2. What Will Happen To You While You Are In This Research Study?

If you agree to be in the study, you will be asked to participate in the following:

To participate in this study, you should not have known cardiovascular or pulmonary diseases, hypertension, diabetes or an orthopedic limitation keeping you from walking at a fast speed. You should be a non-smoker and have a body mass index (an index of your weight according to your height) below 36. Other restrictions for this study are, 1) no involvement in any other research studies while involved in the present study, 2) not starting any new medications, 3) avoid heavy exercise on your study dates and 4) your primary exercise should be that which is prescribed as a part of this study. You will also be asked to come to some of the study visits fasting (visit 1 and 5 only), which means you are to eat nothing after midnight before your study visit.

Visit 1: During your 1st visit, we will measure your height, weight, body fat, your maximal walking speed, the ability of your body to use oxygen, body balance, and we will do a blood draw. For women of child bearing potential, this blood sample will also be analyzed to ensure that you are not pregnant. You will have 2 tablespoons of blood drawn to measure your hemoglobin levels (the oxygen-carrying portion of your blood), lipids (fat in your blood), and your fasting glucose (the amount of sugar in your blood). We will measure your blood pressure and if it is high, you will be encouraged to see your doctor and you will not be able to participate in the study. Peak walking speed and body balance will be measured using a device called the JD-Mate that is like a watch placed on your waist. This device will also have a sensor that is attached to your right ear lobe to measure heart rate and the amount of oxygen in your blood.

Part of this study will be the collection of genetic material. Because the genetic tests in this study are not used for regular medical care, you will not be told the results of the test(s). The test results will not be put in your medical record either.

You will be asked to do an interval exercise program. You will do 5 sets or more of low intensity walking at 40 percent of your maximal exercise capacity for 3 minutes. You will also do 5 sets or more of high intensity walking greater than 70 percent of your maximal exercise for 3 minutes. You will do 3 minutes at 40 percent followed by 3 minutes at 70 percent. You will continue with this pattern until all 10 sets are completed. This will take 30 minutes.

Training Period: You will continue to do this interval exercise program 4 or more times per week at home or in the Dan Abraham Healthy Living Center (DAHLC) using the JD-Mate device and return for your second visit in 2 months.

Visit 2 to 4: You will be asked to visit the DAHLC every 2 weeks so that data from your tracking device are transferred to a computer (2, 4 and 6 weeks into the training

program). You will then receive instruction by a trainer in the DAHLC to make sure that you achieved a good training intensity.

Visit 5: This will be a repeat of all the measures of visit 1.

3. How Long Will You Be in This Research Study?

You will be in the study for 2 months.

4. Why You Might Want To Take Part In This Research Study

This study is designed primarily for research purposes, although the investigators believe and previous research has shown that exercise training like that proposed in this study will help decrease the likelihood of age associated disability and disease, promote independence and enhance quality of life.

5. What Are the Risks Of This Research Study?

Exercise: The risks from exercise include leg pain and fatigue (feeling tired), shortness of breath and general tiredness. Rarely, there is the risk of abnormal heartbeats, severe shortness of breath and heart attack or stroke. In large groups of patients checked by clinical exercise testing, there is a very small risk of death, of heart attack, and of complications requiring hospitalization. These risks generally increase with age.

Blood draw: The risks of drawing blood include pain, bruising, or rarely, infection at the site of the needle stick.

Genetic Testing: This study involves testing the genes you inherited from your parents (also known as genetic testing). If a researcher finds that results obtained from the genetic testing performed on your samples may be useful for your health care, you will be contacted and given the choice to learn the test results. At this time, you will be given general information on the potential risks, benefits, and costs of choosing to learn the test results. The risks of learning genetic test results may include emotional upset, insurance or job discrimination, and/or family conflicts from learning unknown information about your parents or blood relatives. No test results will be put into your medical record unless you choose to learn the results of the testing. Sometimes results should be released only through a genetic counselor who can help explain the possible risks and benefits of learning this information.

Pregnancy and Birth Control:

1) Will women of child-bearing-potential (able to become pregnant) be allowed to participate in this study?
Yes: Women of child-bearing-potential will be able to participate in this study because the risk to an unborn child appears to be very small

2) Will pregnant, and/or nursing women be allowed to participate in this study?
No: There is not enough medical information to know what the risks might be to an unborn child carried by a woman who takes part in this study.

3) Do you need to have a pregnancy test done to be part of the study?
Yes: As part of this study a pregnancy test is required for all women who are able to become pregnant.

A blood pregnancy test will be done by taking blood from your arm.

You will be told the results of the pregnancy test. If the pregnancy test is positive, you will not be able to take part in the study.

4) Will men who are able to father a child be allowed to participate in this study?
Yes: Men who are able to father a child are allowed to take part in this study.

5) What types of birth control are acceptable?
Surgical sterilization
Approved hormonal contraceptives (such as birth control pills, Depo-Provera)
Barrier methods (such as a condom or diaphragm) used with a spermicide
An intrauterine device (IUD)

Risk summary
The risks of this research study are minimal, which means that we do not believe that they will be any different than what you would experience at a routine clinical visit or during your daily life.

6. What Other Choices Do You Have If You Don't Take Part In This Research Study?

This study is only being done to gather information. You may choose not to take part in this study.

7. Are There Reasons You Might Leave This Research Study Early?

Taking part in this research study is your decision. You may decide to stop at any time. You should tell the researcher if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the researchers may stop you from taking part in this study at any time:

- if it is in your best interest,
- if you do not follow the study rules,
- if the study is stopped.

8. Will You Need To Pay For Any Of The Tests And Procedures?

You will not need to pay for tests and procedures which are done just for this research study. These tests and procedures are:

-blood tests
-body composition testing

If you have study related questions regarding billing, insurance or reimbursement, stop by or call:
Rochester: Admission and Business Services office, or call Patient Account Services at (507) 287-1819

9. Will You Be Paid For Participating In This Research Study?

You will have free DAHLC membership during the study and for one more month after the study, which represents a monetary value of 75$.

10. What Happens If You Are Injured Or Ill Because You Were In This Research Study?

If you have side effects from taking part in this study, you need to report them to the researcher and your regular physician, and you will be treated as needed. Mayo will give medical services for treatment for any bad side effects from taking part in this study. Such services will be free if not covered by a health plan or insurance. No additional money will be offered.

11. What Are Your Rights If You Are In This Research Study?

Taking part in this research study will not change your rights and benefits. Taking part in this research study does not give you any special privileges. If you decide to not participate in this study, or stop in the middle of the study, no benefits are taken away from you. Specifically, you do not have to be in this research study to receive or continue to receive medical care from Mayo Clinic.

You will be told of important new findings or any changes in the study or procedures that may affect you or your willingness to continue in the study.

12. What About Your Privacy?

Authorization To Use And Disclose Protected Health Information

Your privacy is important to us, and we want to protect it as much as possible. By signing this form, you authorize Mayo Clinic and the investigators to use and disclose any information created or collected in the course of your participation in this research protocol. This information might be in different places, including your original medical record, but we will only disclose information that is related to this research protocol for the purposes listed below.
This information will be given out for the proper monitoring of the study, checking the accuracy of study data, analyzing the study data, and other purposes necessary for the proper conduct and reporting of this study. If some of the information is reported in published medical journals or scientific discussions, it will be done in a way that does not directly identify you.

Information Disclosed to Study Sponsor
The study data sent by the study doctor to the sponsor does not include your name, address, social security number, or other information that directly identifies you. Instead, the study doctor assigns a code number to the study data and may use your initials. Some study data sent to the sponsor may contain information that could be used (perhaps in combination with other information) to identify you (e.g., date of birth). If you have questions about the specific health information that will be sent to the sponsor, you should ask the study doctor.

This information may be given to other researchers in this study, including those at other institutions, representatives of the company sponsoring the study, including representatives in the USA or other countries, or private, state or federal government parties or regulatory authorities in the USA and other countries responsible for overseeing this research. These may include the Food and Drug Administration, the Office for Human Research Protections, or other offices within the Department of Health and Human Services, and the Mayo Clinic Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

This authorization lasts "forever".

You may stop this authorization at any time by writing to the following address:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

If you stop authorization, Mayo may continue to use your information already collected as part of this study, but will not collect any new information.

13. What Will Happen to Your Samples?

Your samples will be used and stored as described for this study.

Mayo has the right to end storage of the sample without telling you

Your sample of blood will be kept at Mayo for use in this study. Researchers at Mayo who are not involved with this study may ask to use your sample for more research. You have a say in how your stored sample is used in future research. You can still take part in the data collecting study without giving your sample for future use.

Exceptions when your samples may be used without your permission:

1) When government rules allow your sample to be used without identifying you, even with a code.
2) When use of the sample is not considered human subject research.

At all other times:
-You can let Mayo use your sample.
-You can say NO to have your sample used by Mayo.

Identification information:
If you agree to allow your sample to be used for further research, the sample may be stored forever. The sample will be stored at Mayo and would be given a code (instead of your name) while it is stored and when it is used in research. This code allows your sample to be used without anyone knowing that it is your sample just by looking at the label.

There is a very small chance that some commercial value may result from the use of your donated sample. If that happens, you will not be offered a share in any profits.

Risks:
Some future studies may be for testing the genes you inherited from your parents (also known as genetic testing). If a researcher finds that future test results may be useful for your health care, you will be contacted and given the choice to learn the test results. At that time, you will be given general information on the potential risks, benefits, and costs of choosing to learn the test results. The risks of learning genetic test results may include emotional upset, insurance or job discrimination, and/or family conflicts from learning unknown information about your parents or blood relatives. Test results will only be put into your medical record if you chose to learn the results. Sometimes results should be released only through a genetic counselor, who can help explain the possible risks and benefits of learning the results.

Please read the following statements and mark your choice:

1. I permit my sample to be stored and used in future research of exercise and physical fitness is lifestyle associated diseases in middle-aged and older people at Mayo:

☐ Yes ☐ No Please initial here: _____Date: _____

2. I permit my sample to be stored and used in future research at Mayo to learn about, prevent, or treat any other health problems:

☐ Yes ☐ No Please initial here: _____Date: _____

Who will use your sample?
If you agree to give your sample, it will be the property of Mayo and may be used for research by Dr. Bruce Johnson and other staff at Mayo Clinic. Researchers at other institutions may also ask for a part of your sample for future studies.

How do researchers from other institutions get the sample?
Researchers from universities, hospitals, and other health organizations conduct research using blood. They may contact Mayo and request samples for their studies. If you approve release of your sample by checking 'Yes' below, Mayo may send the blood sample(s) and some information about you to researchers who request them, but Mayo will not send your name, address, phone number, social security number, or any other identifying information with the sample. If you allow your sample to be given to researchers at other institutions, it will be given to them with a code number rather than your name. If these researchers use the sample for future research and decide that a test result may be useful for your health care, they may contact the Mayo Clinic and Mayo would then contact you to offer you the choice to learn the test results.

I permit Mayo to give my sample to researchers at other institutions:
Please mark one box:

☐ Yes ☐ No Please initial here: _____Date: _____

Mayo has the right to end storage of the sample without telling you.

If you want your sample destroyed at any time, write to:

Dr. Bruce Johnson
Cardiovascular Diseases
Gonda 5-368
200 First Street SW
Rochester, MN 55905

If you move please send your new address to

Mayo Clinic Rochester
Section of Registration
200 First Street Southwest
Rochester, MN 55905

14. What Is The Institutional Review Board (IRB) And How Does It Protect You?

The Mayo Clinic IRB is made up of:
- Physicians and Scientists
- IRB Specialists
- Allied Health Employees
- Local Community Members
- Visitors (Lawyers, Compliance, Administration, and others)

The IRB reviews human research studies. It protects the rights and welfare of the people taking part in those studies. You may contact the IRB if you have questions about your rights as a participant or if you think you have been treated unfairly.

15. Who Can Answer Your Questions?

You can call …	At …	If you have questions or concerns about …
Principal Investigator: Dr. Bruce Johnson, PhD **Other Study Contact:** Minelle Hulsebus (study coordinator)	**Phone:** **(507) 284-4375** **(507) 538-9278**	**Questions about the study tests and procedures** **Research-related injuries or emergencies** **Any research-related concerns or complaints**
IRB Administrator: **Marcia Andresen-Reid**	**Phone:** **507-266-4000** **Toll-Free:** **866-273-4681**	**Rights of a research subject** **Use of protected health information** **Any research-related concerns or complaints**
Research Billing	**Rochester:** **507-287-1819** **Jacksonville:**	**Billing / Insurance Questions**

	904-953-7058 **Arizona:** **480-301-8000**	

16. Summary and Enrollment Signatures

You have been asked to take part in a clinical trial, also called a research study, at Mayo Clinic. The information about this study has been provided to you to inform you about the nature of this IRB approved study.

- I have read the whole consent form, and all of my questions have been answered to my satisfaction.
- I know that joining the study is voluntary and I agree to join the study.
- I know enough about the purpose, methods, risks, and possible benefits of the study to decide that I want to join.
- I know that I can call the investigator and research staff at any time with any new questions or to tell them about side effects.
- I understand that a copy of this form will be put in my medical records and that I will be given a copy of this completed form.
- I understand that I may withdraw from the study at any time.

Please sign and date to show that you have read and understand all of the above guidelines. Please do not sign unless you have read the entire packet of information. If you do not want to sign, you don't have to, but if you don't you cannot participate in this research study.

(Date/ Time)	(Printed Name of Participant)	(Clinic Number)
	(Signature of Participant)	
(Date / Time)	(Printed Name of Individual Obtaining Consent)	
	(Signature of Individual Obtaining Consent)	

（資料2） Yale 大学 医倫理委員会(IRB)申請書

HIC#_____

YALE UNIVERSITY SCHOOL OF MEDICINE
YALE NEW HAVEN HOSPITAL
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Research

Title of Research Project: Effects of interval walking on physical fitness and life-style associated diseases in middle-aged and older people. (Pilot Study)			
Principal Investigator: Loretta Di Pietro, PhD, MPH		**Yale Academic Appointment:** Associate Professor - EPH	
Campus Address: John B. Pierce Laboratory, 290 Congress Avenue, New Haven, CT 06519			
Campus Phone: 562.9901 x203	**Fax:** 624.4950	**Pager:**	**E-mail:** ldipietro@jbpierce.org
Protocol Correspondent Name & Address: Same			
Campus Phone:	**Fax:**	**E-mail:**	

SECTION I: PRINCIPAL INVESTIGATOR/FACULTY ADVISOR AGREEMENT

As the Principal Investigator or Faculty Advisor of this research project, I certify the following:

- The information provided in this application is complete and accurate.
- That I assume full responsibility for the protection of human subjects and the proper conduct of the research.
- That subject safety will be of paramount concern, and every effort will be made to protect subjects' rights and welfare.
- That the research will be performed according to ethical principles and in compliance will all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- That all members of the research team will be kept apprised of research goals.
- That I will obtain approval for this research study and any subsequent revisions prior to initiation.
- That I will report to the HIC any serious injuries or other unanticipated problems involving risk to participants.

_____

Signature Date

SECTION II: FUNDING, TRAINING AND PROTOCOL-RELATED CONFLICT OF INTEREST

1. **Funding Source:** Please describe the funding source(s) for this study. Check all boxes that apply:

   ☒External* ☐Department Name ☐Investigator Initiated: _____ ☐Other: _____

   *For externally funded studies, please supply the following information:

   - PI of Contract or Grant: **Loretta DiPietro, PhD**
   - Funding Source: **Shinshu University Graduate School of Medicine**
   - Contract or Grant Title: **Effects of interval walking on physical fitness and life-style associated diseases in middle-aged and older people.**
   - Contract or Grant #: **TBA**

2. **Human Subject Protection Training:** All investigators and study personnel (persons involved in the design and/or conduct of research involving human subjects) are required to complete human subject protection training (HSPT). This training requirement can be met through the Yale web-based program at http://info.med.yale.edu/irbtraining/ or the NIH program at http://www.cancer.gov/clinicaltrials/learning/page3. Please note that investigators who have not completed this training requirement cannot participate in study activities until this training is completed.

3. **Conflict of Interest Statement:** All investigators and study personnel (those persons involved in the design and/or conduct of the research involving human subjects) are required to read a copy of the Yale Human Investigation Committee Policy on Protocol-Related Conflict of Interest ("HIC COI Policy" – see http://info.med.yale.edu/hic/policy/index.html). ***Please note that the HIC COI Policy addresses protocol-related conflict of interest, and is distinct from the annual disclosure required by the Yale University Policy on Conflict of Interest and Conflict of Commitment.***

   All investigators and study personnel are required to sign their name in the space provided below. Those who have answered "no" to all screening questions asked in the HIC COI Policy should indicate below that no Protocol-Related COI exists. Those who answered "yes" to any question in the HIC COI Policy should download a copy of the Protocol-Related Conflict of Interest Disclosure Form, which must be submitted to the HIC along with this Application.

Indicate under Affiliation whether the Investigator or Study personnel are part of the Yale Faculty or staff or part of the faculty or staff of a collaborating institution.

	Name	Signature ***	Protocol-Related COI?	HSPT Completed?	Affiliation
Principal Investigator	Loretta DiPietro, PhD		Yes X No	☒ Yes ☐ No	John B. Pierce Laboratory
Co-Investigator(s)			☐ Yes ☐ No	☐ Yes ☐ No	
			☐ Yes ☐ No	☐ Yes ☐ No	
			☐ Yes ☐ No	☐ Yes ☐ No	
Study Personnel	Andrew Grabarek, BS		Yes X No	☒ Yes ☐ No	John B. Pierce Laboratory
	Vahid Mohsenin, MD		Yes X No	X Yes ☐ No	John B. Pierce Laboratory
	Fred Urbano, MD		☐ Yes X No	X Yes ☐ No	John B. Pierce Laboratory

***** My signature here indicates that I have read and am in compliance and will continue to be with the HIC's Protocol-Specific Conflict of Interest Policy.**

4. **Department Chair's Conflict of Interest Statement (to be completed by the Chair of *each department* with which the Principal Investigator and co-investigators are affiliated and/or which the research affects):** Do you know of any real or apparent institutional conflict of interest (e.g., Yale University ownership of a sponsoring company) that might compromise this research?

   Yes ☐ No ☐

_____ _____

*Signature of Chair**** *Department*

***My signature here indicates that I have read and am in compliance with the HIC's Protocol-Related Conflict of Interest Policy. I further agree to submit a Protocol-Related Conflict of Interest Disclosure Form if I am aware of any real or apparent institutional conflict of interest.

HIC#_____

APPROVED FOR SUBMISSION TO HIC:

_____	_____	_____
Signature of Primary Reviewer	*Please Print Name of Primary Reviewer*	*Date*

For HIC Use Only

_____	_____
Date Approved	**Human Investigation Committee**

Protocol is valid until: _____

SECTION III: GENERAL INFORMATION

1. **Choose all that apply:** *(* See indicated section in HIC Guidelines for Investigators)*

☐ Children/minors* (Section E.1)
☐ Decisionally impaired* (Section E.2)
☐ Females of childbearing potential
☐ Radioactive Materials
☐ IND # _____
☐ IDE # _____A or B (Section C.1)
☐ Pregnant women/fetuses/placenta
☐ Prisoners
☐ Non-English Speaking
x☐ Use of Employees
☐ Students

2. **Location of study:** Please identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research.

☐ Yale University ☐ Yale-New Haven Hospital ☐ APT Foundation, Inc. ☐ West Haven VA
☐ Community Consultation Board, Inc. ☐ Haskins Laboratories ☒ John B. Pierce Laboratory, Inc.
☐ Connecticut Mental Health Center ☐ Hill Health Corporation ☐ Comprehensive Cancer Center
☒ Yale Center for Clinical Investigation ☐ MR-TAC ☐ Other locations, **Specify:** _____

Please indicate the location(s) within the hospital and/or Medical School where the research will take place:
____Hospital research Unit (HRU), JBP_____

Please note: when other institutions are engaged in the research, it may be necessary to secure the approval of their Institutional Review Boards (IRB) and/or to insure that the institution has obtained a Federal Wide Assurance (FWA). Institutions may not list the Yale HIC as their IRB of record unless the Federal government has approved their FWA and they have in place a fully executed IRB Authorization Agreement between their institution and Yale University.

3. **Probable Duration of Project**: Please state the expected duration of the project, including all follow-up and data analysis activities. *[Please answer this and all other questions on the form with font size 12.]*

One year

4. **Number of Subjects:** Please state the number of subjects to be enrolled at Yale. For multi-center studies, indicate the total number of subjects to be enrolled across all sites. If different subject populations will participate, state the anticipated number in each group.

10 men and 10 women, ages 50-70

SECTION IV: RESEARCH PLAN

1. **Statement of Purpose:** What are the scientific aims of the study, or the hypotheses to be tested?

The purpose of this *pilot study* is to test the health effectiveness of a higher-intensity interval walking program in middle-aged and older people (50-70 y). Specific aims are:

1. *To determine the feasibility of a tri-axial accelerometer for use in training for middle- aged and older men and women.*

A tri-axial accelerometer (JD-mate) with the capacity to guide the user to correct exercise intensities while walking has been developed and tested in Japan. Although this device has been used successfully among Japanese adults, it has not been tested in people living in other countries.

We hypothesize that at least 80% of middle- and older-aged adults instructed in the use of the JD-mate will use it successfully over a period of four weeks.

2. *To determine the feasibility of a short-term higher-intensity interval walking program, guided by the JD-mate, for middle- and older-age men and women.*

A recently-published paper among Japanese people (5) reports very good (>75%) *retention* over 5 months to a walking program comprising 5 or more sets of interval walking [3-min at 40% VO_{2peak}/3-min at 70% VO_{2peak}] on 4 or more days/week [120 min or more per week]. *Adherence* to the training protocol (≥120 min/week of the interval walking) over the entire study period was approximately 50%. We propose that middle-aged and older people living in Connecticut can achieve >95% retention and >90% adherence to the same training protocol over a *shorter* study (pilot test period) lasting 4 weeks.

3. *To determine the effects of a 4-week interval training program for walking on specific health variables in this same population.*

Although moderately paced (~6 km/hour) walking has been broadly recommended for middle-aged and older people to maintain health, this intensity may not be adequate to *increase* specific aspects of physical fitness such as maximal aerobic capacity (VO_{2max}) or quadriceps strength (two primary factors in the age-associated decline of physical function). We hypothesize that 4 weeks of interval walking training will significantly improve a number of factors associated with health and function through middle-age and beyond – namely, body composition (BMI, % body fat), peak walking velocity, body balance, VO_{2peak}, blood pressure, and plasma concentrations of glucose, C-reactive protein (CRP), lipids (total-, HDL-, and LDL-cholesterol and triglycerides), and self-reported depression and health condition scores.

2. Background: Describe the background information that led to the plan for this project. Please provide references to support the expectation of obtaining useful scientific data. When available, previous work in animal and/or human studies should be included.

With an ever-increasing elderly population in the United States and throughout the world, exercise is becoming increasingly important for the maintenance of a healthy and independent life. For most middle-aged and older segments of the population, walking is a very common method of exercise. Although regular walking is an effective strategy for health maintenance (especially with regard to body weight maintenance (1,2)), the intensity of the walking must be considered in order to achieve improvements in health and physical fitness – especially after possibly several decades of disuse. Indeed, walking at the recommended "moderate" pace (~60% VO_{2peak}) may be short of the intensity required to produce significant gains in muscle strength and aerobic capacity.

Higher walking intensities (~70-85% VO_{2peak}) have recently been shown effective in older people (3,4), and this may be necessary to producing the intended long-term benefits of exercise for this particular segment of the population. Nemoto and colleagues (5) recently reported significant gains in thigh muscle strength (17%; $p<0.001$) and VO_{2peak} (8%; $p<0.001$) and reductions in blood pressure ($p<0.01$) in middle- and older-age Japanese adults performing interval walking training. Moreover, these improvements were significantly greater than those observed among adults performing sustained moderate-intensity walking. We (3) and others (4) have also demonstrated the benefits of higher-, relative to moderate-intensity walking for long-term

improvements in insulin sensitivity and other cardiovascular risk factors in previously untrained older people.

Unfortunately, higher-intensity aerobic activity is often difficult to recommend in untrained middle-aged and older people due, in part, to the risk of injury or cardiovascular events resulting from this type of unaccustomed exercise. Interval training has distinct advantages over a constant-intensity training regime, however, as short bursts of high-intensity activity are separated by periods of lower- or moderate-intensity to allow for "recovery" of several physiologic systems. *Repetition of these intervals over weeks and months will no doubt result in shorter required recovery times, indicating successful adaptation to the higher-intensity challenge*. Thus, the cardiorespiratory and metabolic "value" of a 30-min period of exercise increases markedly with interval, compared with constant-intensity aerobic training.

In most field studies of exercise training, heart rate (HR) and rate of perceived exertion (RPE) have been used to monitor relative exercise intensity. However, trainers using HR and RPE methods might find it difficult to instruct participants to perform higher-intensity interval walking because they would not be able to determine absolute walking intensity to a high resolution of one minute. For example, after a change in walking speed it takes 1 to 2 minutes for HR to reach a steady-state equivalent to the new level of exercise intensity. Moreover, HR and RPE responses are influenced by adaptation to training, environmental conditions, and the physical condition of participants. For these reasons, the JD-mate triaxial accelerometer (5) is highly recommended to monitor exercise intensity during interval training.

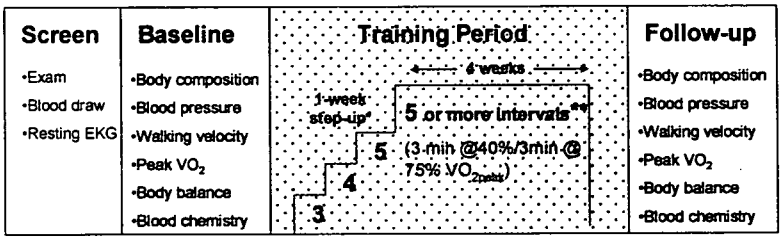
2. **Research Plan:** Please provide an orderly scientific description of the study design and research procedures as they directly affect the subjects.

2.1 Study subjects

Middle-aged and older volunteers (10 men/10 women; 50-70 years) will be recruited from the general New Haven area, as well as from the University community. Potential subjects will be non-smokers, without Class I obesity (BMI < 30 kg/m^2), and free of any untreated chronic diseases. In addition, eligible subjects will be free of anti-hypertensive medication or any glucose or lipid-lowering agents. Female subjects will be free of oral contraceptive use or hormone replacement therapy. All interested volunteers will undergo an initial screening at the Yale Center for Clinical Investigation (YCCI): Hospital Research Unit (HRU) at Yale University-New Haven Hospital, which will include an examination by a physician associated with the HRU, a fasting blood sample (a CBC with automated differential to rule out intercurrent infection, and a basic metabolic panel), and a resting EKG in order to identify health issues contraindicative to exercise. The EKG will be read by the physician co-investigator listed on the protocol. (Vahid Mohsenin, MD). In addition, volunteers will be administered the Physical Activity Readiness Questionnaire (PAR-Q) to assess overall cardiovascular and musculoskeletal symptoms that may contraindicate their ability to exercise (see Section 2.3.2 below). All interested study subjects will provide informed written consent prior to the collection of any screening or baseline data.

2.2 Overall study protocol (Figure 1)

Figure 1. – Overall and training protocol.



*** During the 1-week step-up period, subjects will exercise on 3 days and will increase the number of intervals performed on those days from 3 to 5. **5 or more intervals per session on 4 days per week (or 120 min or more per week).**

2.2.1 *Baseline.* On the day of baseline testing, eligible study subjects will report to the HRU at approximately 8:00 AM following an over night fast. Height, weight, and overall body composition will first be measured (see Methods below). A small blood sample (15 cc) then will be obtained for the determination of plasma glucose, insulin, HbA1c, total-, LDL-, and HDL-cholesterol, triglyceride and C-reactive protein (CRP) concentrations.

2.2.1.a Single Nucleotide Polymorphisms (SNPs) - An additional 5 mL of blood will be drawn and processed immediately for the study of various single nucleotide polymorphisms (SNPs) associated with hyperglycemia and dislipidemia and their ability to be altered by interval walking exercise. Processed bloods for the SNPs analysis will be stored frozen for the duration of the study (~one year) and then *de-identified* samples shipped to Shinshu University Graduate School of Medicine for analysis. The total amount of blood drawn for the baseline testing is 20 mL.

In addition, seated systolic and diastolic blood pressure and resting heart rate will be measured and recorded. Following these measurements, subjects will be fed breakfast and will sit quietly for one hour. Next, peak walking velocity and peak aerobic capacity will be determined according to the methods described below. Also, subjects will answer a health interview and two questioners; Center for Epidemiologic Studies depression scale (CES-D), and Questioner about current health condition and pain (see Section 2.3.4 and 2.3.5 below). Subjects will then be instructed in the use of the JD-mate. They will also have the exercise training protocol explained to them in detail before leaving the HRU.

2.2.2 *Exercise training protocol (Figure 1).* Subjects will perform the exercise on 4 or more days per week over the course of the 4-week study. The interval walking protocol comprises 5 or more sets of lower- intensity walking (~40% of the pre-training VO_{2peak}) for 3 minutes followed by higher-

intensity walking (~70 -85% VO_{2peak}) for 3 minutes for a total of 30 or more min per session (120 or more min per week). Prior to the start of the actual 4-week training period, subjects will undergo a one-week *supervised* step-up period, during which they will gradually increase the number of walking intervals performed from 3 to 5 per session as the exercise trainer guides them in the proper techniques. The intensity and number of steps performed each session will be monitored with the JD-mate. The subject is alerted by a signal when a change of walking intensity is required. Following the step-up period, subjects will perform the formal 4-week exercise program *on their own during a self-chosen time each day.* Walking can be performed outdoors on a track or sidewalk or indoors on a track or treadmill.

Intensity of exercise is monitored by the JD-mate by acceleration and by heart rate and this information is stored via a micro-processor chip. Every week the subjects are expected to visit the John B. Pierce Laboratory so that data from the tracking device can be transferred to a central server computer via the internet. The data are instantly analyzed and subjects then receive instruction by a trainer as to whether their target levels of intensity have been achieved. Using this approach to subjects, the trainer judge whether the targets are met, and if not, subjects are encouraged to increase their effort to achieve it.

2.2.3 *Follow-up testing.* After 4 weeks of training and within 48 h of the last exercise session, subjects will return to the HRU for follow-up testing. Procedures for obtaining these follow-up data will be performed by the same investigator and will be identical to those performed at baseline.

2.3 Instruments

2.3.1 *The JD-mate* (Kissei-Comtec, Matsumoto, JAPAN) is worn on the waist and monitors acceleration (ambulation) in three directions (forward/back; right/left; up/down). When combined with its ability to measure heart rate, this device provides a valid measure of absolute exercise intensity (6). The JD-mate has a built-in timer and thus can then alert the subject when to change walking intensity by a beeping signal. At weekly intervals, data from each accelerometer are downloaded to a central server computer in the Pierce Laboratory through the internet. The data are instantly analyzed and subjects then receive instruction by a trainer as to whether their target levels of intensity have been achieved. Using this approach to subjects, the trainer judge whether the targets are met, and if not, subjects are encouraged to increase their effort to achieve it.

2.3.2 *The Physical Activity Readiness Questionnaire (PAR-Q).* The PAR-Q is a commonly-used questionnaire designed to assess participant readiness and safety prior to starting an exercise program. The questionnaire comprises 7 questions pertaining to physical symptoms associated with overall cardiovascular and musculoskeletal function. An affirmative answer to one or more questions would exclude a volunteer from the study.

2.3.3 *Exercise Self-Efficacy Survey.* This survey assesses a participant's feelings of confidence in performing the exercise protocol under various conditions (bad weather, when tired, on vacation, when anxious, etc). Subjects are asked to provide a confidence rating for each of 15 questions, with ratings of confidence ranging from 0% (not at all confident) to 100% (absolutely confident).

2.3.4 *Center for Epidemiologic Studies depression scale (CES-D).* The CES-D is a widely used questioner for screening for depression of subjects. Subjects are asked to indicate how often they have felt or behaved this way during the past week for each of 20 items by checking the appropriate answer;

Rarely or none of the time (less than 1 day), Some or a little of the time (1-2 days), Occasionally or a moderate amount of time (3-4 days), Most or all of tie time (5-7 days).

2.3.5 *Questioner about current health condition and pain.* This questioner assesses a participant's feeling of severity and frequency of pain in legs, lumber, chest, neck, and shoulders, and palpitation and dizzy. Subjects are asked to provide a rating for each of 6 items for severity with 1 (Not at all) to 4 (Severe), and for each of 6 times for frequency with 1 (< 1 day), 2 (1-2 days), 3 (3-4 days), 4 (5 days ≤).

2.4 Physiologic outcome measures

2.4.1 *Body composition.* Height will be measured on a stadiometer and weight measured on a digital scale. Percent body fat will be estimated using skinfold measurements taken by the same investigator (LDP) before and after training at six sites (triceps, subscapula, chest, umbilicus, iliac, and anterior thigh) according to methods described by the American College of Sports Medicine (7). Body fat will also be estimated by bio-impedance absorptiometry using a Tanita scale.

2.4.2 *Blood pressure.* Systolic (SBP) and diastolic (DBP) blood pressures will be measured by auscultation after a 10-minute seated rest, prior to the measurements of peak aerobic capacity.

2.4.3 *Peak walking velocity.* Peak walking velocity at the distance of 25 meter will be measured using the JD-mate in a basement hospital hallway. The device measures vertical displacement as the ratio of vertical to total acceleration counts, with higher vertical acceleration for a given walking velocity indicative of lower walking efficiency (6). There is evidence to confirm that peak walking velocity as measured by the JD-mate is significantly correlated with knee extension force in middle-age and older people (5). All walking will take place at Yale-New Haven Hospital or the John B. Pierce Laboratories. Subjects will be accompanied by the Principal Investigator and her research associate.

2.4.4 *Peak aerobic capacity by graded walking.* Subjects will begin walking with the JD-mate on their waist on a flat surface (basement hospital hallway) at three graded self-paced velocities (slow, moderate, and fastest) for 3 min at each velocity. Three-dimensional acceleration will be measured by the JD-mate, while HR is measured with the sensor placed on the right ear lobe by the infra-red method at 10-millisecond intervals. Heart rate will be recorded every 5-seconds as averaged values. The total impulse from the accelerometer is transferred to a computer and converted to oxygen consumption rate by using an equation reported previously (6). Peak aerobic capacity and peak HR for walking will be the values obtained for the last minute at maximal walking velocity. All walking will take place at Yale-New Haven Hospital or the John B. Pierce Laboratories. Subjects will be accompanied by the Principal Investigator and her research associate

2.4.5 *Body balance.* Body balance is estimated by integrating the forward-back and right-left accelerations during up-right standing at rest for 2 min using the JD-mate. Lower acceleration counts indicate better balance (a proxy indicator of leg strength).

2.4.6 *Blood chemistry.* All blood samples will be placed in pre-chilled test tubes. Blood for the analyses of DNA sequencing variation (SNPs analysis) will be processed according to standard procures and frozen. Upon completion of the study, these de-identified samples will be shipped to Shinshu University Graduate School of Medicine for analysis. All other samples will be centrifuged at 4° C and

the plasma stored at -70° C until analyzed in the Core Laboratory of the YCCI. Plasma glucose concentrations will be analyzed using the glucose oxidase method (YSI 2300; Yellow Springs Instruments Co., Yellow Springs, OH). Insulins will be analyzed by double antibody RIA (HI-14K) (Millipore, St.Charles, MO). Plasma cholesterol and triglyceride concentrations will be determined by standard microflourimetric procedures (Sigma, St. Louis, MO). Measures of interest are total-, high-density lipoprotein (HDL)- and LDL-C (LDL=total-C-(TGs/5 + HDL-C)). Glycosylated hemoglobin (HbA1c) will be determined using an Ames DCA 2000 analyzer (Miles, Inc., Eikhart, IN) and CRP levels will be measured using the ultrasensitive assay (Kamiya Biomedical).

3. **Statistical Considerations:** Describe the statistical analyses that support the study design. This section should include:

A total of 20 subjects will be study in this *pilot* protocol. Univariate statistics (mean±SD) first will be generated for all physiologic variables (except for SNPs) for men and women separately. Differences in the physiologic variables of interest before and after 4 weeks of training will be determined by the paired t-test, first among the pooled sample and then separately by sex. Although a study sample of 10 men and 10 women will not provide adequate statistical power to test all of the hypotheses, we will be able to obtain measures of effect size with corresponding standard deviations for use in a large-scale RO1 application.

4. References:

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