSSC active duty military personnel, and the active duty population located at other military organizations, to include, but not limited to coordination with the NSSC Soldier and Squad Optimization and Integration Team (SSO-IT).

Civilian volunteers and other active duty personnel will be recruited by "word of mouth", posted flyers, or electronic distribution of the "off-site" flyer to include a text-only, approved version of the flyer. Recruiting materials will be distributed around NSSC, surrounding community and organizations, and on bulletin boards (physical and virtual/electronic) at surrounding universities or other organizations. The text-based flyer will be posted on various USARIEM social media sites and may be used in distribution media requiring a text format (e.g., electronic newsletters). Recruiting may also be conducted through information meetings presented to college classes, clubs, sports teams, or other organizations. Approvals from the requisite parties will be obtained prior to any recruitment activities.

C1.2 Recruitment Process

Consent briefings will be conducted either in-person or virtually using a video communication platform (i.e., Microsoft Teams, Zoom, Facetime, or similar).

For Soldiers in the U.S. Army Natick Soldier System Center Human Research Volunteer (HRV) Program, the Principal Investigator will furnish a copy of the consent form to the Human Research Volunteer Program Coordinator or designee. The HRV Coordinator will schedule the consent briefing for the HRV platoon.

Volunteers from other military organizations (i.e., non-HRV military) may be recruited through coordination with NSSC SSOIT or similar avenues. Non-HRV military may be recruited around NSSC. The NSSC SSO-IT Coordinator, or respective Unit Leadership, may schedule the consent briefing for the non-HRV military. Non-HRV military coming to Natick to participate in research on temporary duty station (TDY) permissions, will receive transportation and lodging paid for by the study. Additionally, compensation for meals will be provided at the local per diem rate for days that research volunteers are not following the controlled study diet.

An ombudsperson will be present during all group briefings for military personnel.

Superiors of Service members (e.g., unit officers, senior NCOs, and equivalent civilians) / supervisors of DoD civilians (e.g., military and civilian supervisors or anyone in the supervisory structure) shall not be present at any recruitment sessions or during the consent process in which members of units under their command / personnel under their supervisions are afforded the opportunity to participate as human subjects of research.

For civilian volunteers, the Principal Investigator will provide a copy of the informed consent document to the individual. The Study Coordinator will schedule the consent briefing for the civilian volunteers. An ombudsperson will not be present during either individual or group briefings.

C1.3 Eligibility

Following the completion of written consent, volunteers will also undergo the OMSO medical clearance process for participation in accordance with USARIEM procedures outlined for screening volunteers for research involving exercise. This includes completing a two part medical clearance: 1) general medical clearance to confirm general health status and 2) specific medical clearance to confirm specific eligibility for the current study, to include an ECG. Screenings will be conducted and reviewed by a licensed physician or physician assistant from OSMO. OMSO will travel to the site of study recruitment to conduct study-specific medical clearances. Final eligibility will be determined by the Principal Investigator using

information from the OMSO medical clearances. All volunteers will undergo the required clearances in order to participate. Volunteers recruited through NSSC SSO-IT, or equivalent, may undergo medical clearance at their home duty station prior to arrival at USARIEM (clearances will be coordinated between the Principal Investigator, OMSO, and the unit's Brigade Surgeon or similar).

Volunteers will be screened for anemia. Final classification of anemia, and therefore eligibility to participate, will be determined by OMSO.

All volunteers must be willing to consume only the food and beverages provided by study staff during the controlled feeding portions of this research study, and they must be willing to adhere to exercise and physical activity prescriptions and restrictions.

If the results of all screening tools reveal the volunteer fits the screening criteria, they will be eligible to participate in the study.

C1.4 Consent Process

Prior to providing informed consent, discussions with potential volunteers (such as over the telephone) will involve answering general questions, but will not involve formal screening nor the collection of any personally identifiable information besides their name, email, and telephone number. No study procedures will occur prior to any volunteer giving their informed consent.

The Principal Investigator, an Associate Investigator or the Study Coordinator will brief potential volunteers about the nature, purpose, procedures involved, risks, expectations and requirements for participation in the study. Prospective volunteers will be familiarized with the study procedures and informed verbally and in writing of their rights to withdraw from any part of the study without penalty or prejudice. The Principal Investigator or designee will answer all group and private questions. Potential volunteers will have at least one hour after they are briefed to read and review the Informed Consent document. Potential volunteers who are ready to provide their written, informed consent may be encouraged to do so after the briefing. Potential volunteers who would like to take more time to consider whether they wish to participate in the research will be given the option of returning the consent form at a later time (i.e., by early the next morning or in the subsequent days).

Documentation of informed consent may be captured in the form of an electronic signature (i.e. common access card signatures for military or federal personnel). A copy of the informed consent document will be provided to the volunteer with the original kept for study documentation. Volunteers who have already consented will be informed of any new information or changes to the protocol that may affect their willingness and ability to continue participation in the study using an approved consent addendum.

In the case of COVID risk mitigation plans, briefings can be done virtually and consent forms can be emailed and digitally signed.

C1.4.1 Research involving subjects with cognitive impairment or who lack capacity to provide informed consent

N/A

C1.4.2 Research involving non-English speaking subjects

N/A

C1.4.3 Research involving a waiver of the requirement to obtain informed consent OR alteration of the elements of informed consent

N/A

C1.4.4 Research involving a waiver of the requirement for investigator to obtain a signed consent form

N/A

C1.4.5 Waivers of assent or parental permission when the research involves children

N/A

C1.4.6 Research involving data collection for the USAMRDC Volunteer Registry Database

N/A

C2. COMPENSATION FOR PARTICIPATION

Volunteers who participate in the study will receive $50.00 per blood sample (9 samples per CHO tracer trial day; 2 CHO tracer trial days per volunteer equals 18 samples for entire study), for a total of $900.00 for completing the study. Volunteers will not be compensated for blood draws completed during the screening/medical clearance visit. Should volunteers withdraw, they will be compensated for any blood samples they complete except for the initial blood sample during the medical screening. Payments will processed when the volunteer concludes the study (end of study period or voluntary withdrawal), as a total lump-sum payment, received as a direct deposit.

Note: Participants who receive more than $600 in a calendar year will have this income reported to the Internal Revenue Service.

C3. WITHDRAWAL FROM RESEARCH PARTICIPATION

Volunteers may withdraw from the study at any time without penalty or loss of benefits to which they would otherwise be entitled. They will do so by informing the Principal Investigator, Associate Investigator, or a staff member of their intent to withdraw verbally or in writing (electronic or paper/pencil). They will then be asked to verbally discuss their choice to withdraw with the Principal Investigator so that reasons for withdrawal can be documented. However, volunteers will not be required to discuss why they withdrew, as the volunteer may choose to discontinue participation without providing reason. An Investigator may stop an individual's participation in the study if the volunteer is unwilling or unable to complete study procedures. If an individual needs or wants to withdraw when in the altitude chamber, the volunteer will descend to SL in a separate part of the chamber with a study staff member, and be released from the altitude chamber at the time of withdrawal. An Investigator may also withdraw a volunteer if the individual becomes ill or injured or it would not be in the volunteer's best interest to continue. If the participant is withdrawn by the Investigator or decides to voluntarily withdraw him/herself, all further data collection will discontinue. Any de-identified data and biospecimens collected up until the point of withdrawal will be retained for future use and stored with all other study data. Volunteers will be compensated for any blood samples they completed up until that point, and they will be asked to return any remaining food items that were provided, in addition to any wrappers and diet/activity logs that they had completed up to the point of withdrawal.

C4. PRIVACY FOR SUBJECTS

To protect the volunteer's privacy, all of their research-related records will be labeled or "coded" with an assigned research volunteer number that will not include their name or any other form of identifiable information. The principal investigator or study coordinator will keep the link between volunteer number and the volunteer's research records in a locked cabinet. Any documents that will require the volunteer's name, such as the consent form, will be kept in a locked cabinet separate from any research documents that contain the volunteer's ID number. The principal investigator and study coordinator are the only people who will be able to match the research volunteer number with any of their personal identifying information.

When the results of the research are published no information will be included that would reveal the volunteer's identity to others. Photographs, videos, or audio-tape recordings of volunteers will only be used, if the volunteer grants permission through the Audio/Visual Image Release form. Each volunteer will also be asked to grant permission for his/her name to be included on his/her photo or video image or in writing connected to his/her image. If a volunteer does not grant permission through the Audio/Visual Image Release, then no photos or other visual recordings will be taken of him/her. In the event that it is discovered that an individual has been inadvertently photographed or visually recorded without his/her permission, the materials will be immediately destroyed. Permission through the Audio Visual Image Release form will be confirmed before any photographs or other visual recordings are used.

C5. CONFIDENTIALITY PROCEDURES FOR RESEARCH RECORDS, DATA, HUMAN BIOLOGICAL SPECIMENS

Complete confidentiality cannot be promised to military participant because information bearing on the military participant health may be required to be reported to appropriate medical or command authorities.

All data and medical information obtained will be considered privileged and held in confidence. A unique study ID number will be assigned to each participant that will not contain any personal identifiers such as name, social security number, address, date of birth, zip code, etc. and that only this study participant ID number will be used on all data collection instruments, to include questionnaires, data collection forms, computer records, etc. A number will be assigned as each volunteer is medically cleared for participation. The master key linking ID numbers to individuals will be destroyed when the study is closed. When the results of the research are published or discussed in conferences, no information will be included that would reveal identity.

C6. RISKS OF HARM, MEASURES TO REDUCE THE RISKS OF HARM, AND BENEFITS OF PARTICIPATION

C6.1 Risks of Harm

Research Procedure Name: Dual energy X-ray absorptiometry (DEXA) scan
Research Procedure Description: Volunteer will lay face-up on the DEXA densitometer table in shorts, t-shirts, and stocking feet. Volunteers will be asked to remain motionless for the 8-10 min scan.
Research-related Risks: Exposure to less than 0.6 mrem Xirradiation in a slowspeed (up to 20 minutes) wholebody scan is possible. This dose is equivalent to approximately 1/500 of normal annual background radiation (300 mrem/year), 1/6 of the radiation received in a transatlantic flight (3.825 mrem), or 1/3 of the radiation received in a chest X-ray (2 mrem).
Measures to Minimize Risks of Harm: A quality assurance check will be completed on the DEXA each day prior to its use; the software will not allow the use of the DEXA densitometer if the quality assurance check fails. Volunteers must be advised that the health effects of very low level exposures to ionizing radiation, if any, are unknown (thought to be minimal).

Research Procedure Name: Indwelling Catheters
Research Procedure Description: A needle will be used to guide a catheter into the antecubital vein of the volunteer. The catheter will be attached to saline to keep the line patent for multiple blood samples.
Research-related Risks: The risks of blood sampling are small and usually limited to local bruising or swelling. Also sometimes volunteers feel faint or may faint. If the volunteer has had problems with fainting during blood sampling in the past, they may be more prone to them during future procedures. If the catheter, the tube that is left in the arm after the needle is removed, becomes clogged at any time during the protocol, we will have to replace this to continue blood sampling. This will require another needle to be inserted into your arm. In addition, the catheter can cause irritation, bruising, swelling, infection, or an allergic reaction.
Measures to Minimize Risks of Harm: Trained technicians will use sterile techniques to place the catheter; however, in spite of being careful there is a chance that the site may become infected. Subjects will be briefed on the signs and symptoms of potential complications and given information on

who to contact for medical assessment and/or treatment. Volunteers should not donate blood for eight weeks before or after this study.

Research Procedure Name*:* 6,6-[$^2H_2$] glucose infusions
Research Procedure Description: 6,6-[$^2H_2$] glucose will be infused using a indwelling catheter.
Research-related Risks: The primary risks associated with tracer studies are those related to venous catheterization. The catheter can cause irritation, bruising, or infection. There are no known risks or reported side effects associated with administration of 6,6-[$^2H_2$] glucose infusions to humans during clinical or experimental studies. The risks associated with the infusion include volume overload, infection, and allergic reaction to the infused substance. There have been no occurrences of volume overload, no occurrences of infection or allergic reaction attributable to iodine used prior to venipuncture in any of the 200 infusion protocols that the investigators have been involved. To minimize the likelihood of these events occurring, infusion rate will be closely monitored and maintained at less than 30 ml/hr throughout the entire infusion experimental trial day.
Measures to Minimize Risks of Harm: All staff who directly participate in the infusion studies will be properly trained how to safely monitor (i.e., infusion pumps and IV lines) infusion studies from Dr. Margolis, who has extensive experience with infusion studies. In addition, infusates will be prepared sterile, pyrogen-free, and in the proper dosages by a licensed pharmacist. If sign of allergic reaction, rash or redness, infusion will be stopped immediately.

Research Procedure Name: Venipuncture
Research Procedure Description: A needle will be used to obtain single blood samples of the antecubital vein.
Research-related Risks: Venipuncture is a routine clinical procedure the medical community commonly uses to obtain blood samples. The immediate complications may be slight pain during the entry of the needle into the skin, possible dizziness, and syncope. Dizziness or syncope constitutes no long-term harm, and immediate relief is achieved by having the subject put their head down between their knees or lie down. Additionally, a hematoma may result from the venipuncture, but this is more unsightly than risk producing. Late complications might include thrombosis of the vein due to trauma or infection. These complications are extremely rare.
Measures to Minimize Risks of Harm: Participant monitoring, aseptic technique, including sterile disposable blood collection apparatus and adherence to standard medical precautions reduce risk. Trained technicians will perform all venipuncture.

Research Procedure Name: Exercise
Research Procedure Description: Exercise testing will occur on a cycle ergometer or treadmill. Exercise will be at various levels of intensity based on exercise protocol.
Research-related Risks: Exercise per se rarely provokes cardiovascular events in healthy individuals with normal cardiovascular systems. The prevalence of fatal events in the U.S. is approximately 1:133,000 to 1: 185,000 for men and 1:769,000 to 1:1,500,000 for women who are competitive high school and college athletes. For middle-aged and older adults the relative risk rises to 1:15,000 to 1:18,000 for individuals without a prior history of cardiovascular events. Current civilian and military guidelines state that individuals less than 40 years of age who have no symptoms of or known presence of heart disease or major coronary risk factors have a low risk for cardiac complications during vigorous exercise. All volunteers in this study fall into this low risk category. Local muscle discomfort and fatigue may occur in active muscles during and shortly after exercise. Muscle soreness, ranging in intensity from mild to severe, may persist for 1 to 7 days.
Measures to Minimize Risks of Harm: *(Precautions, safeguards):* Studies have confirmed the safety of maximal exercise testing, particularly among apparently healthy persons without significant cardiovascular risk factors. As a precaution, there will be at least one spotter during all exercise sessions, and heart rate will be monitored in real time during testing. In addition, exercise monitors and test administrators will be CPR-certified.

Research Procedure Name*:* Oral Pioglitazone administration

Research Procedure Description: Participants will be administered 15 mg of PIO, an insulin sensitizing agent, for five days during one arm of the research trial. Study staff will observe research volunteers when PIO is administered.
Research-related Risks: The most common side effects of PIO include cold-like symptoms (upper respiratory tract infection), headache, sinus infection, muscle pain, and sore throat. Rare but possible side effects can include hypoglycemia, fluid retention which leads to swelling (edema), and weight gain. Chronic use of PIO has been associated with increased risk of bladder cancer.
Measures to Minimize Risks of Harm: (Precautions, safeguards): To minimize side effects the study will use a low dose of PIO, 15 mg, which is considered a starting dose when medication is initially prescribed. PIO will be administered for short-term use (5 days), which is significantly less dose exposure time for side effects, and particularly more rare side effects to occur. Additionally, PIO will be administered as a monotherapy, without presence of other hypoglycemia agents such as insulin, and diet and exercise will be controlled which will nearly eliminate risk of hypoglycemia. The study will recruit healthy, male volunteers who will be medically screened to eliminate volunteers with known comorbidities which could exaggerate side effects. OMSO will be responsible for administering PIO to volunteers and medical oversight of volunteers.

Research Procedure Name: Altitude Exposure
Research Procedure Description: Participants will exercise at 4300 m simulated altitude.
Research-related Risks*:* Hypoxemia, lightheadedness, AMS, HAPE, HACE, ear pain/discomfort, and peripheral/facial edema.
Measures to Minimize Risks of Harm*: (Precautions, safeguards):*

- During hypobaric exposures, volunteers will always be accompanied by one or more trained staff that will monitor signs and symptoms of research-related risks. To additionally minimize risk, volunteers will be familiarized with safety procedures and equipment in the hypobaric chamber. Additionally, medical staff (OMSO) will be onsite while volunteers and staff are in the simulated altitude chamber.
- *Hypoxemia*- Exposure to hypobaric hypoxia introduces the hazard of hypoxemia. Depending upon the degree and duration of hypobaric exposure, hypoxemia may be well tolerated without consequences, or may present a full spectrum of clinical entities ranging from mild discomfort to potentially lethal conditions. Participants will be monitored by study staff and participants will be allowed to terminate testing early if they desire.
- *Lightheadedness*- Exercising at altitude can cause an increased risk of lightheadedness which may occur just after stopping heavy exercise. Lightheadedness increases the risk that the volunteer could fall. This risk (rare) will be minimized by having at least one staff member in close proximity to the volunteer (as is done for all exercise tests regardless of location) so that he/she will not fall off. If a volunteer becomes lightheaded and feels faint, he/she will be assisted off the treadmill and instructed to lay down with feet up until fully recovered. Volunteers will be assisted on and off exercise and other experimental apparatus as needed.
- *AMS*- AMS is a self-limited syndrome that is common when unacclimatized lowlanders are exposed to higher altitudes. Symptoms of AMS include headache, nausea, anorexia, lethargy, dizziness, tiredness, weakness, insomnia, and sometimes vomiting. The prevalence and severity of AMS increase directly in proportion to ascent rate and elevation. With rapid ascent, and no beneficial treatment (e.g., breathing supplemental O_2) symptoms commonly appear within four to six hours. Symptoms of AMS will reach their severity within 18 to 24 hrs of altitude exposure, and be the most severe after awakening. With rapid ascent to the altitudes of 3000 m and 4000 m for a 24-hr period, more than 30-90% of the untreated volunteers will likely experience AMS, with symptoms ranging from mild (10 to 40%), to moderate (20 to 40%) to severe (10 to 20%). Due to the duration (~7-8 hrs) of the altitude exposure in the current study, AMS may occur but should not reach its most severe symptoms.
- *HAPE/ HACE*- The incidence of clinical HAPE in unacclimatized sea-level residents being exposed to high altitude for at least a few days appears to be less than 1%. As a clinical condition, the possibility of HACE occurring when sea-level residents are exposed to high altitude appears to be even lower than the incidence of HAPE. If either or both are recognized early and treated by descent, they are completely reversible. With regard to the proposed study, the risk of any

volunteer developing either HAPE or HACE is considered remote and is not expected. If a volunteer shows developing symptoms of HAPE or HACE they will be removed from the simulated high altitude environment.

- *Ear Pain/Discomfort*- Changes in ambient gas pressure during induction of or return from hypobaric conditions can cause untoward effects due to gas trapped in the body. At the relatively slow rates of pressure change during normal operation (simulated ascent and/or descent) of the hypobaric chamber, some individuals may experience discomfort in their ears, paranasal sinuses, teeth, or abdomen. In healthy individuals, pressure in the middle ear and sinuses can be equalized with ambient atmospheric pressure by swallowing, yawning, or tensing the muscles of the throat, procedures that contract the pharyngeal muscles and open the Eustachian tubes, thereby ventilating the middle ear and sinus. Another more effective means of ventilating the middle ear and sinuses is forced expiration against a closed nose and mouth. Adjunctive therapy may also include the use of short-acting antihistamines or decongestants to reduce swollen Eustachian tubes and sinus openings due to inflammation or infection. During recompression (descent), moderately severe ear pain may be experienced by volunteers who cannot maintain Eustachian tube patency. If ear or sinus pain occurs, recompression will be stopped and the affected volunteer will be decompressed until the pain resolves; recompression will then be restarted at a slower rate. Damage to eardrums is likely only under the unusual situation where deliberately rapid recompression is necessitated by dire emergency.
- *Peripheral/Facial Edema*- Peripheral and facial edema occurs in some individuals when initially exposed to high altitude. It is characterized by pronounced edema of the face and upper extremities, decreased urine output and weight gain. Although uncomfortable, it is a benign condition. The peripheral and facial edema resolves with descent.

C6.2 Incidental or Unexpected Findings

All incidental or unexpected health findings identified by OMSO during the screening process or throughout the research process will be documented and disclosed to the volunteer. The volunteer will be encouraged to make an appointment with their primary care provider for a full evaluation of any identified problems. Volunteers with evidence of any physical, mental, and/or medical conditions that would make the proposed studies relatively more hazardous will be excluded.

Any results perceived to be clinically relevant will not be shared with participants, as biological samples will not be analyzed until after participants have finished data collection.

Body composition, exercise performance measures, and baseline dietary intake data may be shared upon request at the individual level once the requesting individual completes all study procedures.

C6.3 Potential Benefits

There are no direct health or other benefits related to participation in this study. Information gathered from this research may benefit society in the future.

C7. DATA AND SAFETY MONITORING

The Principal Investigator will be responsible for monitoring the data collected. The Principal Investigator, with the assistance of the Study Coordinator, will ensure that the data is being collected according to the methods described in the protocol. Electronically collected data will be downloaded daily and checked for quality. This also includes continuous evaluation of the following: recruitment, the informed consent process, adverse events, protocol adherence, and protocol deviations. This will occur continuously in order to identify unanticipated problems or risks to the volunteers associated with the research. The Principal Investigator will ensure that the number of volunteers recruited for this study complies with the protocol. If adverse events occur, the Principal Investigator will submit a monthly summary of the related adverse events to OMSO to determine whether the number of adverse events is excessive for the risks outlined in the research protocol. The Principal Investigator and Study Coordinator will be responsible for ensuring that the appropriate regulatory and IRB documentation is on file and up to date.

C8. REPORTABLE EVENTS

C8.1 Expected adverse events

An adverse event is defined as any untoward or unfavorable medical occurrence in a human research participant, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the individual's participation in the research, whether or not considered related to the individual's participation in the research.

Expected adverse events which are not serious are reported to the IRB at the time of continuing review of the protocol. These events include some level of AMS under HA conditions, local bruising and soreness from blood sampling, and muscle soreness or pain from exercise.

All medical events that the USARIEM Office of Medical Support and Oversight (OMSO) evaluates will be reported to the ORQC.

C8.2 Unexpected adverse events and unanticipated problems

A serious adverse event is any adverse event temporally associated with the subject's participation in research that is fatal, life-threatening, permanently disabling, requires inpatient hospitalization, or results in congenital anomalies/birth defect, overdose or cancer, or based on appropriate medical judgment, may jeopardize the participant, or may require medical or surgical intervention to prevent one of the above outcomes.

All medical events will be reported to USARIEM's Office of Medical Support and Oversight (OMSO). OMSO staff will retain a copy of the report in the subject's OMSO medical file as a means of tracking and analyzing trends in medical events.

All unanticipated problems involving risk to subjects or others, serious adverse events that are unexpected and determined to be at least possibly or definitely related to study participation, will be promptly reported within one working day by phone (508-206-2371/2200) or email (usarmy.natick.medcom-usariem.mbx.usariem-rqc-protocol@health.mil) to the USARIEM ORQC and the Commander. These events will also be reported to the HQ USAMRDC IRB within one working day by phone (301-619-6240), or by e-mail (usarmy.detrick.medcom-usamrdc.other.irb-office@mail.mil)

Adverse events assessed by the PI as not serious and serious adverse events that are deemed to be unrelated to participation in the study will be reported to the IRB at the time of continuing review of the protocol.

In the event of a medical emergency at facilities on the Natick Soldier Center, the local Emergency Medical Services (EMS) will be contacted immediately by dialing 5911. The installation security personnel will direct the ambulance to the proper location on the installation. While awaiting their arrival, Basic Life Support will be rendered by study personnel or on-site medical coverage. EMS response time to USARIEM is approximately 5 minutes. Transport time to definitive care is approximately 8 minutes.

C8.3 Adverse device effects

N/A

C8.4 FDA-regulated research under IND and IDE

The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication of use or any other significant labeling or advertising for the drug. The investigation will be conducted in compliance with the requirements for institutional review per 21 CFR Part 56 and the requirements for informed consent per 21 CFR Part 50. The drug will not be promoted as safe or effective and the study will be conducted in compliance with 21 CFR Part 312.7.

SECTION D: REFERENCES

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SECTION E: ABBREVIATIONS AND ACRONYMS

U.S. Army Research Institute of Environmental Medicine; USARIEM, LC-Tandem Mass Spectrometer; LC-MS/MS, Isotope Ratio Mass Spectrometer; IRMS, Pennington Biomedical Research Center; PBRC, College of American Pathologists; CAP, High Altitude; HA, Sea Level; SL, Placebo; PLA, Glucose Rate of Appearance; Ra, Glucose Rate of Disappearance; Rd. Metabolic Clearance Rate; MCR, Multi-Domain Operations; MDO, Modular Ration Enhancement Operations HA; MORE-HA, Pioglitazone; PIO, Biomedical Performance Enhancement; BPE, Delegation of Authority; DOA, Dual Energy X-ray Absorptiometry; DEXA, Peak Oxygen Uptake; VO_{2peak}, Identification; ID, Human Research Volunteer; HRV, Solider and Squad Optimization and Integration Team; SSO-IT, Office of Medical Support and Oversight; OMSO, Acute Mountain Sickness; AMS, High Altitude Peripheral Edema; HAPE, High Altitude Cerebral Edema; HACE

SECTION F: DoD PRIVACY RULE AND PROTECTED HEALTH INFORMATION (HIPAA)

NA – will not use or disclose protected health information