Interdisciplinary Research (IDR) Origination Awards

Cover Page

Project Title

Title: **Heat-Induced Skeletal Muscle Mitochondrial Biogenesis, and Vascular Remodeling: A Countermeasure for Human Disuse Atrophy and Aging.**

Principal Investigator(s) (full-time faculty)

Name (PI listed first)	Department	College
Robert Hyldahl	Exercise Science	Life Sciences
Jayson Gifford	Exercise Science	Life Sciences
John C. Price	Chemistry and Biochemistry	Physical and Mathematical Sciences
Chad Hancock	Nutrition, Dietetics and Food Science	Life Sciences

Track

Track one

Abstract

Human aging leads to an overall decline of muscle mass (sarcopenia) and function (dynapenia). The progression of sarcopenia and dynapenia can be attenuated with physical exercise by improving muscle oxygen delivery, mitochondrial energetics and protein turnover. Individuals who would benefit from exercise interventions are often unable to do so due to co-morbidities associated with aging. We have recently developed a paradigm of muscle-targeted heat treatment, which improves mitochondrial and vascular function and limits disuse-induced atrophy in young sedentary human skeletal muscle. The overall objectives of this grant are to characterize the effect of Repeated Exposure to Heat Stress (REHS) on mitochondrial adaptation (respiratory function and mitochondrial proteomics), vascular function and exercise performance in aged adults. The proposed studies will also allow us to test the efficacy of REHS on the two major contributors of sarcopenia and dyspnea; age and inactivity.

Project narrative

Human aging is a dynamic, multi-faceted process that leads to an overall decline of biological function. One of the most evident and disabling changes associated with aging is a progressive and steady loss of muscle mass (sarcopenia) and muscle exercise tolerance (dynapenia) (9, 15). Sarcopenia is exacerbated by periods of muscle disuse that can result from sickness, injury, surgery, or immobilization (casting). Currently, the only effective intervention shown to slow the progression of sarcopenia is physical exercise, which works in large part by improving muscle oxygen delivery, mitochondrial energetics and protein turnover. However, individuals who would benefit the most from such interventions are often unable to do so due to co-morbidities associated with aging. In these cases, an alternative or adjunct therapy to target loss in skeletal muscle function is sorely needed.

Our research group has recently developed a paradigm of muscle-targeted passive heat treatment, which we have shown to improve mitochondrial (6) and vascular function in young sedentary human skeletal muscle. The impact of these findings would be of greater significance if they were also found to be true in aged skeletal muscle. Furthermore, given the relationship between mitochondrial function and muscle wasting, it is possible that passive heating may also provide some protection from disuse-related muscle atrophy and related dysfunction. Therefore, the *overall objectives* of this grant are to characterize the effect of **R**epeated **E**xposure to **H**eat **S**tress (REHS) on mitochondrial adaptation, vascular function and exercise performance in aged adults, and the maintenance of skeletal muscle mass during a period of immobilization (Figure 1). The second objective will use adults (19-35 yrs), not aged subjects as a proof of principle for efficacy in aged individuals, as the risk of doing such a study in aged individuals is too high. Our ***scientific premise*** is built on our extensive human data showing that REHS: 1) improves mitochondrial respiratory capacity and vascular function in sedentary young adults, and 2) preserves vascular function, and reduces muscle wasting in response to limb disuse.(6, 13) **The design of the proposed studies will allow us to test the efficacy of REHS on the two major contributors of sarcopenia – age (Aim 1) and physical inactivity (Aim 2; Figure 1).**

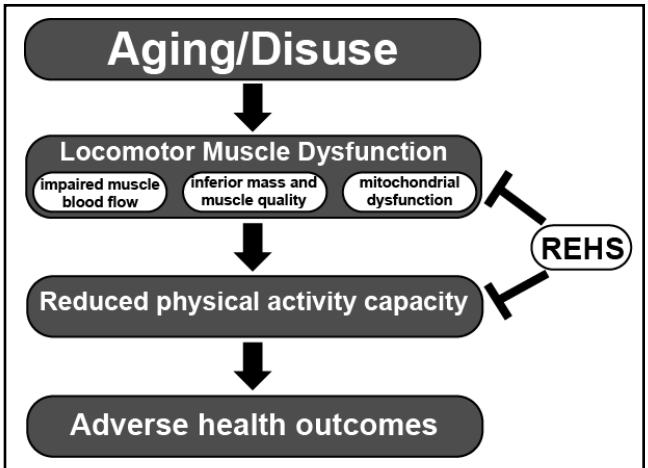


Figure 1: Schematic of the working hypothesis

Aim 1: Determine if REHS improves muscle mitochondrial function, vascular dynamics and exercise performance in aged adults relative to exercise training (ET)

Hypothesis 1: REHS will improve muscle mitochondrial function, promote mitochondrial biogenesis, and improve exercise performance, though to a lesser extent than exercise training

Hypothesis 2: REHS will improve vascular function and microvascular remodeling similarly to exercise training

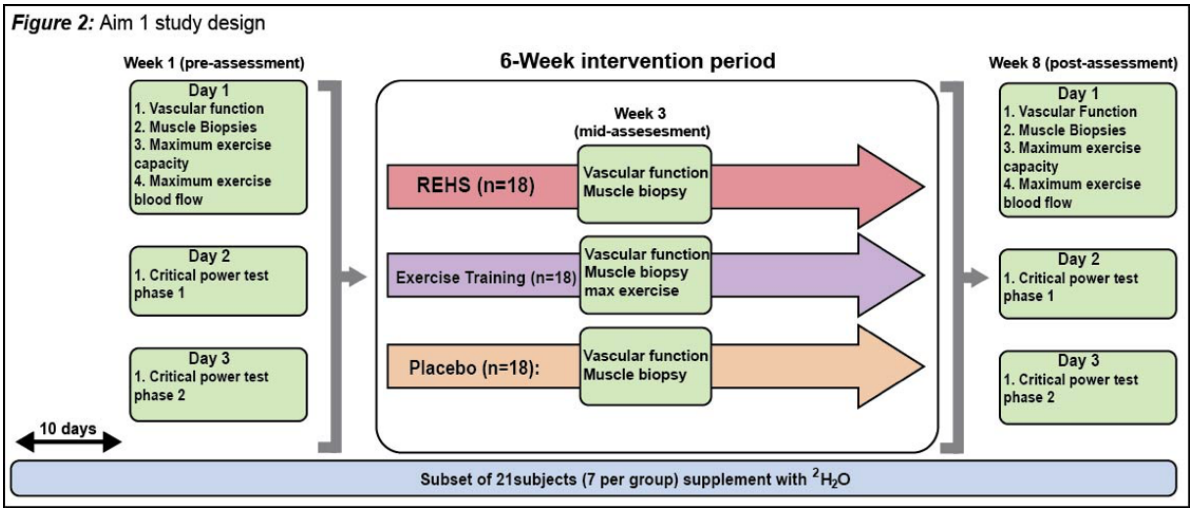
Approach

Study ethics and recruitment: Ethical permission for Aim #1 via the Brigham Young University IRB has already been obtained (IRB 2020-023).

Subjects A total of 54 participants (Men = 27, Women =27), aged >65 years will be recruited from the Provo, UT and surrounding communities, and randomly allocated into one of 3 groups (18 per group).

Research design: This study will be a randomized, single-blinded, placebo-controlled study to determine the efficacy of REHS on outcomes related to lower extremity skeletal muscle function and vascular health relative to exercise training. Subjects will be divided equally (N=18) into three groups: REHS, exercise training (EX), and a sham, or placebo heat treatment group (PLA). The study will consist of 3 phases – a

pre-training testing period, 6-week intervention, and a post-training intervention period. Presented in Figure 2 is a schematic of the study design.



Methodologies:

<u>*REHS and SHAM groups:*</u> 18 subjects will undergo a 6-week passive heat treatment regimen (2h/treatment, 3x/week). As per our previous studies, we will heat the *vastus lateralis* muscle using 2 ME300 AutoTherm SWD (Mettler Electronics Corp) short-wave diathermy units with a 22-cm induction coil drum. We have used this method to successfully raise intramuscular temperature by 4°C(5). A strength of the diathermy model is that we will be able to placebo control the heat treatments, which is important for the functional (exercise tolerance) outcomes. For the SHAM condition (18 aged subjects), we place the diathermy drums over the knee extensors, but will not turn them on. Because little heat is produced on the surface of the skin, subjects are generally unaware of whether their muscle is being heated. In fact, many subjects in prior sham controlled heating trials report sensations of heat(5).

<u>*Exercise Training Group:*</u> In order to put the REHS findings within a context of exercise-induced adaptation, we will train a group of 18 subjects (9 Women and 9 Men) with isolated knee extension exercise 3 times per week for 6 weeks. The single leg knee extension exercise is an exercise that is specific to the muscles that will be biopsied and heat treated (3, 4). We will use a high intensity interval training (HIIT) protocol that we have shown effectively increases muscle mitochondrial respiratory capacity and vascular function

Primary Outcomes:

<u>*Mitochondrial Function and Biogenesis:*</u> The ability to sustain exercise and maintain muscle mass is critically dependent upon the ability of the mitochondria (*i.e.* the powerhouse of the cell) to consume oxygen and produce energy. We will use high-resolution respirometry of biopsied muscle cells to quantify the ability of muscle mitochondria to consume oxygen and provide energy (5-7, 16). As mitochondrial function is dependent upon the amount of mitochondria present within the muscle, we will also use a proteomic technique to determine the rate at which mitochondrial proteins are created in the muscle (*i.e.* mitochondrial biogenesis) (11, 12). We hypothesize that mitochondrial function and biogenesis will be decreased by aging and disuse and improved by heat therapy. This technique, which involves the use of stable isotope tracers will also allow us to measure the turnover rates and concentration of more than 1000 individual proteins in the muscle tissue. Thus, a subset of 21 subjects (7 per group) will begin supplementing with ^2H_2O to facilitate this measurement (Figure 2; See John Price Description and references (10-12)).

<u>*Exercise Tolerance:*</u> Exercise tolerance of the treated quadriceps muscle will be determined by measuring the amount of time that different intensities of single leg knee extension exercise can be sustained. Similar in

concept to single-leg cycling, the single-leg knee extension exercise is isolated to the quadriceps muscle which will undergo all of the interventions in this study. A series of exercise tests to fatigue will be used to determine the subjects' maximum aerobic exercise capacity, and the highest submaximal intensity that can be sustained for prolonged periods of time without quickly inducing severe muscle fatigue (*i.e.* Critical Power, CP). These measures of exercise tolerance are known to be negatively impacted by aging and disuse and improved by exercise training. We hypothesize that heat therapy will help maintain or improve exercise tolerance in cases of muscle disuse and aging.

Vascular Function: The ability to sustain exercise without fatiguing is extremely dependent upon the delivery of fresh blood to the exercising muscle. We will use ultrasound to measure the ability of the treated muscle and vasculature to deliver blood during submaximal and maximal intensity single leg knee extension exercise. Quantification of vascular-related protein (*e.g.* number of capillary structures around a muscle cell) in biopsied muscle samples will complement the aforementioned *in vivo* measurements of vascular function.

Statistical Approach: Sample size estimates have been determined using our own published data for changes in mitochondrial respiration with heat treatment over 6 days (6) using $\beta = 0.85$ and $\alpha = 0.05$. Comparisons between groups will be performed using univariate statistics (ANOVA or its non-parametric equivalent). Proteomic data will undergo multivariate data analysis using principal component analysis (PCA) to describe the underlying variance within the data. Ontology enrichment analyses will also be used to compare concerted changes in functionally related proteins. Benjamini-Hoffman multiple testing corrections will be applied to these functional analyses.

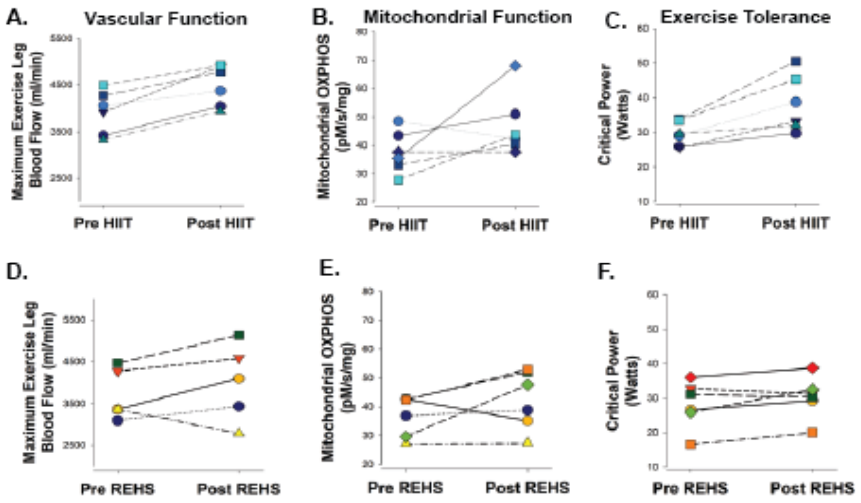


Figure 3. *Data in Support of Aim1:* We have conducted a pilot study in young, sedentary individuals to establish the scientific premise and feasibility of REHS as a therapeutic intervention for aged adults. Both 6 weeks of exercise training (A-C) and REHS (D-E) improved vascular function (A,D), muscle mitochondrial function (B,E) and exercise tolerance (C,F)) in most subjects.

Aim 2: Determine whether REHS attenuates atrophy, corrects the cardiometabolic dysfunction and reduces the decrement in functional performance caused by 2 weeks of limb disuse

Hypothesis 1: REHS will attenuate disuse-induced losses in muscle mitochondrial respiratory capacity, vascular function and functional performance.

Hypothesis 2: REHS will attenuate disuse-induced losses in myofibrillar and mitochondrial protein synthetic rates and will reduce muscle atrophy.

The *scientific premise* of Aim 2 is based on our recently published work and extensive preliminary data, which demonstrate that REHS prevents the loss of mitochondrial function, reduces muscle atrophy and prevents the loss of vascular function that resulted from 10-days of lower limb immobilization (5, 6). Our *approach* will be to use young subjects as a proof of principle for efficacy in aged individuals, as the risk of doing such a study in aged individuals is too high. See figure 4 for a broad overview of the approach we will use to accomplish this AIM.

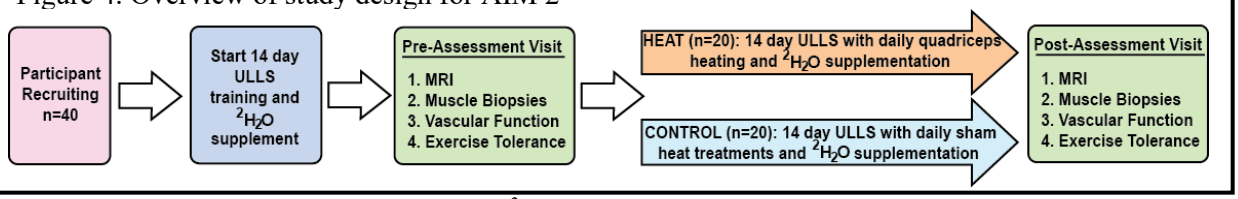
Approach:

Study ethics and recruitment: Brigham Young University IRB approval for Aim 2 will be considered pending proposal funding. Nonetheless, we have received IRB approval for our previously published study using similar methods and do not anticipate any problems obtaining IRB approval for this second aim.

Subjects: A total of 40 young (aged<35 years) participants (Men = 20, Women =20), will be recruited from the Provo, UT and surrounding communities, and randomly allocated into one of 2 groups.

Study Design: All recruited subjects will undergo 14 days of unilateral lower limb suspension (ULLS) and be randomized into a REHS or SHAM treatment group. Measurements will be taken prior to immobilization and following the 14 days of immobilization. A schematic of the study design can be found in Figure 4.

Figure 4. Overview of study design for AIM 2



Methodologies: *Diathermy Treatments and ^2H_2O supplementation methodologies are described in Aim 1*

Unilateral lower limb suspension (ULLS) Subjects will use short-length crutches with handgrip and forearm support. The right foot will be placed in a shoe with a 10cm thick sole to unload the right limb. Subjects will live at home and maintain their normal daily tasks through the experimental period. Compliance will be monitored by daily laboratory visits. In a prior study, we demonstrated that this approach results in measureable muscle atrophy and is feasible, with only 1 out of 24 total subjects withdrawing from the study (5). To reduce the risk of possible falls, and to improve compliance, a 2-week training period will be implemented prior to the initiation of unloading (Figure 4). Also, to reduce the chance of thromboembolism, we will perform passive exercises during each daily visit.

Primary Outcomes: *Mitochondrial function, vascular function and exercise tolerance methodologies are described in Aim 1*

Protein synthesis: Muscle protein fractional synthetic rate (FSR) is decreased by ULLS (1, 14). Similar to our previous studies (8, 11, 12), stable isotope tracers (^2H_2O) will be used to quantify long-term muscle protein synthesis (MPS), DNA and RNA synthesis and protein breakdown in response to disuse and heat treatment. We will use the same methods described for Aim 1 to measure total and mitochondrial protein synthetic rates.

Myofiber Morphology: Myofiber area will be determined by fiber type using histochemical staining of muscle cross sections as per our previously published studies (5).

MRI: MRI will be used to assess whole muscle cross sectional area of the *vastus lateralis*. Participants will be scanned while laying supine in a 3.0 Tesla MRI scanner (Siemens). A stock Siemens 2-D multi-slice gradient-recalled echo (GRE) MRI pulse sequence will be used. Images will be takin in slices every 5mm, resulting in a total sequence time of approximately 2-min. This will provide cross-sectional images of the *vastus lateralis* from the base of the femur (distal condyles) up to the groin. We have used the protocol to successfully measure *vastus lateralis* CSA before and after disuse (5).

Data in Support of Aim1: In a preliminary study, we investigated the effect of 10 days of REHS on immobilization-induced muscle and vascular dysfunction. Subjects wore an immobilization brace for 10

days, during which time half the subjects received a sham treatment for two hours per day (SHAM) and half the subjects received the proposed diathermy treatment (REHS). REHS effectively completely or partially attenuated the negative effects of immobilization.

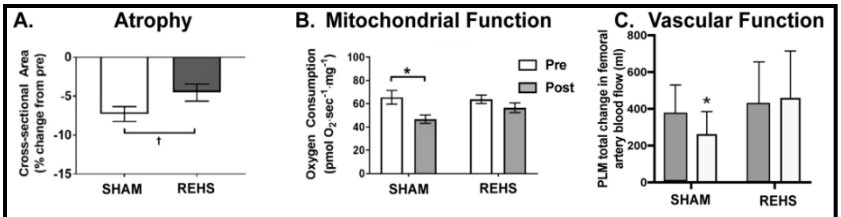


Figure 5. Ten days of immobilization caused muscle atrophy (A), loss of muscle mitochondrial function (B), and reduced vascular function (C). all of which were completely or partially restored when heat was applied each day during the 10 days of immobilization

Funding support from the IDR grant: In 2017/18, our group submitted an R01 application and subsequent revision to the National Institute of Health/National Institute of Arthritis and Musculoskeletal and Skin Diseases with much of the same approaches described in this application, but not directed at the aging question. While the grant did not ultimately get funded in 2019, the decision was close to the funding payline (our application was scored at the 26th percentile and the payline for early stage investigators was the 22nd percentile). Since this application, our group has published some of the research we proposed on younger individuals (2, 5, 6). The results of the research described in this application will have great impact on understanding potential approaches to improve quality of life in older individuals. If awarded, the funds from the IDR grant will be used to gather more preliminary data in an aged cohort (AIM 1), which improve the strength of our next applications, focused on aging. We already have the IRB approval for AIM 1, however a major limitation to obtain more preliminary data to support this research in older subjects (<65 years), is the great expense of **subject compensation** and cost of the **research on the mitochondrial protein biogenesis and turnover (Cost per subject is estimated to be ~$1200)**. These funds would also be used to support graduate student research on these projects. Currently, we are planning for one of our new PhD students to submit an NIH, F31 (predoctoral national research service award) in April of this year. It should be noted that all of these research projects involve a great number of undergraduate students, and these funds would at least indirectly support the research activities for these students as well.

Research Team Members and Roles

Robert D. Hyldahl, PhD (College of Life Sciences: Department of Exercise Science). Dr. Hyldahl has had extensive experience in muscle response to inflammation and, adaptations to heat treatment. Dr. Hyldahl directs and performs all of the human muscle biopsies as well as directly overseeing the heat therapy and exercise modalities that are the focus of the studies in this proposal.

Jayson R. Gifford, PhD (College of Life Sciences: Department of Exercise Science). Dr. Gifford is an expert in evaluating vascular health and adaptations in response to the heat therapy as well as the exercise modalities utilized in these studies. He directs the studies aimed at evaluating knee extensor function, vascular health and muscular performance.

John C Price, PhD (College of Physical and Mathematical Sciences; Departmetn of Chemistry and Biochemistry). Dr. Price will direct the evaluation of mitochondrial biogenesis and proteomic assessments. Dr. Price is an expert in using stable isotope tracers to determine metabolic adaptations at the molecular/proteomic level. Dr. Price's expertise is central to this novel approach to gain an understanding of the proteomic and metabolomic adaptations to REHS.

Chad R Hancock, PhD (College of Life Sciences: Department of Nutrition, Dietetics and Food Science). Dr. Hancock's research focus and expertise is in the area of mitochondrial adaptations in skeletal muscle. Dr. Hancock will direct the functional evaluation of mitochondrial respiration in response to the heat treatment and exercise modalities described in this proposal.

1. **de Boer MD, Selby A, Atherton P, Smith K, Seynnes OR, Maganaris CN, Maffulli N, Movin T, Narici MV, and Rennie MJ**. The temporal responses of protein synthesis, gene expression and cell signalling in human quadriceps muscle and patellar tendon to disuse. *The Journal of physiology* 585: 241-251, 2007.
2. **Deyhle MR, Hafen PS, Parmley J, Preece CN, Robison M, Sorensen JR, Jackson B, Eggett DL, Hancock CR, and Hyldahl RD**. CXCL10 increases in human skeletal muscle following damage but is not necessary for muscle regeneration. *Physiological reports* 6: e13689, 2018.
3. **Gifford JR, Garten RS, Nelson AD, Trinity JD, Layec G, Witman MA, Weavil JC, Mangum T, Hart C, Etheredge C, Jessop J, Bledsoe A, Morgan DE, Wray DW, Rossman MJ, and Richardson RS**. Symmorphosis and skeletal muscle VO2 max : in vivo and in vitro measures reveal differing constraints in the exercise-trained and untrained human. *The Journal of physiology* 594: 1741-1751, 2016.
4. **Gifford JR, Trinity JD, Layec G, Garten RS, Park SY, Rossman MJ, Larsen S, Dela F, and Richardson RS**. Quadriceps exercise intolerance in patients with chronic obstructive pulmonary disease: the potential role of altered skeletal muscle mitochondrial respiration. *Journal of applied physiology (Bethesda, Md : 1985)* 119: 882-888, 2015.
5. **Hafen PS, Abbott K, Bowden J, Lopiano R, Hancock CR, and Hyldahl RD**. Daily heat treatment maintains mitochondrial function and attenuates atrophy in human skeletal muscle subjected to immobilization. *Journal of applied physiology (Bethesda, Md : 1985)* 127: 47-57, 2019.
6. **Hafen PS, Preece CN, Sorensen JR, Hancock CR, and Hyldahl RD**. Repeated exposure to heat stress induces mitochondrial adaptation in human skeletal muscle. *Journal of applied physiology (Bethesda, Md : 1985)* 125: 1447-1455, 2018.
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8. **Mathis AD, Naylor BC, Carson RH, Evans E, Harwell J, Knecht J, Hexem E, Peelor FF, 3rd, Miller BF, Hamilton KL, Transtrum MK, Bikman BT, and Price JC**. Mechanisms of In Vivo Ribosome Maintenance Change in Response to Nutrient Signals. *Molecular & cellular proteomics : MCP* 16: 243-254, 2017.
9. **Melton L, Khosla S, and Riggs B**. Epidemiology of sarcopenia. *Mayo Clin Proc* 75 Suppl: S10-12; discussion S12-13, 2000.
10. **Naylor BC, Porter MT, Wilson E, Herring A, Lofthouse S, Hannemann A, Piccolo SR, Rockwood AL, and Price JC**. DeuteRater: a tool for quantifying peptide isotope precision and kinetic proteomics. *Bioinformatics (Oxford, England)* 33: 1514-1520, 2017.
11. **Price JC, Holmes WE, Li KW, Floreani NA, Neese RA, Turner SM, and Hellerstein MK**. Measurement of human plasma proteome dynamics with (2)H(2)O and liquid chromatography tandem mass spectrometry. *Analytical biochemistry* 420: 73-83, 2012.
12. **Shankaran M, King CL, Angel TE, Holmes WE, Li KW, Colangelo M, Price JC, Turner SM, Bell C, Hamilton KL, Miller BF, and Hellerstein MK**. Circulating protein synthesis rates reveal skeletal muscle proteome dynamics. *The Journal of clinical investigation* 126: 288-302, 2016.
13. **Tamura Y, Kitaoka Y, Matsunaga Y, Hoshino D, and Hatta H**. Daily heat stress treatment rescues denervation-activated mitochondrial clearance and atrophy in skeletal muscle. *The Journal of physiology* 593: 2707-2720, 2015.
14. **Tesch PA, Lundberg TR, and Fernandez-Gonzalo R**. Unilateral lower limb suspension: From subject selection to "omic" responses. *Journal of applied physiology (Bethesda, Md : 1985)* 120: 1207-1214, 2016.
15. **Thompson L**. Age-related muscle dysfunction. *Exp Gerontol* 44: 106-111, 2009.

16. **Tueller DJ, Harley JS, and Hancock CR**. Effects of curcumin and ursolic acid on the mitochondrial coupling efficiency and hydrogen peroxide emission of intact skeletal myoblasts. *Biochemical and biophysical research communications* 492: 368-372, 2017.

Budget justification

The largest limitation to completing the projects outlined in this proposal are the large costs of both subject compensation and the costs associated with the mitochondrial protein synthesis/turnover analysis. The second of these involves using stable isotope tracers to interrogate the turnover of specific proteins from muscle biopsies.

Subject compensation costs:

For the first part of AIM 1, the plan is to include 18 subjects per group (3 groups). To participate in these somewhat invasive and time-intensive studies, subjects are compensated $500.

Subject compensation for 3 groups*18 subjects*$500= $27,000.

For the second part of AIM 1, the experiments involving the protein measures using stable isotope tracers on a subset of subjects (7 in each group) will cost $1200/subject.

Stable isotope experiments for 21 subjects*1200/subject = $25,200

Supplies for respiration experiments = $2000

For the AIM 2, the costs will be similar in terms of subject compensation. We plan for 40 subjects at $500/subject = $20,000

Similarly, a minimum of 10 from each group will also involve the stable isotope studies subset of each group = $24000

Category		
Subject compensation	$27,000	$20,000
Stable isotope supplies	$25,200	$20,000
Supplies for respiration experiments (substrates, inhibitors, buffers, etc.)	$1000	$1000
Undergraduate research assistant $10/hr for 40 wks	$6,000	
Partial Graduate Student support	$7,500	$7,500
Supplies biopsy proceedures and exercise training and heat protocols	$1,500	$1,500
Student travel expenses and registration fees to present at a meeting		$1,800
	Year One Total: $60,700	Year Two Total: $59,300

Summary of Plans for External Funding

As noted in the project narrative, In 2017/18, our group submitted an R01 application and subsequent revision to the National Institute of Health/National Institute of Arthritis and Musculoskeletal and Skin Diseases with much of the same approaches described in this application, but not directed at the aging question. While the grant did not ultimately get funded in 2019, the decision was close to the funding payline (our application was scored at the 26th percentile and the payline for early stage investigators was the 22nd percentile). Since this application, our group has published some of the research we proposed on younger individuals. The results of the research described in this application will have great impact on understanding potential approaches to improve quality of life in older individuals. If awarded, the funds from the IDR grant will be used to gather more preliminary data in an aged cohort (AIM 1), which improve the strength of our next applications, focused on aging. The renewed focus on aging will give the grant greater impact and allow us to submit to the National Institute of Aging, which has a higher payline than NIAMS. Thus, the aging preliminary data is critical. We have received positive reviews from NIA program officers.

The current study we are conducting is a registered clinical trial.

Brigham Young University IRB Protocol Record F2020-023, Heat Therapy and Muscle Function Study, is registered and posted on the ClinicalTrials.gov public website.

The primary focus will be to apply for the R01 grant at the NIH, NIA. In addition, we believe the proposed research can be tailored for an R21 application if that appears to be the better route, or applying our current work to a NASA research grant (HERO award) aimed at developing countermeasures for risks posed by prolonged spaceflight and microgravity.

Program	Agency	Solicitation Date
NIH R01 application PA-20-185	National Institute of Aging	June 5th, with a likely re-submission November 5th or March 5th.
F-31 Diversity predoctoral training grant PA-16-308	National Institute of Aging	August 8th with a possible re-submission Dec. 8th.
NASA HERO award	NASA	Dates have not been announced for this year yet. We would aim to focus our research for this award in 2022.
NIH PA-18-850: Prevention Research in Mid-Life Adults	National Institute of Aging	R-21 mechanism for exploratory research programs, not renewable, but may be a good approach depending on the review of the R01 application. Due date Oct. 16th.

Current and Pending support

John C. Price

Ongoing Research Support

R15GM132852 (Griffitts) 6/2019-5/2022 0.3 calendar
NIH GMS $75,000
The structural basis of spatially constrained enzymatic promiscuity

The major goals of this project are to biochemically and structurally investigate the mechanism of thiazole installation by micrococcin biosynthetic proteins

R01AG066874 (Price) 05/2020-02/2025 2 calendar
NIH $2,961,000
Biochemical consequences of regiospecific metabolic bias in the brain

The goal of this project is to build our unique capabilities to monitor in vivo flux of protein to develop similar capabilities to monitor lipid flux and use the combined capabilities to monitor how Alzheimer's disease risk factors change metabolism in differentially modify metabolism in regions of the brain.

1R01AI156382-01 (Morris, contact PI, Golden, PI, Christensen, PI, Werbovetz, PI)
09/20-08/30/2024 1.0 summer
NIH/NIAID ~$41,240
Development of a multiplexed assay in kinetoplastid parasites to identify probes for glycolysis

The objectives of this proposal is to identify inhibitors of glucose metabolism using live parasites expressing multiplexed biosensor probes for metabolic intermediates to develop probes for understanding regulation of glycolysis.

Completed Research Support (last 3 years)

N/A (Anderson) 12/2016-12/2020 0 calendar
Fritz B. Burns Foundation $7,000,000
Establishing the Fritz B. Burns Cancer research center at BYU

The major goals of this project are to purchase instrumentation and supplies for a core cancer research lab to investigate mechanisms of metabolic regulation that chemo-resistant cancers use to thrive and grow in the presence of chemotherapeutics. No salary support is provided.

Jayson Gifford

Ongoing External Awards

- Veteran's Administration Merit Award (2017-2021)
  - $1,100,000
    - Title- Vascular Endothelial Function in Alzheimer's Disease: A Potential Therapeutic Target
    - Role: Co-Investigator

Ongoing Internal Awards

- Graduate Mentor Ward (2019) $14,000
  - Title- Effects of Different Exercise Modes on Vascular Endothelial Function
  - Role: Primary Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Hyldahl, Robert D.

eRA COMMONS USER NAME (credential, e.g., agency login): robhyldahl

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Utah, Salt Lake City, UT	B.S.	2003	Exercise Sciences
University of Massachusetts Amherst, Amherst, MA	Ph.D.	2011	Kinesiology

A. Personal Statement

Skeletal muscle is an enormous, fascinating, and spectacularly adaptive organ system, whose vitality has an enormous impact on human health and well-being. Though appreciated most widely for its role in human motor activity, skeletal muscle accounts for over 50% of whole body protein metabolism, 80% of glucose disposal, and is emerging as an exquisitely important endocrine organ. Importantly, epidemiological data have shown quite clearly that reduced skeletal muscle mass and function have widespread deleterious effects on human health including frailty, metabolic dysfunction and mortality. Accordingly, the overarching goal of my laboratory is to contribute in a meaningful way to the development of crucial and much-needed clinical interventions to maintain skeletal muscle vitality through the lifespan by studying the biologic mechanisms that underlie muscle adaptation to stress (e.g. disuse, damage, exercise and injury) in healthy, aged or clinical populations. To achieve this goal, we strive primarily to undertake studies in *human* skeletal muscle through innovative and novel approaches, supplementing, where appropriate, with mechanistic experiments in relevant animal and *in vitro* models. Of particular interest are: 1) The role of extracellular matrix remodeling in muscle repair and adaptation to exercise; 2) The effect of externally applied modalities (e.g., heat/cold stress, massage etc.) on muscle metabolic and regenerative function, and their potential as therapeutic interventions; and 3) How inflammation and inflammatory related intracellular signaling affects muscle adaptation and reparative outcomes in aging.

Pursuant to these goals, I have established a track record in human skeletal muscle and aging research that puts me in an ideal position to be the primary investigator for the proposed experiments. I have amassed significant experience managing human subject research, and I am proficient in taking skeletal muscle biopsies and performing molecular laboratory techniques on skeletal muscle samples. I have successfully taken over 600 skeletal muscle biopsies (including over 100 in individuals >65 years of age), and have published the results of those studies in high impact peer reviewed outlets. Additionally, I have developed a stable publication record as a principal investigator, evident in a steady accumulation of publications featuring students as primary authors.

Positions and Employment

2018 – Associate Professor, Department of Exercise Sciences, Brigham Young University
2019 Visiting Researcher, Department of Biomedical Sciences, Institute of Sports Medicine, University of Copenhagen
2012-2018 Assistant Professor, Department of Exercise Sciences, Brigham Young University
2011–2012 Visiting Assistant Professor, Department of Health and Exercise Science, University of New Mexico
2006–2011 Graduate Research Assistant, Lab of Dr. Priscilla Clarkson, Department of Kinesiology, University of Massachusetts Amherst
2004–2006 Certified Athletic Trainer, Registered Physical Therapists, Sandy, UT
2002–2005 Laboratory Technician, Department of Neurobiology and Anatomy, University of Utah
1999–2002 Undergraduate Research Assistant, Department of Biology, University of Utah

Experience and Professional Memberships

2008- Member, American Physiological Society
2008- Member, American College of Sports Medicine
2000- Member, National Athletic Trainers Association

Contributions to Science

My early publications as a graduate student focused on understanding the molecular mechanisms of skeletal muscle adaptation to damaging exercise and disuse. I was trained in a laboratory with broad expertise in both human subject research and *in vitro* models. My training has allowed me to take an integrative, translational approach to my research questions.

Capitalizing on my success using molecular analyses of human skeletal muscle biopsy samples, my work at Brigham Young University has primarily focused on understanding the mechanisms of muscle adaptation and dysfunction in aging. This line of research has led to an additional interest in how dysregulation of extracellular matrix remodeling and inflammation influence muscle adaptation in older individuals. Additionally, pursuant to the goals of this proposal, we have begun to develop a publication record and research program targeted at the effects of repeated heat stress on muscle mitochondrial and vascular function. Pulications are listed in order of relevance to the proposed project

1. **Hyldahl RD**, Peake JM. Combining cooling or heating applications with exercise training to enhance performance and muscle adaptations. *J Appl Physiol.* Aug 2020; 1:129(2):353-365
2. Hafen PS, Abbott K, Bowden J, Lopiano R, Hancock CR, **Hyldahl RD**. Daily heat treatment maintains. Mitochondrial function and attenuates atrophy in human skeletal muscle subjected to immobilization. *J Appl Physiol.* Jul 2019 ; 1:127(1):47-57
3. Hafen PS, Sorensen JR, Preece CN, Hancock CR, **Hyldahl RD**. Deep tissue heating increases mitochondrial respiratory capacity of human skeletal muscle. *J Appl Physiol.* Nov 2018; 125(5):1447-1455
4. Sorensen JR, Kaluhiokalani JP, Hafen PS, Deyhle MR, Parcell AC, **Hyldahl RD**. Altered pro- and anti-inflammatory macrophage response in aged skeletal muscle following damage: implications for skeletal muscle repair. *FASEB J.* Sep 2019; 33(9):10353-10368
5. Sorensen JR, Skousen CB, Holland A, Williams K, **Hyldahl RD**. Acute extracellular matrix, inflammatory and MAPK response to lengthening contractions in elderly human skeletal muscle. *Exp Geront.* Feb 2018; 106:28-38.
6. Deyhle MR, Carlisle M, Parmley J, Sorenson JR, Hafen PS, Jesperson K, Hancock C, **Hyldahl RD.** Accumulation of skeletal muscle T-cells and the repeated bout effect in rats. *Med Sci Sports Exerc.* Jun 2020; 52(6):1280-1293
7. Deyhle MR, **Hyldahl RD**. The role of T lymphocytes in skeletal muscle repair from traumatic and contraction-induced injury. *Front. Physiol.* Jun 2018; 9:768.
8. Sorensen JR, Fuqua JD, Dehyle MR, Parmley J, Skousen CB, Hancock C, Parcell, AC, **Hyldahl RD**. Preclinical characterization of the JAK/STAT inhibitor SGI-1252 on skeletal muscle function, morphology and satellite cell content. *Plos One.* Jun 2018; 13(6).
9. Deyhle MR, Hafen P, Parmley J, Robison M, Sorenson JR, Jackson B, Hancock C, Parcell AC, **Hyldahl RD**. CXCL10 increases in human skeletal muscle following damage but is not necessary for muscle regeneration. *Physiol. Rep.* Apr 2018; 6(8).
10. **Hyldahl RD**, Evans A, Kwon SK, Ridge ST, Robinson E, Hopkins JT, Seeley MK. Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations. *Euro J Appl Physiol.* Dec 2016; (11-12): 2305-2314
11. Deyhle MR, Gier AM, Evans KC, Eggett DL, Nelson WB, Parcell AC, **Hyldahl RD.** Skeletal muscle inflammation following repeated bouts of lengthening contractions in humans. *Front. Physiol.* Jan 2016; 12(6): 424
12. **Hyldahl RD**, Nelson B, Xin L, Welling T, Groscost L, Hubal MJ, Chipkin S, Clarkson PM, Parcell AC. Extracellular matrix remodeling and its contribution to protective adaptation following lengthening contractions in human muscle. *FASEB,* Jul 2015; 29(7): 2894-904

URL to full list of published work (My Bibliography, US National Library of Medicine):
https://pubmed.ncbi.nlm.nih.gov/?term=Hyldahl%2C+Robert+D%5BAuthor%5D&sort=date

BIOGRAPHICAL SKETCH

NAME: **Jayson R. Gifford**

eRA COMMONS USER NAME (credential, e.g., agency login): JAYSONGIFFORD

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brigham Young University, Provo, Ut	B.S.	08/2009	Exercise Science
Brigham Young University, Provo, Ut	M.S.	08/2011	Exercise Physiology
University of Utah, Salt Lake City, Ut	Ph.D.	05/2015	Exercise Physiology
Salt Lake Veteran's Administration, Salt Lake City, Ut	Fellowship	07/2017	Geriatrics

A. Personal Statement

This proposed grant will investigate the impact of heat therapy, exercise training and muscle disuse on various aspects of muscle function in human subjects. As an exercise physiologist with a track record of investigating the effect of mitochondrial and vascular function on exercise tolerance in young and older populations, I will oversee the *in vivo* measurements of vascular function, exercise blood flow and exercise tolerance. As highlighted in the "Contributions to Science" section, I am very qualified to do this. For example, I recently authored the peer-reviewed guidelines for measuring resistance artery function with Doppler ultrasound (a major outcome in the proposed grant) and, have lead and published many human-based studies investigating the impact of different interventions or conditions on vascular function and exercise tolerance (4 senior-author and 7 co-author publications on the subject in the last year alone).

Having gained considerable experience with long-term intervention studies (*e.g.* exercise training) in older adults during my postdoctoral fellowship in Geriatrics at the Salt Lake Veterans Hospital, I will also help oversee the day-to-day logistics of heat and exercise training interventions.

Over the past year I have dedicated approximately 30% of my effort to collecting the pilot data seen in this proposal and will be able to continue this level of effort, or more, if awarded this grant. I have performed all of the proposed exercise and heating interventions, vascular assessments and exercise tolerance measurements many times on young and old subjects and have no hesitations regarding the feasibility of these measurements or interventions. I am confident in our team's ability to conduct this study and am excited to do so.

Professional Experience

2008-2011 Research Assistant, Brigham Young University
2009-2011 Teaching Assistant, Brigham Young University
2011-2015 Research Assistant, University of Utah
2015-2017 Postdoctoral Fellow, Salt Lake City, Veterans Administration Hospital
2017-Present Assistant Professor, Brigham Young University

Honors and Awards

Outstanding Reviewer Award, Journal of Experimental Biology and Medicine. 2018
Advanced Fellowship in Geriatrics, Veterans Administration, 2015-2017
Nielson Exercise Science Graduate Student Scholarship: University of Utah, 2014
Graduate School Travel Award, University of Utah, 2013-2015
Graduate Student Research Award, Brigham Young University, 2011

Magna Cum Laude, Brigham Young University, 2009
B.Y.U. Academic Scholarship, Brigham Young University, 2006-2009

Contributions to Science (selected from a total of 40 publications)

1.) The determinants of exercise tolerance have far reaching implications influencing functional capacity, independence and longevity. My research has contributed significantly to the understanding of the underlying physiology of exercise intolerance in young, aged and diseased populations. Using a combination of *in vitro* and *in vivo* techniques I have helped describe the roles of vascular oxygen supply and mitochondrial oxygen demand in exercise intolerance various populations.
   a. **Gifford JR,** Garten RS, Trinity JD, Nelson A, Richardson RS. Symmorphosis and skeletal muscle VO_{2max}: In vivo and in vitro measures reveal differing constraints in the exercise-trained and untrained human. Journal of Physiology. 2015.
   b. **Gifford JR**, Collins J. Critical Power throughout aging: insights from the world masters championships. Medicine and Science of Sport and Exercise. 2021.
   c. **Gifford JR,** Hanson BE, Proffit M**.** Indices of resistance artery function are independently related to cycling $\dot{V}O_2max$. Physiological Reports. 2020.
   d. **Gifford JR,** Garten RS, Trinity JD, Richardson RS. Altered skeletal muscle mitochondrial phenotype in COPD: Disease vs. Disuse. Journal of Applied Physiology 2017.

2.) It is becoming increasingly apparent that arterial endothelial dysfunction contributes significantly to the age and disease-related downward spiral that includes mobility limitation, frailty, and ultimately cardiovascular disease (CVD). My efforts have contributed significantly to the understanding of the role of endothelial dysfunction, and decreased NO bioavailability in aged and diseased populations.
   a. **Gifford JR**, Richardson RS. CORP: Ultrasound Assessment of Vascular Function with the Passive Leg Movement Technique. Journal of Applied Physiology. 2017.
   b. Hanson BE, Proffit M, **Gifford JR.** Vascular function is related to blood flow during high-intensity, but not low-intensity, knee extension exercise. Journal of Applied Physiology. 2020.
   c. Park SY, Ives S, **Gifford JR**, Richardson RS. Impact of age on vasodilatory function in human skeletal muscle feed arteries. AJP Heart and Circ. In Press. 2015.
   d. Park SY, Rossman MH, **Gifford JR**, Symons JD, Able D, Riehl C. Effect of exercise training on vascular mitochondrial function. American Journal of Physiology. Heart and Circ. 2017

3.) Skeletal muscle arteries will constrict or dilate in response to different conditions (*e.g.*, exercise, heat stress, oxidative stress) to match blood and oxygen demand downstream. Improper matching by the arteries can result in an energy crisis, hypoxia and diminished functional capacity. Utilizing *in vivo* and *in vitro* techniques, I have helped determine the impact of exercise byproducts, like heat, acid and shear stress, on artery function.
   a. **Gifford JR**, Ives SJ, Park S, Andtbacka R, Mueller M, Richardson RS. (2014) α1-and α2-Adrenergic responsiveness in human skeletal muscle feed arteries: the role of TRPV ion channels in heat-induced sympatholysis. American Journal of Physiology: Heart and Circulation 307:9:H1288-H1297.
   b. **Gifford JR**... Mack GW. Changes in Dermal Interstitial ATP Levels during Local Heating of Human Skin. Journal of Physiology. 2012.
   c. Ives SJ, Andtbacka RHI, Noyes RD, Morgan RG, **Gifford JR**, Park S-Y, Symons JD, and Richardson RS. α1-Adrenergic responsiveness in human skeletal muscle feed arteries: the impact of reducing extracellular pH. Experimental Physiology 98: 256-267, 2013.
   d. Hydren JR, **Gifford JR**, Jarret CL… Richardson RS. Vascular function in continuous-flow left ventricular assist (LVAD) device recipients: Effect of a single pulsatility treatment session. American Journal of Physiology: Regulatory, Integrative and comparative. 2021.

BIOGRAPHICAL SKETCH

NAME: Price, John C., Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): JCPrice

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Utah State University, Logan Utah	B.S.	2001	Chemistry
Pennsylvania State University, University Park	Ph.D.	2005	Biochemistry
University of California, San Francisco	Post-Doc	2010	Neurodegeneration

Personal Statement

In the proposed research we will be using LCMS and stable isotopes to measure the concentrations and kinetics of a variety of biomolecules *in vivo*. I have extensive experience in the use of isotope labels to investigate biological processes. As a graduate student at the Pennsylvania State University, I used isotopically labeled substrates to isolate transient intermediates at key points in enzymatic processes. As a postdoctoral scholar I expanded the use of isotopic labels into metabolic labeling, conducting the first reported large scale measurement of protein turnover (~2500 individual proteins) *in vivo*. Then, as Director of Proteome Research at KineMed Inc., I developed new isotope labeling methods that allowed me to lead the first large scale investigation of protein kinetics in humans. I then applied these methods to investigate human disease. In my current position as Assistant Professor of Biochemistry at BYU, I am teaching these methods and concepts to students.

Specifically, in the course of the proposed research, my graduate students and I will measure the kinetics and relative concentration of proteins in muscle. I will work closely with Drs. Hyldahl and Hancock in experimental design and sample collection. We have already participated in similar metabolic labeling experiments on the BYU campus. We are finding that the combination of our kinetic proteomic methods with classical quantitative proteomics gives us the capability of calculating individual synthesis and degradation rates for every detectable protein in the cell.

Positions and Employment

1999-2001 Teaching Assistant, Dept. of Chemistry, Utah State University, Logan, UT
2005-2010 Fellow, Institute for Neurodegenerative Disease, Univ. Calif., San Francisco, CA
2010-2011 Scientist, KineMed Inc., Emeryville CA
2011-2013 Director of Proteome Research, KineMed Inc., Emeryville CA
2013-2018 Assistant Professor of Biochemistry at Brigham Young University
2018-present Associate Professor of Biochemistry at Brugham Young University

Experience and Professional Memberships

2002-2013 Member, American Chemical Society,
2011- Member, American Soc. for Biochemistry and Molecular Biology,
2013- Member, American Society for Mass Spectrometry,
2013- Member, Human Proteome organization

Honors

2001 American Institute of Chemists Foundation Undergraduate Award
2003 Paul Berg Prize in Molecular Biology
2007 Larry L. Hillblom Foundation Postdoctoral Fellow

Contribution to Science

I have developed a number of mass spectrometry based assays that interrogate the biological activity of proteins and peptides. I published the first large scale mass spectrometry-based proteomics and stable isotopes study to monitor the turnover rate of protein *in vivo*. This was the first published study showing that *in vivo* turnover could be measured at the proteome scale. I then modified the methods to allow simple and safe labeling in humans so that kinetic proteomics methods could be used in clinical research. The papers listed below mark the important points in this development.

A. **Price JC**, Guan S, Burlingame A, Prusiner SB, Ghaemmaghami S.*, **2010**, "Analysis of proteome dynamics in the mouse brain." **Proc. Natl. Acad. Sci. USA, 107,** 14508-13. PMID: 20699386
B. Guan S*, **Price JC**, Prusiner SB, Ghaemmaghami S, Burlingame AL, **2011**, "A data processing pipeline for mammalian proteome dynamics studies using stable isotope metabolic labeling." **Mol. Cell Prot.**, (12):M11.010728. PMID: 21937731. PMCID: PMC3237081
C. **Price JC***, Holmes WE, Li KW, Floreani NA, Neese RA, Turner SM, Hellerstein MK, **2011** "Measurement of human plasma proteome dynamics with 2H2O and liquid chromatography tandem mass spectrometry." **Ana. Biochem**., 420(1):73-83. PMID: 21964502
D. Cooney I, Han H, Stewart M, Carson RH, Hansen DT, Iwasa JH, **Price JC**, Hill CP, Shen PS* **2019** "Structure of Cdc48 segregase in the act of unfolding an authentic substrate" **Science** Vol 365, Issue 6452, pg 502-505

Our current projects study cellular control of protein homeostasis. Changes in protein turnover and protein homeostasis have been proposed to be key events during whole organism aging. Calorie restriction is the gold standard intervention to slow aging, and controlling changes in protein homeostasis are thought to be key in modulation of aging. Using our newly developed method for monitoring in vivo protein kinetics in combination with well-established methods for protein quantitation via mass spectrometry. I lead the first study to measure individual protein synthesis and degradation rates for hundreds of proteins *in vivo*. One important contribution showed that calorie restriction dramatically slows protein synthesis and degradation, ending years of controversy on this subject. The papers below highlight these contributions.

A. Naylor BC, Porter MT, Wilson E, Herring A, Lofthouse S, Hannemann A, Piccolo SR, Rockwood AL and **Price JC* 2017** "DeuteRater: a Tool for Quantifying Peptide Isotope Precision and Kinetic Proteomics", **Bioinformatics**, May 15;33(10):1514-1520. doi: 10.1093/bioinformatics/btx009 PMID: 28093409
B. Mathis AD, Naylor BC, Carson RH, Evans E, Harwell J, Knecht J, Hexem E, Peelor FF, Miller BF, Hamilton KL, Transtrum MK, Bikman BT, **Price JC* 2017** "Mechanisms of in vivo ribosome maintenance respond to nutrient signals", **Mol. Cell. Prot.**, 2017 Dec 8. pii: mcp.M116.063255
C. Thompson A, Bruss MD, **Price JC**, Khambatta CF, Harrison DE, Hellerstein MK, **2016** "Reduced in vivo hepatic proteome replacement rates, but not cell proliferation, rates predict maximum lifespan extension in mice" **Aging Cell**, Feb;15(1):118-27. PMID: 26541492. PMCID: PMC4717272
D. **Price JC***, Khambatta CF, Li KW, Bruss MD, Shankaran M, Dalidd M, Floreani NA, Roberts LS, Turner SM, Holmes WE, and Hellerstein MK, **2012** "The effect of long-term calorie restriction on hepatic proteostasis and mitochondrial dynamics in mice" **Mol. Cell. Proteomics**, 11.12, pg: 1801-1814. PMID: 22984287. PMCID: PMC3518108

During the proposed research we will be developing novel mass spectrometry based assays. Over the years I have successfully developed and applied mass spectrometry to diverse problems such as the measurement of peptide hormones, imaging using mass spectrometry, as well as the measurement of *in vivo* kinetics.

A. Speirs MMP, Swensen AC, Chan TY, Jones PM, Holman JC, Harris MB, Maschek JA, Cox JE, Carson RH, Hill JT, Andersen JL, Prince JT, and Price JC* **2019**, "Imbalanced sphingolipid signaling is maintained as a core proponent of a cancerous phenotype in spite of metabolic pressure and epigenetic drift", Oncotarget. 10:449:479
B. Swensen AC, Finnell J, Orozco CM, Gross AJ, Prince JT, Watt RK, **Price JC* 2017** "Whole Blood and Urine Bioactive Hepcidin-25 Determination using Liquid Chromatography Mass Spectrometry" **Analytical Biochem**., 517C pp. 23-30
C. Carson RH, Lewis CR, Erickson MN, Zagieboylo AN, Naylor BC, Li KW, Farnsworth PB, **Price JC* 2017** "Imaging Regiospecific Lipid Turnover in Mouse Brain with Desorption Electrospray Ionization Mass Spectrometry", **J. Lipid Res**. 58:(9) 1884-1892. PMID: 28743728 PMCID: PMC5580901
D. Shankaran* M, King CL, Angel TE, Holmes WE, Li KW, Colangelo M, **Price JC**, Turner SM, Bell C, Hamylton KL, Miller BF, Hellerstein MK*, **2016** "Circulating protein synthesis rates represent muscle proteome dynamics "virtual biopsy"", Journal of Clinical Investigation, 126(1):288-302. doi:10.1172/JCI79639

URL to full list of published work (My Bibliography, US National Library of Medicine):
http://www.ncbi.nlm.nih.gov/sites/myncbi/john.price.1/bibliograpahy/47992968/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

NAME: Hancock, Chad R.

eRA COMMONS USER NAME (credential, e.g., agency login): CHANCOCK

POSITION TITLE: Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	START DATE MM/YYYY	END DATE MM/YYYY	FIELD OF STUDY
Brigham Young University	B.S.		04/1998	Physical Education (Exercise Physiology)
UNIVERSITY OF MISSOURI - COLUMBIA, Columbia, MO	Ph.D.		05/2005	Physiology/Muscle Physiology
Washington University in St. Louis, St. Louis, MO	Postdoctoral Fellow	05/2005	07/2008	Exercise/Nutrition Metabolism

Personal Statement

A primary focus of this project is aimed at evaluating mitochondrial adaptations and changes that occur with repeated exposure to heat stress. I bring a strong background of research on different aspects of energy metabolism and specifically mitochondrial biogenesis and function. I have authored/co-authored 32 original research manuscripts involving different aspects of cellular energy metabolism and muscle function. My involvement in this project will be to help guide and direct the measurement and evaluation of mitochondrial respiratory function from human muscle biopsy samples. Along with Dr. Hyldahl, we recently published a manuscript in which we evaluated mitochondrial function following heat exposure and the work proposed in this application builds on some of those findings. I have also recently published work on the role of PPAR-delta in exercise induced mitochondrial biogenesis as well as papers examining mitochondrial function in pancreatic beta cells as well as skeletal myocytes. I have also helped with the design and collection of data from experiments that have led up to this application. My specific contributions may include the following: assist with experimental design and writing, protein assessment by activity assays and western blot analysis, and assessment of mitochondrial bioenergetics.

Positions and Employment

2008 - 2014 Assistant Professor, BRIGHAM YOUNG UNIVERSITY
2014 - Associate Professor, Brigham Young University, Provo, UT

Other Experience and Professional Memberships

2000 - Member, American Physiological Society
2007 - Member, Editorial Board-American Journal of Physiology-Endocrinology and Metabolism
2008 - Member, American Diabetes Association

Contribution to Science

My work as a graduate student was focused on gaining a better understanding of the management of high-energy phosphates in skeletal muscle during high intensity muscle contractions. As part of this project, we used a model of skeletal muscle adenylate kinase deficiency and with very intense muscle contractions, were able to drive the concentration of ADP higher than had ever been measured before. This work illustrated multiple mechanisms that are normally at play in muscle to prevent a dramatic rise in ADP concentrations and by extension, preserve a viable energy state for continued muscle function.

My work at Washington University in St. Louis as a Post-Doctoral Fellow was focused on evaluating mechanisms that were responsible for (or not responsible for) changes in mitochondrial content following high fat feeding, caloric restriction and iron deficiency. This work has resulted in several findings that have been of great impact in this area of study. We were able to more fully explore the role of PPAR transcription factors in regulating mitochondrial biogenesis. Furthermore, we just had a manuscript published this year in *Cell Metabolism* that was an extension of this work.

Ultimately we were able to show that 1) changes in mitochondrial content were not necessarily associated with changes in insulin resistance/sensitivity, 2) PPAR-delta plays a critical role in the maintenance of mitochondrial content in skeletal muscle and is also critical for the normal increase in in mitochondrial content that occurs in response to exercise.
1. Garcia-Roves, P., Huss, J. M., Han, D. H., Hancock, C. R., Iglesias-Gutierrez, E., Chen, M., and Holloszy, J. O. (2007) Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. *Proc Natl Acad Sci U S A* 104, 10709-10713

2. Wende, A. R., Schaeffer, P. J., Parker, G. J., Zechner, C., Han, D. H., Chen, M. M., Hancock, C. R., Lehman, J. J., Huss, J. M., McClain, D. A., Holloszy, J. O., and Kelly, D. P. (2007) A role for the transcriptional coactivator PGC-1alpha in muscle refueling. *J Biol Chem* 282, 36642-36651

3. Hancock, C. R., Han, D. H., Chen, M., Terada, S., Yasuda, T., Wright, D. C., and Holloszy, J. O. (2008) High-fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proc Natl Acad Sci U S A* 105, 7815-7820

4. Han, D. H., Hancock, C. R., Jung, S. R., Higashida, K., Kim, S. H., and Holloszy, J. O. (2011) Deficiency of the mitochondrial electron transport chain in muscle does not cause insulin resistance. *PLoS One* 6, e19739

5. Hancock, C. R., Han, D. H., Higashida, K., Kim, S. H., and Holloszy, J. O. (2011) Does calorie restriction induce mitochondrial biogenesis? A reevaluation. *FASEB J* 25, 785-791

6. Koh, J. H., Hancock, C. R., Terada, S., Higashida, K., Holloszy, J. O., and Han, D. H. (2017) PPARbeta Is Essential for Maintaining Normal Levels of PGC-1alpha and Mitochondria and for the Increase in Muscle Mitochondria Induced by Exercise. *Cell Metab* 25, 1176-1185 e1175

During my tenure here at BYU as a faculty member, my work has continued to focus on skeletal muscle changes in mitochondrial content in response to high fat feeding, metformin treatment, selenium and/or isoflavone intake. In addition we have done work examining the importance and role of AMPK and LKB-1 in collaboration with Dave Thomson and Will Winder with some of these projects. These projects have also expanded to more broadly study the mitochondrial response to high fat feeding in liver. Most recently we have been working to better evaluate changes in not just mitochondrial content but function by directly measuring respiration using high-resolution respirometry. This has opened up several lines of research on pancreatic beta cell mitochondrial function and other cell culture models. The project that is described in this application is an exciting extension to the current work we have been doing evaluating changes in mitochondrial function to different stimuli, specifically repeated exposure to heat stress. It is worth noting that the work done at BYU has included a tremendous amount of training with undergraduate students actively doing lab work and publishing their work in respectable journals. Related to this emphasis on undergraduate research, BYU ranks near the top in the nation in terms of the number of its baccalaureate's who end up pursuing PhD programs.

1. Stallings, M. T., Cardon, B. R., Hardman, J. M., Bliss, T. A., Brunson, S. E., Hart, C. M., Swiss, M. D., Hepworth, S. D., Christensen, M. J., and Hancock, C. R. (2014) A high isoflavone diet decreases 5' adenosine monophosphate-activated protein kinase activation and does not correct selenium-induced elevations in fasting blood glucose in mice. *Nutr Res* 34, 308-317

2. Reynolds, M. S., Hancock, C. R., Ray, J. D., Kener, K. B., Draney, C., Garland, K., Hardman, J., Bikman, B. T., and Tessem, J. S. (2016) beta-Cell deletion of Nr4a1 and Nr4a3 nuclear receptors impedes mitochondrial respiration and insulin secretion. *Am J Physiol Endocrinol Metab* 311, E186-201

3. Tueller, D. J., Harley, J. S., and Hancock, C. R. (2017) Effects of curcumin and ursolic acid on the mitochondrial coupling efficiency and hydrogen peroxide emission of intact skeletal myoblasts. *Biochem Biophys Res Commun* 492, 368-372

4. Hafen PS, Preece CN, Sorensen JR, Hancock CR, and Hyldahl RD. Repeated exposure to heat stress induces mitochondrial adaptation in human skeletal muscle. *J Appl Physiol (1985)* 2018.

5. Hafen, P.S., Abbott, K., Bowden, J., Lopiano, R., Hancock, C.R., and Hyldahl, R.D. (2019). Daily heat treatment maintains mitochondrial function and attenuates atrophy in human skeletal muscle subjected to immobilization. J Appl Physiol (1985) 127, 47-57.
My full bibliography can be access using the following link
https://www.ncbi.nlm.nih.gov/sites/myncbi/1VCwb51aEVrQh/bibliography/40445234/public/?sort=date&direction=ascending