**Human and Animal Physiology is committed to challenging and inspiring teaching for BSc, MSc and PhD students.**

HAP has a substantial role in curricular teaching at Wageningen University. We teach basic and advanced physiology and molecular physiology for a variety of programs (MSc and BSc), especially Nutrition and Health, Biology, Animal Sciences, Biotechnology, Molecular Life Science, but also Food Technology and Plant Sciences. We aim to place our advanced physiology and molecular physiology teaching in the context of our research. In addition, we intensively train BSc, MSc and PhD students.
More detailed information about the courses provided by Human and Animal Physiology can be found on HAP Education and for all Wageningen University courses see the Study Handbook.

HAP offers MSc thesis projects which are directly involved in (ongoing) molecular and physiological research. HAP research is focused on energy metabolism including intermediary metabolism as well as mitochondrial metabolism and physiology, which are central components of diet- and age-related diseases and affect organ and tissue function. With this research HAP aims I) to gain comprehensive mechanistic and physiological insights in energy metabolism related to health and organ functioning and, II) to obtain methodology and data to substantiate the efficacy of interventions, compounds and foods to improve metabolic health. To achieve this, HAP performs experimental research in humans, model animals, tissues and cells, using state-of-the-art physiological, molecular, biochemical, histological and bioinformatics tools.

For more information on BSc or MSc theses you see the list of topics below. Please contact office.hap@wur.nl to plan an appointment with Sander Grefte to discuss the latest thesis possibilities.

For more information on general educational matters and MSc internship you can contact Dr Katja Teerds.

**MSc thesis topics**

- **Substrate-specific effects on primary NNT$^{-}$/$_{-}$ cells.** NNT is a protein involved in regulating the anti-ROS defence systems, which functioning is related to several diseases and aging. It has been shown that NNT is specifically active during low substrate availability, when other systems involved in clearing ROS are working at lower pace. However, we do not know exactly when NNT is active. We have created unique cell lines from mice that have or do not have a functional NNT gene, which can be cultured to be able to specifically research NNT functioning. To investigate vulnerability of NNT$^{-}$/$_{-}$ cells to specific substrates inducing either glycolysis or (parts of the) TCA cycle, compared to NNT$^{+}$/$_{+}$ cells, by researching the rate of cell growth and cell viability. If time allows, RNA or protein can be isolated from these cells to measure either gene or protein expression, or measure oxygen consumption of the cells. **Aims:**
    - Can we measure a decreased cell growth in NNT$^{-/-}$ vs NNT$^{+/+}$ primary fibroblasts cultured with specific energy substrates? (technique: cell culture & nuclei staining)
    - Can we measure an increased ROS levels in NNT$^{-/-}$ vs NNT$^{+/+}$ primary fibroblasts cultured with specific energy substrates? (technique: cell culture & immunocytochemistry)
    - Is the protein or gene expression for proteins/genes that regulate substrate use or redox homeostasis increased/ decreased in NNT$^{-/-}$ vs NNT$^{+/+}$ primary fibroblasts cultured with specific energy substrates? (technique: cell culture, protein/ RNA isolation & Western blot/ RT-qPCR)
    - Is oxygen use increased/ decreased in NNT$^{-/-}$ vs NNT$^{+/+}$ primary fibroblasts cultured with specific energy substrates? (technique: cell culture & oroboros or seahorse)
    - Does a hypoxia challenge increase or decrease the differences in e.g. gene expression in the NNT$^{-/-}$ vs NNT$^{+/+}$ primary fibroblasts cultured with specific energy substrates? (technique: cell culture & e.g. RT-qPCR or Western blot)

- **Mitochondrial Health in Male and Female Reproduction.** Disturbance of metabolic homeostasis (e.g. negative energy balance due to lactation) and/or oxidative stress may affect the delicate microenvironment of reproductive organs subsequently leading to inappropriate germ cell maturation and fertility problems due to for example reduced follicle and oocyte quality and by more variation in follicle and oocyte quality. Although altered mitochondrial function has been suggested to be crucially involved, the underlying mechanisms are largely unknown.
    - **Aim 1:** To study the functional and mechanistic role of mitochondria and more specifically nicotinamide nucleotide transhydrogenase (NNT) in germ cell development in rodents. MSc topics within this project may include the following techniques and materials: rodent materials and general molecular physiological, biochemical, immunohistochemical analyses.
    - **Aim 2:** To study the functional and mechanistic role of mitochondria in oocyte quality in dietary intervention studies performed in pigs. MSc topics within this project may include the following techniques and materials: pig materials and general molecular physiological, biochemical, immunohistochemical analyses.

- **Role of derailed energy metabolism and mitochondrial remodelling in ageing- and metabolic risk factors-related cardiac dysfunction and arrythmia.** Atrial fibrillation (AF) is the most common progressive cardiac arrhythmia. It is associated with increased mortality, serious complications, high hospitalization rate and high socioeconomic burden. The lifetime risk of AF is 1 in 3 and is increasing with advancing age. AF can result in static atrial blood, promoting the formation of atrial thrombi and triggering fatal symptoms such as stroke and heart failure. Unfortunately, there are no curative or preventive measures for AF due to incomplete understanding of the molecular mechanisms of arrhythmogenesis underlying AF pathogenesis. Notably, risk factors play a prominent role in the AF pathogenesis. Over 98% of AF is associated with risk factors such as ageing, obesity, diabetes mellitus (DM), metabolic syndrome and hypertension. AF is mostly associated with and probably caused by these risk factors that promote the arrhythmogenesis underlying AF. Many studies have described the potential mechanistic links between AF risk factors and AF susceptibility. Although a great deal of knowledge has been obtained, the precise mechanism by which risk factors induce arrhythmogenesis remains unclear and further research is needed to be able to ultimately develop preventive/curative interventions for AF. In particular, the molecular mechanisms underlying the association between these risk factors (ageing, obesity, DM, metabolic syndrome) and AF are largely unclear, hampering the development of effective preventive and therapeutic interventions for these risk factors-induced AF development.
    - **Aim:** Accumulating evidence suggests that **derailed energy metabolism** is a common phenomenon in AF as well as in the AF risk factors including obesity, DM, metabolic syndrome and ageing. Our recent studies aim to study the role of **mitochondria dysfunction and oxidative stress, dysregulated lipid metabolism, dysregulated microbiota, the depleted nicotinamide adenine dinucleotide** (NAD, a coenzyme central to energy metabolism) as key elements in the dysregulation of energy metabolism in the *in vitro* cellular model and the *in vivo Drosophila* model and in vivo mice model for AF.
    - Major techniques include but not limited to: high-speed imaging, high-resolution (confocal) microscopy, cell culture, qPCR, western blot, histology, Oroboros/seahorse respirometry, RNAseq, metabolomics (in collaboration), microbiota (in collaboration), electrical burst pacing, gene editing/engineering, electronic microscopy (in collaboration) ect.

- **Metabolic Health.** Metabolic health is crucial in human and mammalian function and a distortion of metabolic health will result in (metabolic) diseases like insulin resistance/type 2 diabetes, obesity, and cardiovascular diseases. Nutrients in our diet can have a major role, not only as a direct effect on energy metabolism at whole-body and tissue/cellular levels, but also via so-called nutritional programming. This captures the effects induced by specific nutrients given in early life which affect physiology in adulthood. Specific focus is on intestinal tissue and function using mouse as an animal model, as well as other metabolic organs including adipose tissue and liver, the inter-organ communication, and elucidation of underlying molecular mechanisms. Some recent work started also using fruit flies as an animal model. MSc topics within this overarching topic may include the following techniques and materials: rodent/fly materials and general molecular physiological, biochemical (QPCR, Western, ELISA, etc.), and immunohistochemical analyses.

- **Mitochondrial metabolism of intestinal cells.**
  Mitochondria are often called the powerhouses of the cells. They are organelles within cells that are responsible for the generation of cellular energy in the form of ATP. Mitochondrial function has gained more and more interest in recent years, since it became clear that mitochondrial function is not only a consequence of a cells functioning, it can actually drive cellular processes including cell proliferation and differentiation. (Bottje and Carstens 2009)Since the intestine is not yet fully matured at weaning (Moeser et al., 2017), cell differentiation is even more important during this stage of the piglet's life for further intestinal development and maturation. Insults to mitochondrial function, for example caused by feed refusal due to weaning stress or by metabolites formed through fermentation of protein, may thus contribute to intestinal malfunction
    - **Aim 1:** In vivo assessment of mitochondrial functioning in the intestine of pigs. MSc topics within this project may include the following techniques and materials: work with the animals and general molecular physiological, biochemical, immunohistochemical analyses.

- **The role of muscle regeneration in human and mouse muscle-aging.** Age-related loss of muscle mass and strength, or sarcopenia, is a fundamental cause of frailty, functional decline and disability. Due to the rapidly increasing proportion of older people, sarcopenia presents a huge potential public health issue worldwide with high impact on healthcare costs. Unfortunately, little is known at the molecular level of the underlying mechanisms that lead to sarcopenia. Aged mice are frequently used as an animal model for anti-sarcopenia interventions. However, the translatability of mice for human muscle-aging has been poorly investigated. In addition, recent publications suggest that the pathophysiology of muscle-aging and frailty could be highly sex-specific. New research is required to investigate the translatability of mice for human muscle-aging and to investigate the role of sex (females vs. males) in muscle-aging. In the current project, we have different materials at our disposal:
    - At WUR a large human study has been performed, including both male and female young and old participants.
    - At TNO (Leiden) a large mouse study has been performed in which both male and female mice were included.

  In both studies, different parameters for muscle weakness were analyzed. Muscle tissue was collected in both old and young mice and humans and needs to be analyzed on histological level to assess the progression of several markers of muscle-aging. In this project, immunofluorescence will be utilized to explore the role of (among others) muscle regeneration in muscle-aging in mice and humans, and comparisons between male and female muscle-aging will be made as well. The student will gain comprehensive lab experience, with a focus on histology and microscopy. Possibly, two students can work on this project simultaneously (e.g. one on mouse samples, one on human samples).

- **The effect of aging and hypoxia on muscle mitochondrial function.** One of the hallmarks of ageing is the progressive loss of muscle mass and function, which significantly increases the risk of morbidity and mortality. A strong relation with mitochondrial dysfunction has been shown. Specifically for the neuromuscular junctions, the main role of mitochondria is to provide sufficient energy for signal transmission and to buffer the high calcium load needed for proper muscle-nerve interactions. Interestingly, in aged rats, mitochondrial swelling, formation of megamitochondria, and markers of apoptosis (cytochrome c release, activated caspase 3 were observed in NMJ.
    - **Aim 1:** To investigate the effect of age on the muscle molecular signature. In this project you will assist in ongoing study measuring extra cellular flux analysis of human biopsies and or human cells. MSc topics within this project may include the following techniques and materials: human biopsies or culturing primary cells, extra cellular flux analysis and standard molecular physiological, biochemical, immunohistochemical and analytical analyses
    - **Aim 2:** To investigate the role of age and oxygen of the formation of neuromuscular junctions. MSc topics within this project may include the following techniques and materials: mouse tissues, cell culture, hypoxia, ex vivo immunohistochemical analysis and standard molecular physiological, biochemical, immunohistochemical and analytical analyses.
    - **Aim 3:** To investigate the effect of aging on in vivo or in vitro muscle function.

- **Energy for life.** Most tissues and cells rely on oxygen to drive mitochondrial metabolism to sustain cellular functions. In contrast, cancer cells rely on glycolytic metabolism even in the presence of oxygen, the so called Warburg effect. Within these extremes, other different cell types may rely more or less on glycolytic or mitochondrial metabolism.
    - **Aim 1**: To setup and study novel extra-cellular flux analysis (seahorse) to characterize mitochondrial different healthy and disease cell types. MSc topics within this project may include the following techniques and materials: cell culture, treatment with (pharmacological) compounds and extra-cellular flux analysis (seahorse).
    - **Aim 2:** To investigate how nutrient composition affect cellular metabolism and mitochondrial communication. MSc topics within this project may include the following techniques and materials: cell culture, treatment with (pharmacological) compounds, extra-cellular flux analysis (seahorse), standard molecular physiological, biochemical, immunohistochemical and analytical analyses

- **Inflammatory modelling of the human gut.** The human intestine is composed of three main parts; the epithelial cells, the mucosal immune cells and the microbiota. Using both 2D (transwells) and 3D (gut-on-a-chip) models, our aim is to combine these components in vitro in a way that mimics the gut as realistically as possible. Next, we challenge these models with a priming stimulus (TNF, LPS), which elicits an inflammatory response. These primed models can then be used to evaluate the effects of a broad array of compounds, e.g. food components, nutraceuticals, supernatant of probiotic bacteria and anti-inflammatory drugs (infliximab, tofacitinib). Read-outs that are commonly used to assess these models are barrier integrity, NF-κB activation, cytokine secretion and metabolic profiling.
    - **Aim 1:** establish a 2D transwell model of intestinal inflammation using Caco-2 intestinal epithelial cells and THP-1-Lucia NF-κB reporter cells to be used for screening of anti-inflammatory compounds
    - **Aim 2:** establish a 3D gut-on-a-chip model (Mimetas OrganoPlate®) of intestinal inflammation using Caco-2 intestinal epithelial cells and THP-1-Lucia NF-κB reporter cells to be used for screening of anti-inflammatory compounds

**The impact of short-term muscle disuse with or without neuromuscular electrical stimulation on metabolic health and muscle mass in healthy individuals with and without type 2 diabetes.** Short periods of muscle disuse, e.g. bedrest during illness or limb immobilization during the recovery from injury, lead to the rapid loss of muscle mass and metabolic health. Despite decades of research, the underlying cause of muscle atrophy and insulin resistance during periods of physical inactivity remain to be established. Furthermore, the effect of such periods in individuals with type 2 diabetes (T2D), who are already insulin resistant, are unknown. Lastly, effective intervention strategies to preserve muscle mass and metabolic health are currently lacking. In this project, students will be able to contribute to one of two ongoing human intervention studies, i.e. a forearm immobilization and a bed rest study, both due to start approximately September 2024. In these studies we will use detailed *in vivo* nutritional physiology techniques including forearm balance, stable isotope tracers, insulin clamps, and repeated blood and muscle sampling to determine the uptake and use of amino acids, glucose and fatty acids in inactive muscles tissue. Students will have the opportunity to actively contribute to all aspects of high-quality muscle physiology research.

- **Aim 1:** Determine the temporal changes in of forearm glucose and amino acid uptake and kinetics in response to forearm immobilization in healthy volunteers with or without T2D.
- **Aim 2:** Investigate the effect of bedrest, with or without repeated daily neuromuscular electrical stimulation combined with protein intake, in healthy volunteers

**BCAAs, friend or foe for our muscles?! The effect of dietary amino acid restriction on muscle protein synthesis and insulin sensitivity.** The branched-chain amino acids (BCAAs) leucine, isoleucine and valine have been suggested to play a crucial role in the amount of muscle mass we have, and are currently commonly used by resistance training athletes to support muscle growth. Despite this, it is unknown whether BCAAs are essential for muscle protein synthesis. Importantly, BCAAs are also implicated in the development of insulin resistance. As such, crucial mechanistic research is needed to investigate if BCAAs are required for muscle mass maintenance while not hampering metabolic health. We are currently investigating this in an ongoing study, which uses state-of-the-art metabolic techniques to directly measure muscle protein synthesis and muscle insulin sensitivity in healthy young volunteers. A motivated student will be able to assist in participant recruitment, screening, data collection, and sample/data analyses, thereby gaining crucial experience in all aspects of a mechanistic human physiology study.

- **Aim 1:** To investigate the impact of dietary BCAA ingestion vs omittance on skeletal muscle protein synthesis.
- **Aim 2:** To quantify the effect of ingestion of a full amino acid supplement vs a supplement without BCAAs on skeletal muscle insulin sensitivity.

**The metabolic effects of a hyperglycaemic meal in lean and obese individuals using a glucose microtracer approach**. Obesity is an important risk factor for several lifestyle-related diseases, representing a significant burden on public health and healthcare systems. Different conditions promote the development of obesity, being diet and/or dysregulation of metabolic pathways included. Dysregulation of metabolic pathways can also (further) stimulate the progression of obesity and its associated comorbidities. The polyol pathway is a metabolic pathway in which glucose is converted to fructose. In this study, which will be conducted mid 2024-mid 2025, we will assess the effect of a hyperglycaemic meal on metabolic health (i.e. insulin sensitivity, polyol pathway activity) in lean and obese individuals via administration of a single oral dose of radioactive labelled glucose isotopes. With research participants staying in the clinical facility for 3.5 days, students contributing to this project will be able to contribute to recruitment, screening, data collection, and sample and data processing, alongside day-to-day interaction with research participants.

- **Aim 1:** To assess the effect of a hyperglycaemic meal on metabolic health and polyol pathway activity in individuals with a normal weight and obesity.

**Bioavailability of microplastics in humans: quantifying the absorption, distribution, metabolism and excretion of microplastics via ingestion of $^{14}$C-labelled microplastics**. In recent years, the potential negative health effects of micro- and nano-plastics have received increased attention (e.g. https://nos.nl/artikel/2504270-bij-drinken-uit-wegwerpflesje-krijg-je-talloze-stukjes-nanoplastic-binnen). Although it is known that microplastics accumulate in

various tissues, it is unknown how orally ingested microplastics are being processed and taken up in the human body. As such, at the end of 2024 we will conduct the first ever human study using a microdose of microplastics, radio-isotopically labelled, to study how these microplastics are being absorbed, distributed, metabolized, and excreted in healthy young volunteers. A student will have the unique opportunity to contribute to participant recruitment, screening, and sample/data collection on this highly relevant topic for overall human health.

- **Aim 1:** To determine the uptake, bioavailability, and excretion of orally ingested micro- and nano-plastic particles in humans