PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVI and XXXVII cycles)

Name of PhD student: Loredana Rao
Title of PhD research: Sarcopenia, insulin resistance and metabolic syndrome
Name of PhD supervisor: Prof. Andrea Frontini
Research lab name: Laboratory of Morphological and functional Biology, DISVA
Cycle:
XXXVII

PhD Curriculum:
Biomolecular Sciences

DISVA instrumentation labs/infrastructure eventually involved in the project:
[ ] Actea Mobile Laboratory
[X] Advanced Instrumentation lab
[ ] Aquarium
[ ] MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ] Other. Please, indicate: .....

ABSTRACT (1000 characters, including spaces):
Sarcopenic obesity is an aging-related condition characterized by a reduced muscle mass and function and an increased amount of visceral and ectopic adipose tissue. Excess in fat accumulation promotes a series of molecular and metabolic modifications as impairment in mitochondrial fatty acid oxidation, oxidative stress, and recruitment of immune cells with the release of cytokines leading to the development of insulin resistance and type 2 diabetes.
During physical exercise, muscular fibres produce myokines that have both an autocrine effect on muscle itself and endocrine effect on other organs like adipose tissue. The first year of my PhD was dedicated to the improvement of the methodologies involved in the morpho-functional analysis to be then applied to the main core of the project. We aim to better characterised the function of specific myokines (i.e. irisin) in modulating energy homeostasis by acting on muscle or on other tissue such as fat. The experimental settings rely on *in-vitro* system that simulates physical exercise and on *in-vivo* model.

Part 1. Scientific case of the PhD Research (2 to 3 pages, including figures)
BACKGROUND:
Sarcopenia is an aging-related degenerative condition characterized by the gradual loss of skeletal muscle mass and function. It is associated with multiple adverse outcomes such as comorbidities, physical disability, higher risk of falls and increased hospitalization, functional decline, and increased mortality (1).
Alongside the loss of muscle mass, a critical aging-related transformation is the pronounced redistribution of adipose tissue: in fact, a continuing loss of subcutaneous adipose tissue with age is accompanied by increased visceral obesity and accumulation of adipocytes and lipids in different location, such as in liver and in skeletal muscle (myosteatosis) (2).
Notably, obesity in aged individuals stimulates sarcopenia by altering skeletal muscle lipid metabolism, insulin resistance, and inflammatory pathways.
Both intermuscular adipose tissue and intramuscular lipid metabolites can lead to marked impairment in mitochondrial fatty acid oxidation, increased lipolysis, and increased oxidative stress. These events can promote lipotoxicity, insulin resistance, and inflammation in skeletal muscle, resulting in decreased muscle mass and muscle fibre contractility. Excess fat accumulation, especially in central obesity, promotes

inflammatory pathways through adipocyte hypertrophy, hypoxia and cell death (necrosis). The consequent recruitment of immune cells including macrophages, mast cells, and T lymphocytes in the tissue leads to a condition of low-grade chronic inflammation with the production of pro-inflammatory cytokines both by stressed adipocytes and activated immune cells. This is the reason why central (visceral) obesity is more dangerous than peripheral (subcutaneous) fat accumulation. Since all the molecules produced in this detrimental condition are drained directly into the liver by the portal system and then reach and impact on the metabolism of other organs/tissue such as skeletal muscle in which one of the major effects is to reduce insulin sensitivity. Moreover, increased secretion of leptin from adipose tissue upregulates the production of pro-inflammatory cytokines such as tumour necrosis factor (TNF)α and interleukin (IL)-1β. Elevated TNFα directly impairs adiponectin signalling, thus inhibiting mitochondrial biogenesis and myogenesis in human skeletal muscle cells (3).
The combination of all these factors, especially the increase of fat mass and the parallel decrease of muscle mass, leads to the development of a comorbidity call Sarcopenic Obesity (SO), which has worse consequences than sarcopenia or obesity alone.
Between all the possible treatment for SO, physical activity, especially resistance training, seems to be the best option to mitigate the effects of this detrimental process. Besides increasing of muscle hypertrophy and number/quality of muscle fibres, exercise is well known to stimulate mitochondrial oxidative capacity and lipid oxidation, increase number of mitochondria and mitochondrial enzymes activity, also improving insulin sensitivity and glucose tolerance (4). All these physiological and metabolic adaptations induced by exercise are mostly caused by humoral factors secreted within extracellular vesicles, by the muscle fibres during contraction. These vesicles contain many factors and some of these are myokines: the autocrine and paracrine effects of myokines are mostly involved in the regulation of muscle physiology, such as muscle growth, glucose and/or lipid metabolism, which can provide a feedback loop for the muscle to adapt to exercise training. In contrast, the endocrine effect of myokines is important in mediating the whole-body effect of exercise and it's the beneficial effects on the key organ involved in the regulation of energy homeostasis (5).
A fair number of myokines have been already characterized in their secretion pattern and mechanism of action. Some of them are of particular interest because of their endocrine effect on adipose tissue. Among these irisin is particularly appealing as potential candidate to be further investigated in the cross-talk with adipose tissue and in the modulation of energy homeostasis to prevent the establish of unfavourable conditions.
This implies that these molecules secreted during physical activity could act on modulating the molecular and physiological mechanisms that are impaired specific tissues of people with Sarcopenic Obesity.

SCIENTIFIC AIMS:
The aim of this project is to find a way to curb the detrimental process of Sarcopenic Obesity by recurring to lifestyle intervention meaning physical exercise and diet supplementation.
For this purpose, in the first place I will focus on specific myokines secreted from the muscle during physical activity to characterize their mechanisms of secretion, which are still not completely defined, and to study their effects on specific organs, in particular those involved in energy homeostasis, as adipose tissue; it will be also studied the crosstalk between myocytes and adipocytes to define all the possible interactions and the humoral factors secreted from these two cellular types.
To do this, for the next six-eight months, I will work basically on *in-vitro* model, to develop a system to simulate physical exercise on muscular fibres to study the mechanism of secretion of myokines like irisin and their behaviour as autocrine factors. I'm also planning to work on co-cultures of myocytes and adipocytes to evaluate the endocrine effect of myokines on adipose tissue, simulating particular conditions such as insulin resistance, oxidative stress or mitochondrial disfunction classically found in metabolic syndrome.
As a final goal, the project will move on *in-vivo* model to study the effect of myokines on an animal model with sarcopenic obesity (aged trained Vs aged sedentary Vs aged supplemented with irisin).

WORKPLAN AND RESEARCH ACTIVITIES:
-Objectives
The primary goal of the first year of my PhD program was to acquire a morphological and functional approach to complement the knowledge in the molecular field acquired during my Master thesis.

To acquire and standardize most of the techniques that I will perform during the rest of this PhD project, I spent a training period in the Laboratory of Human Morphology and Anatomy in the Department of Clinical and Experimental Sciences, Univpm, where morphological techniques are routinely performed. Then, I was able to apply some of the techniques learned on a specific project already ongoing in the lab and focused on the phenotypical characterization of fat depots in a genetically modify mouse model. These mice have a selective deletion of a neuropeptide that control lipid metabolism and energy homeostasis and it was a useful model to put into practice the methods acquired.
In more detail, I had the possibility to learn how to identify and classify a tissue based on its appearance and intrinsic characteristics, I performed the main methods used to obtain a good quality and long-lasting sample, the microtome cut of paraffine embedded tissue, the main histological staining and immunohistochemistry and immunofluorescence techniques.
Most importantly, I started to approach a qualitative characterization of cells and tissue and how to evaluate and describe qualitative changes in cell and tissue due to different experimental conditions and or/pathologies.
I also started to work with cell cultures (Myoblasts from murine skeletal muscle, C2C12), on which I will conduct the first in-vitro phase of my experimentation.

-Methods
The techniques I have practiced on this year can mainly be used for in-vivo/ex-vivo analysis and for a morpho-functional characterization of a certain tissue, but they also can be applied for in-vitro analysis. Starting from a sample of a tissue, I learned and practiced all the main methodologies for the histological analysis:

- Formalin fixation
- Paraffin embedding of formalin fixed tissues
- Microtome cutting and preparation of microscope slides
- Histological staining (H&E staining, Masson's trichrome staining)
- Immunohistochemical analysis (3, 3'-diaminobenzidine staining) Fig. 1
- Immunofluorescence assay (using fluorescent markers) Fig. 2

For the interpretation of the results of this morpho-functional analysis:

- Optical microscope (Fig. 1)
- Confocal microscope (Fig 2)
- Software for semiquantitative analysis (ImageJ) Fig. 1

Regarding the *in-vitro* methods, I started to approach C2C12 cell cultures and the main techniques of seeding on plates, maintenance of cell in culture and induction of differentiation.

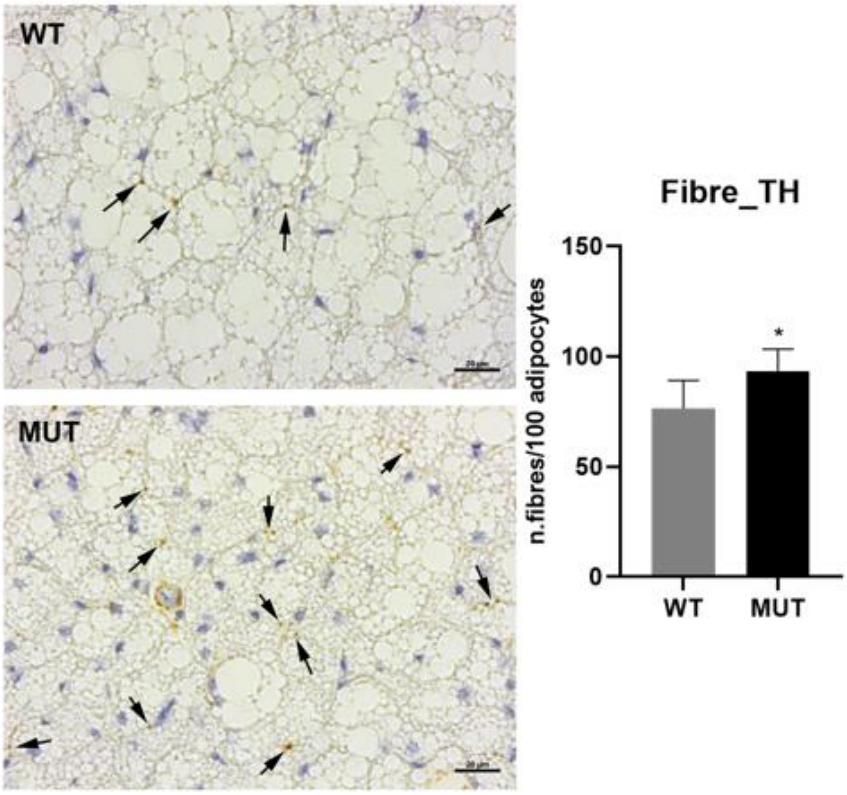


Figure 1: Detection and quantification of noradrenergic fibres in brown adipose tissue

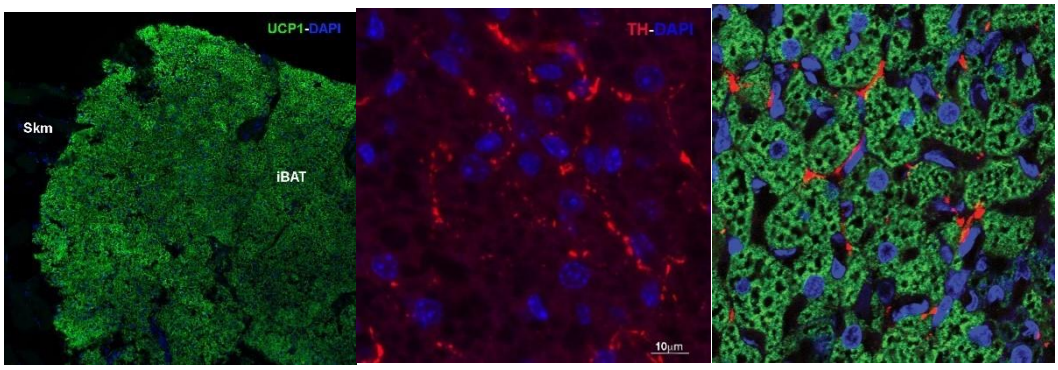


Figure 2: Immunofluorescence for adrenergic fibres and mitochondrial uncoupling protein 1 in brown adipose tissue.

-Expected / obtained Results

As an obtained result from this year, as mentioned in the previous paragraph, there is the acquisition of a methodology that can be applied for morphological and functional analysis.

Regarding the expected results for the next year of PhD, the major aim is to develop an *in-vitro* system that simulates physical exercise on myotubes cultures by Electrical Pulse Stimulation (EPS) to study and characterize the secretion into extracellular vesicles of myokines like irisin. I will also evaluate their effect on myotubes differently treated in order to recreate different physiological condition typical of aging and sarcopenic obesity, insulin resistance, oxidative stress or mitochondrial disfunction, to deepen if physical exercise could improve the management of these conditions by acting on the specific molecular mechanisms (oxidative stress, mitochondrial disfunction etc.) and eventually curb metabolic syndrome.

I will also test if co-cultures of myotubes and adipocytes, can be used as suitable model for EPS experiments, in order to focus also on the cross-talk between these two cellular types and to eventually identify new actors

and/or mechanisms involved in the beneficial effect exerted by physical exercise on adipose tissue metabolism.

REFERENCES:

1. Papadopoulou, Sousana K. "Sarcopenia: A contemporary health problem among older adult populations." *Nutrients* 12.5 (2020): 1293.
2. Bilski, Jan, et al. "Multifactorial mechanism of sarcopenia and sarcopenic obesity. Role of physical exercise, microbiota and myokines." *Cells* 11.01 (2022): 160.
3. Roh, Eun, and Kyung Mook Choi. "Health consequences of sarcopenic obesity: a narrative review." *Frontiers in endocrinology* 11 (2020): 332.
4. Laurens, Claire, Audrey Bergouignan, and Cedric Moro. "Exercise-released myokines in the control of energy metabolism." *Frontiers in physiology* 11 (2020): 91.
5. Huh, Joo Young. "The role of exercise-induced myokines in regulating metabolism." *Archives of pharmacal research* 41.1 (2018): 14-29.

Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

1. Course: Technology Transfer and Innovation, Prof. Donato Iacobucci.
2. Course: Getting Started with R: Environmental Computing, Dr. Giuseppe d'Errico.
3. Course: Assessing oxidative stress in biological systems, Prof. Elisabetta Damiani.
4. Seminar: "Caratteristiche biofisiche dei sistemi viventi (e come guardarli)", Prof. Ranieri Bizzarri, Università di Pisa.
5. Seminar: Shot of Science, "Time restricted eating (TRE) and exercise: a good strategy for disease prevention", Prof. Jedrzej Antosiewicz.
6. Seminar: "The impact of sleep deprivation in the brain: ER stress and myelin modification", Amina Aboufares El Alaoui.
7. Seminar: "The resolution revolution in Cryo-electron-microscopy, in Structural Biology and in Life Sciences", Martino Bolognesi, Department of Biosciences, University of Milan.
8. Seminar: "Current threats to research ethics and how to cope with them", Marco Seeber, Department of Political Science and Management, University of Agder, Norway.
9. Seminar: "Sfumature rosa in un mondo blu.", Prof. Giorgia Gioacchini.
10. Seminar: Shot of Science, "FTIRM in oncological diseases: a complementary technique for diagnosis and prognosis", Alessia Belloni.
11. Mobility period: 45 days spent in the Laboratory of Human Anatomy and Morphology, Head Prof. A. Giordano, Dipartimento di Scienze Cliniche e Sperimentali, Univpm.

List of conferences/workshops attended and of contributions eventually presented

Attendance to Second DiSVA-MaSBiC Annual Symposium "Protein Structure and Function in Biology, Medicine and Nanotechnology". 13 and 14 Ottobre 2022 at Disva-UNIVPM.

Part 3. PhD student information on publications

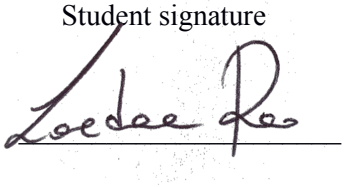
List of publications on international journals

Maria Razzoli, …..... Loredana Rao, Andrea Frontini and Alessandro Bartolomucci. **Selective loss of TLQP-21 results in a paradoxical resistance to diet-induced obesity** *in preparation*

[Date]

14/10/2022

Student signature



Supervisor signature