Official Title: Mitochondrial function in circulating cells and muscle tissue

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Study Title:	**Mitochondrial function in circulating cells and muscle tissue**
Principal Investigator:	Elisabet Borsheim, Ph.D.
Office:	501-364-3053
Cell:	409-392-4203 (cell)
Co-Investigators:	Eugenia Carvalho, Ph.D. Richard Frye, M.D. Gohar Azhar, M.D. Sean Adams, Ph.D.
Study Physician:	Gohar Azhar, M.D.
Study Location:	Arkansas Children's Nutrition Center (ACNC), Arkansas Children's Hospital Research Institute, 15 Children's Way Little Rock, AR 72202 and University of Arkansas for Medical Sciences Reynolds Institute on Aging, Little Rock, AR 72205
Funding:	Arkansas Children's Nutrition Center (ACNC) United States Department of Agriculture (USDA)

Background

Data from human and animal models strongly suggests that reduced tissue mitochondrial function can be an early marker for detection of dysfunctional metabolism [1]. It is unclear if tissue mitochondrial dysfunction can be detected using less invasive methodology. In this project, we plan to study mitochondrial bioenergetics in circulating cells and paired muscle biopsies in two metabolic disparate groups of women: healthy lean vs. overweight/obese and/or insulin resistant women of similar age.

Mitochondria in circulating blood cells sense oxidative stress during capillary passage and react by producing reactive oxygen species (ROS) [2]. Bioenergetic profiling of readily available circulating cells has recently been proposed to reflect systemic mitochondrial health/fitness, physical function and inflammation [3, 4]. Thus, the mitochondria function in circulating cells may potentially inform on the overall metabolic health in an individual. The "bioenergetic health index" (BHI) in peripheral blood mononuclear cells (PBMC) and/or platelets has therefore been described as a potential new biomarker for assessing patient health [3, 5, 6].

Studies to date have, however, not compared results of mitochondrial function in circulating cells and in muscle tissue in the same persons. Instead, a relation has been shown indirectly by an association between the bioenergetics of PBMC and physical function and inflammation in overweight/obese older adults [4]. Thus, there is a need for establishing this relation by comparing bioenergetics in circulating cells and in muscle tissue from the same individual, and in individuals with contrasting metabolism.

By studying healthy lean and overweight/obese and/or insulin-resistance women of the same age, we expect to discover differences in muscle mitochondrial function in the two groups, with compromised function in the overweight/obese and/or insulin resistant group. Obesity is associated with metabolic impairments in peripheral tissues and dysfunctional adipose tissue unable to buffer the flux of dietary lipids, leading to ectopic lipid deposition and lipotoxicity in other organs. Lipid accumulation in skeletal muscle is associated with insulin resistance and overall cellular dysfunction, with mitochondrial dysfunction as one of the underlying mechanisms [7]. We hypothesize that PMBC mitochondrial function in the overweight/obese and/or insulin resistant group is compromised compared to the healthy lean group. Finally, we hypothesize that there will be a relation between PBMC and muscle mitochondria function across the two groups.

The main goal of this project is to determine whether mitochondrial function in circulating cells is related to that measured in the muscle fibers of the same subjects, and if differences can be observed when comparing metabolic dissimilar groups. Considering the difficulty of obtaining muscle biopsies from various populations, especially pediatric populations, it would be a major step if a blood sample can inform about overall/tissue mitochondrial health.

Study Design:

Twenty (20) healthy lean, and 20 overweight or obese and/or insulin resistant women of similar age will be recruited, with the goal of 15 women/group completing the study.

Inclusion criteria:

- Women.
- 25-35 years of age.
- Either healthy lean (BMI<25), or overweight (25≤BMI<30) or obese (BMI≥30) and/or insulin resistant.

Exclusion criteria:

- Pre-existing medical conditions (e.g., chronic renal failure, bleeding disorders, heart disease, self-reported hepatitis or HIV, thyroid disorders) that can affect study outcomes, as determined by the PI and/or designee.
- Self-reported possibility of current pregnancy.
- Regular smoking.
- Alcohol or drug abuse.

Enrollment/Recruitment: Various advertisements will be distributed by direct mail or to various locations, including but not limited to healthcare offices, weight loss clinics, health fairs, schools/universities, recreational centers, websites (ACH, ACRI, ACNC, UAMS, and others as applicable) and churches. Also, print or digital ads may appear in newspapers, magazines, social media and circulars. Recorded phone messages that play when the phone is answered and the person is on hold, BoomText messages, screensavers, and radio/television advertisements may be used. We will also work with physicians, who have patients that may qualify as potential volunteers. They will in that case administer a flyer to the potential subject and ask them to contact us if they are interested in participation.

A standardized screening script will be used to determine whether potential participants qualify for the study. Potential participants will be asked if their information can be saved for consideration in future studies and their response will be documented. We will protect all personal information obtained during recruitment, enrollment, and testing processes, and maintain this in a closed office.

Informed Consent Process: During the informed consent process, the PI, co-Is, or a research staff member will review all study procedures and the informed consent documents with the potential participant. If eligible and interested in enrollment, informed consent from the participant will be obtained and documented. The participant will be given ample time to review the consent form, ask questions and receive clarification prior to signing any documents.

Study Procedures: The participants that qualify and sign consent for this study will come only once to the Reynolds Institute on Aging at UAMS or to the ACNC where the visit will take place, depending on room and physician's availability.

The visit will take 2-3 hours and the participant will arrive after an overnight fast. At the visit, vitals anthropometrics will be recorded, body composition may be measured, a blood sample will be collected and a muscle biopsy sampled.

- Vitals and Anthropometric measures: The subject's blood pressure, pulse, temperature, body mass, height, and waist and hip circumferences will be obtained using standardized techniques.
- Physical activity questionnaire: A questionnaire (attached) will be administered to record physical activity. This will enable us to control for levels of physical activity between participants.
- Dual-Energy X-ray Absorptiometry (DXA): Body composition may be measured using a DXA device (Hologic Inc, MA, USA). This technology involves very small amounts of radiation. The subject will be asked to lie down without moving during the entire length of scanning (about 5

min). The scanning process will be aborted at the request of the participant or if any movement occurs. This procedure may be repeated if the first one is invalid or is aborted.

- Blood collection: Blood will be collected by a trained phlebotomist. In total, ~50 ml (~3.5 tablespoons) will be drawn for isolation of PBMC and platelets, and for analyses of insulin, glucose, lipid levels, and other analytes such as metabolites or hormones.
- Muscle biopsy: After the blood draw, a muscle tissue biopsy will be taken from m. vastus lateralis, about 4-6 inches above the knee by Gohar Azhar, M.D. Before any incisions are made, the skin will be cleansed with an antiseptic scrub solution and allowed to dry, and the skin and tissue below injected with lidocaine. A small incision about the size of this dash "—" will then be made, through which a needle about the size of the letter "O" is gently pushed into the muscle. A piece of the muscle is then removed with the needle. The skin will thereafter be closed with dermabond glue, and a light dressing applied. The biopsy site will be iced, a biopsy procedure note recorded, and the subject will be given thorough oral and written instructions about how to care for the biopsy site (please see attachments).

  The biopsy will immediately be placed in a BIOPS buffer and analyzed for mitochondrial function within a few hours after sampling. We have established the protocol for determination of mitochondrial function in permeabilized muscle tissue using high resolution respirometry (Oxygraph O2k, Oroboros Instruments, Innsbruck, Austria) [8]. Similarly, the co-investigator Dr. Richard Frye, MD, at ACHRI has established protocols on determination of mitochondrial function in cells, using the Seahorse Extracellular Flux (XF) 96 Analyzer (Seahorse Bioscience, North Billerica, MA, USA).

Sample Storage**:** Blood and muscle samples will be kept at -80°C once the initial processing and analyses have taken place. Samples will be stored in appropriate freezers in the PI's laboratory. These are monitored continuously for proper temperature and working condition. Samples will be destroyed only after all data has been analyzed and reported. All blood and muscle samples will be using a unique study acronym. None of a subject's personal identifiers will be present on any biological sample.

Statistical and Power Analyses: Forty women between 25-35 years old (20 healthy lean and 20 insulin-resistant and/or overweight or obese) will be recruited to participate in this study. Assuming a moderate dropout rate of 25%, we anticipate the final sample size to be 30 (15 healthy lean and 15 insulin resistant

and/or overweight or obese). A two-sample t-test will be used to assess differences between means of the two groups for either the blood or the muscle measurements. In addition, correlation and concordance analyses will be performed between the blood and the muscle results across the two groups to assess their correlation and agreement. Outcome variables will be checked to verify that the distributional assumptions (normality) for the t-test are met. When substantial deviation from assumptions is encountered, and if a suitable data transformation is not found, groups will be compared using the nonparametric Wilcoxin/Mann-Whitney rank-sum test.

Power analysis: Using the 2-sample t-test with equal variance, a sample size of 30 (with 15 subjects per group) achieves 80% power to detect a standardized difference of 1.06 units between the two group means versus the null hypothesis of equal means at a 5% α-level. Alternatively, without any dropout, a sample size of 40 (with 20 subjects per group) achieves 80% power at 5% α- Although the effect sizes are estimated to be moderate to large, it will provide valuable information regarding whether a larger study is warranted, and will form a basis for statistical approaches to other planned follow-up studies.

Data Safety Monitoring Plan

All investigators, co-investigators and study staff will complete and maintain appropriate CITI training. All data and communications will be recorded in standardized case report forms to ensure a paper copy trail. Source documents and CRFs will be stored in a secure area of the PI's laboratory. Access will be limited to study personnel. Documents will be archived according to UAMS/ACH/ACNC policies regarding destruction of research records. At no time shall Protected Health Information be released to non-study personnel.

Research data will be entered into a clinical database. This database will be housed on a shared drive backed up nightly off-site Monday – Friday. Access to our server is password protected, as is access to the study database(s). Access will be limited to study personnel.

Discomforts and Risks

The risks associated with the study procedures are as follow:

- DXA: The DXA instrument uses radiation to measure body composition. The radiation exposure is small (< 0.1 mrem) corresponding to approximately 2 days' worth of the naturally occurring radiation a person is exposed to while living in the Little Rock Area.

- Blood Sampling: The total amount of blood drawn for this study will be approximately 50 ml (~3.5 tablespoons), which is only 1/5 of what is generally given during blood donations. Aseptic technique will be followed which will minimize the risks of infection. However, the risks related to blood draw include pain and bruising.

- Muscle biopsy: The muscle biopsy procedure is performed under local anesthesia (1% lidocaine with a small amount of bicarbonate) for normal pain management, and possible formation of a bruise from local bleeding. Bleeding from the muscle biopsy site is highly unlikely since no major arteries or veins are located in the region. A possible complication of this procedure is infection due to lack of sterility during the procedure. General precautions that will be taken include strict sterility during muscle biopsies. At the end of the study, the small incision at the biopsy sites will be dressed with an antibiotic ointment to minimize risk of infection. Further, pressure bandage and ice will be applied.

Safety Reporting

Definitions

A. Related: For this policy, an event is "related" if it was caused by participation in the research activity or there is a reasonable possibility that the event may have been caused by the procedures involved in the research.
B. Risk: The probability of harm or injury (physical, psychological, social or economic) occurring as a result of participation in a research study.
C. Unanticipated: A problem is "unanticipated" when it was unforeseeable at the time it occurred.
D. Unanticipated Problem Involving Risks to Subjects or Others (UPIRTSO): Any problem, event or new information that is:
   1) Unanticipated or unexpected;
   2) Related to the research; and

3) Involves new or increased risks to subjects or others.

E. Unexpected: An event is "unexpected" when its specificity, nature, severity or incidences are not accurately captured in the approved consent form. Examples include a lower rate of response or a side effect that is more severe than initially expected.

(Serious) Adverse Events handling

Any clinical study event that is judged to be an (S)AE, either reported spontaneously by the subject or observed by the investigator or her/his staff, is recorded on the (S)AE form during the course of the study. The investigator must ensure that this information, including onset, duration and nature of event, severity, and action taken, is captured.

The severity of any (S) AE is scored as follows:

- Mild: transient or mild discomfort; no medical intervention/therapy required.
- Moderate: mild to moderate limitation in activity; some assistance may be needed; and/or minimal medical intervention/therapy required.
- Severe: marked limitation in activity; some assistance usually required; and/or significant medical intervention/therapy/hospitalisation required.

We do not foresee any adverse events associated with this protocol. In addition, testing can be aborted at any time at the participant's request. All procedures are minimally to moderate invasive. However, any adverse effects or unanticipated problems will be reported to the study PI, the IRB, and the study sponsor in accordance with UAMS IRB Policy 10.2 Information that Must Be Reported to the IRB and IRB Actions.

Risks/Benefits

Participants will not directly benefit from this study. Future population may benefit from this research. Expected risks associated with this protocol are described in detail above. All experimental procedures will be performed by appropriately trained and credentialed personnel.. Potential risks from participation in this research protocol include problems associated with blood drawing and muscle biopsies.

Participant Compensation: The reimbursement will be $100 for completing the study. This will be mailed to them after study completion with a completion letter.

Possible Problems and Pitfalls: We expect minimal problems with this study. Both the physician that will perform the biopsies (Dr. Azhar, M.D.), and Dr. Borsheim and her staff have experience in collecting blood samples and performing muscle biopsies.

Future plans: In future studies we plan to use these techniques to determine if children born to overweight and obese mothers or people at high risk for diabetes such as first degree relatives of diabetic subjects will show impaired mitochondrial function in circulating cells relative to control subjects. We may also use the techniques to examine whether physical activity and/or nutritional interventions affect these measurements. The information from the current protocol will inform as to whether or not the use of blood cell energetics measurements can supplant the need for muscle biopsies.

Dissemination of Data

Results of this study may be used for presentations, posters, or publications. The publications will not contain any identifiable information that could be linked to a participant.

LITERATURE

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2. Ijsselmuiden, A.J., et al., *Circulating white blood cells and platelets amplify oxidative stress in heart failure.* Nat Clin Pract Cardiovasc Med, 2008. **5**(12): p. 811-20.
3. Chacko, B.K., et al., *The Bioenergetic Health Index: a new concept in mitochondrial translational research.* Clin Sci (Lond), 2014. **127**(6): p. 367-73.
4. Tyrrell, D.J., et al., *Blood-cell bioenergetics are associated with physical function and inflammation in overweight/obese older adults.* Exp Gerontol, 2015. **70**: p. 84-91.
5. Chacko, B.K., et al., *The Bioenergetic Health Index is a sensitive measure of oxidative stress in human monocytes.* Redox Biol, 2015. **8**: p. 43-50.
6. Kramer, P.A., et al., *Decreased Bioenergetic Health Index in monocytes isolated from the pericardial fluid and blood of post-operative cardiac surgery patients.* Biosci Rep, 2015. **35**(4).
7. Tumova, J., M. Andel, and J. Trnka, *Excess of free fatty acids as a cause of metabolic dysfunction in skeletal muscle.* Physiol Res, 2015.
8. Porter, C., et al., *Mitochondrial respiratory capacity and coupling control decline with age in human skeletal muscle.* Am J Physiol Endocrinol Metab, 2015. **309**(3): p. E224-32.

Muscle Biopsy Procedure Note

Research Muscle Biopsy Procedure

Study Name: ***Mitochondrial function in circulating cells and muscle tissue***

Study PI: Elisabet Borsheim, Ph.D.

Subject Name/ID: _____Date: _____

Location: Vastus Lateralis Biopsy #1 **Side:** _____Right _____Left

Drug Allergies: _____

Anticoagulant Medication:

Heparin ___ No ___ Yes If yes, date/time last taken: _____

Coumadin _No ___ Yes If yes, date/time last taken: _____

Plavix ___ No ___ Yes If yes, date/time last taken: _____

Aspirin ___ No ___ Yes If yes, date/time last taken: _____

Procedure: Muscle Biopsy #1
After sterile preparation and adequate local anesthesia with _____ml 1% lidocaine, the muscle biopsy procedure was performed with a 5 mm Bergstrom needle with/without complications. Pressure was applied to the biopsy site continuously until all bleeding ceased. The incision was bandaged with a sterile compression dressing.

_____
Staff signature

The subject was provided verbal and written instructions regarding wound care, as well as contact numbers in case of questions or problems.

Duties performed, name:

Biopsy: _____ Assistance: _____

Comments: _____

_____

Center for Translational Research in Aging
Biopsy Care Instructions

Activity is good for your muscle after the biopsy; this prevents it from getting stiff. Your muscle may feel sore for a day or two, as if you have performed unaccustomed exercise; however, this is normal and will pass.

A pressure dressing has been applied over the biopsy site. Keep this on until you go to bed tonight. A special glue (Dermabond) was used to keep the incision closed, and a dressing applied over this. Keep this dressing on for 5-7 days following the biopsy. You can get the biopsy site/dressing wet in order to shower; otherwise, try to keep it dry to prevent the dressing from falling off prematurely. Please do not take any pain medication that may affect bleeding (e.g., aspirin). If necessary, clean the site with the alcohol wipes provided, and if the dressing falls off early, replace it with the ones provided.

If you have any questions or problems with severe soreness, bleeding, or if the biopsy site becomes red or warm to the touch, please call Scott Schutzler, R.N. at (501) 388-1025 or Dr. Elisabet Borsheim at (409) 392-4203 (cell).

Name : _____
Date : _____
☐ baseline ☐_____ months

Physical Activity Scale for the Elderly
And Paffenbarger Physical Activity Index

LEISURE TIME ACTIVITIES

1. Over the past 7 days, how often did you participate in sitting activities such as reading, watching TV or doing handcrafts?

[0] never → Go to Q.2
[1] seldom (1-2 days)
[2] sometimes (3-4 days)
[4] often (5-7 days)

1a. What were these activities?
_____

1b. On average, how many hours per day did you engage in these sitting activities <u>on these days</u>?
[1] less than 1 hour [2] 1 but less than 2 hours
[3] 2 – 4 hours [4] more than 4 hours

2. Over the past 7 days, how often did you take a walk outside your home or yard for any reason? For example for fun or exercise, walking to work, walking the dog etc?

[0] never → Go to Q.3
[1] seldom (1-2 days)
[2] sometimes (3-4 days)
[4] often (5-7 days)

2a. On average, how many hours per day did you spend walking <u>on these days</u>?
[1] less than 1 hour [2] 1 but less than 2 hours
[3] 2 – 4 hours [4] more than 4 hours

b) What was the **<u>total</u>** distance (kms/miles/blocks) that you walked in the past 7 days? (1 mile = 12 blocks: 1km = 0.625)

<u>Total</u> number of blocks walked in the past **week** _____, or km _____, or miles _____

[1] less than 1 mile
[2] one but less than 2 miles
[3] two to 4 miles
[4] more than 4 miles

3. How many flights of stairs did you **<u>climb up</u>** in the past 7 days? (one flight = 10 steps)
Total number of steps climbed in the past **week** _____, or flights of steps _____.
[1] less than 1 flight
[2] one but less than 2 flights
[3] two to 4 flights
[4] more than 4 flights

4. Over the past 7 days how often did you engage in **light** sport or recreational activities such as 'light' cycling on an exercise bike, lawn bowls, bowling, water aerobics, golf with a cart, yoga, tai chi, fishing from a boat or pier or other similar activities?

[0] never → Go to Q.5

[1] seldom (1-2 days) / [2] sometimes (3-4 days) / [4] often (5-7 days)

4a. What were these activities?

_____

4b. On average, how many hours per day did you engage in these light sport or recreational activities on these days?

[1] less than 1 hour [2] 1 but less than 2 hours
[3] 2 – 4 hours [4] more than 4 hours

5. Over the past 7 days, how often did you engage in **moderate** sport or recreational activities such as doubles tennis, ballroom dancing, golf without a cart, softball or other similar activities?

[0] never → Go to Q.6

[1] seldom (1-2 days) / [2] sometimes (3-4 days) / [4] often (5-7 days)

5a. What were these activities?

_____

5b. On average, how many hours per day did you engage in these moderate sport or recreational activities on these days?

[1] less than 1 hour [2] 1 but less than 2 hours
[3] 2 – 4 hours [4] more than 4 hours

6. Over the past 7 days, how often did you engage in **strenuous** sport and recreational activities such as jogging, simming, cycling, singles tennis, aerobic dance, skiing (downhill or cross country) or other similar activities?

[0] never → Go to Q.7

[1] seldom (1-2 days) / [2] sometimes (3-4 days) / [4] often (5-7 days)

5a. What were these activities?

_____

5b. On average, how many hours per day did you engage in these strenuous sport or recreational activities on these days?

[1] less than 1 hour [2] 1 but less than 2 hours
[3] 2 – 4 hours [4] more than 4 hours

7. Over the past 7 days, how often did you exercise specifically to increase muscle strength and endurance such as lifting weights or pushups etc?

[0] never → Go to Q.7

[1] seldom (1-2 days)

[2] sometimes (3-4 days)

[4] often (5-7 days)

5a. What were these activities?

_____

5b. On average, how many hours per day did you engage in exercise to increase muscle strength/endurance on these days?

[1] less than 1 hour [2] 1 but less than 2 hours

[3] 2 – 4 hours [4] more than 4 hours

HOUSEHOLD ACTIVITIES

8. During the past 7 days, have you done any light housework such as dusting or washing dishes?
[1] No [2] Yes

9. During the past 7 days, have you done any heavy housework or chores such as vacuuming, scrubbing floors, washing windows or carrying wood?
[1] No [2] Yes

10. During the past 7 days, did you engage in any of the following activities?

	No	Yes
a. Home repairs like painting, wallpapering, electrical, etc.	0	1
b. Lawn work or yard care including snow or leaf removal, wood chopping, etc.	0	1
c. Outdoor gardening	0	1
d. Caring for another person such as a dependent child, dependent spouse or another adult	0	1

WORK-RELATED ACTIVITIES

11. During the past 7 days, did you work for pay or as a volunteer?
[1] No [2] Yes

11a. How many hours per week did you work for pay and/or as a volunteer? _____hours

11b. Which of the following categories best describes the amount of physical activity required on your job and/or volunteer work?

(1) Mainly sitting with light arm movements (eg. Office work, watch maker, seated assembly line worker, bus driver, etc.)

(2) Sitting or standing with some walking (eg. Cashier, general office worker, light tool and machinery worker)

(3) Walking with some handling of materials generally weighing less than 50 pounds (eg. Mailman, waitress, construction worker, heavy tool and machinery worker)

(4) Walking and heavy manual work often requiring handling of materials weighing over 50 pounds (eg. Lumberjack, stone mason, farm or general laborer).

PASE Score

PASE Activity	Score	PASE weight	PASE score
Muscle strength/endurance*	h/d	30	
Strenuous sports*	h/d	23	
Moderate sports*	h/d	23	
Light sports*	h/d	21	
Job involving standing/walking*	h/d	21	
Walking*	h/d	20	
Lawn work or yard care		36	
Caring for another person		35	
Home repairs		30	
Heavy housework		25	
Light housework		25	
Outdoor Gardening		20	
PASE Total			

* Determine the average number of hours/day (h/d) over the 7-day period
1 = engaged in activity during the previous 7 days
0 = did not engage in activity during the previous 7 days

Paffenbarger Score

	Blocks walked	X	8 kcal / block	=	kcal
	Flights climbed	X	4 kcal / flight	=	kcal
	Minutes light sport/recreation	X	5 kcal / min	=	kcal
	Minutes moderate sport/ recreation or muscle strength	X	7.5 kcal / min	=	kcal
	Minutes heavy sport/recreation	X	10 kcal / min	=	kcal
				Total	**kcal/week**