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**FROM BRAIN TO MUSCLE AND BACK:  
NOVEL APPROACHES TO HARNESS  
THE BENEFITS OF EXERCISE**

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# From Brain to Muscle and Back: Novel Approaches to Harness the Benefits of Exercise

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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*“Life is not about how fast you run or how high you climb but how well you bounce.”*

*To those who keep moving.*

*“...we know that it is the search that gives meaning to any find and that one often has to travel a long way in order to arrive at what is near.” José Saramago*

## ABSTRACT

As the world population is growing older and more sedentary every day, the need for new approaches to combat chronic diseases grows steadily. Physical exercise improves health and reduces the risk of developing a plethora of chronic diseases. This body of work aims to investigate the molecular mechanisms underlying the neuronal and muscle function, their interaction and the potential signals that mediate this communication.

To gain more insight into neurodegeneration, in **paper I**, we used a mouse model of Alzheimer's disease and examined the mechanisms generating amyloid plaques. We discovered that Presenilin 1 (PS1), the key player of the enzyme responsible for generating the pathogenic peptides that make up the plaques, can play a dual role. Upon phosphorylation at a specific site, PS1 can facilitate the degradation of the substrate that would otherwise be cleaved to generate toxic amyloid peptides. This function ultimately reduces soluble amyloid peptide levels as well as the plaque burden. Overall this study extends our understanding of neurodegenerative processes and proposes a new target for intervention.

In **paper II**, we investigated the transcriptional signatures of inherent and acquired exercise capacity in the skeletal muscle using uniquely developed rodent models. Our results associate high exercise capacity with angiogenesis and oxygenation while low exercise capacity profile reflects gene programs related to inflammation and cardiovascular disease. We interrogated the transcriptome data for potential upstream regulators and also secreted factors that can mediate exercise capacity and response. Finally, we compared the rat transcriptomic signatures with those of humans and identified an overlapping set of genes.

In **paper III**, we explored the biological function of a muscle-secreted factor called Neurturin (NRTN). Transgenic animals overexpressing NRTN in skeletal muscle are leaner and more glucose tolerant than controls. Their muscles exhibit increased oxidative metabolism and vascularization. We observed a NRTN-induced remodelling in neuromuscular junction morphology and discovered that NRTN can promote a slow motor neuron identity and reduce markers for fast-motor neurons. Functionally, muscle-specific overexpression of NRTN enhances endurance performance and improves motor coordination. Systemic delivery at the adult stage could achieve an improvement in glucose metabolism and also recapitulate the improved motor coordination. We propose NRTN as a myokine with therapeutic promise for metabolic dysfunction and neuromuscular diseases.



## LIST OF SCIENTIFIC PAPERS

- I. Victor Bustos, Maria Pulina, **Yildiz Kelahmetoglu**, Fred Gorelick, Marc Flajolet, Paul Greengard. Bidirectional regulation of A $\beta$  levels by Presenilin 1, *Proceedings of the National Academy of Sciences*, Jul 2017, 114 (27) 7142-7147; DOI: 10.1073/pnas.1705235114
- II. **Yildiz Kelahmetoglu**, Paulo R. Jannig, Igor Cervenka, Lauren G. Koch, Steven L. Britton, Jiajia Zhou, Huating Wang, Matthew M. Robinson, K Sreekumaran Nair, Jorge L. Ruas. Comparative Analysis of Skeletal Muscle Transcriptional Signatures Associated with Aerobic Exercise Capacity or Response to Training in Humans and Rats, *Frontiers in Endocrinology*, 2020, 11(819). DOI=10.3389/fendo.2020.591476
- III. Jorge C. Correia\*, **Yildiz Kelahmetoglu\***, Paulo R. Jannig, Christoph Schweingruber, Dasa Svaikovskaya, Liu Zhengye, Igor Cervenka, Mariana Oliveira, Jik Nijssen, Vicente Martínez-Redondo, Michael Stec, Naveen Khan, Johanna Lanner, Sandra Kleiner, Eva Hedlund and Jorge L. Ruas. Muscle-secreted neurturin couples fiber oxidative metabolism and slow motor neuron identity (Manuscript).

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## LIST OF ABBREVIATIONS

|              |   |
|--------------|---|
| ACh          | Acetylcholine                               |
| AChR         | Acetylcholine receptor                      |
| AD           | Alzheimer's disease                         |
| A $\beta$    | Amyloid $\beta$                             |
| ALS          | Amyotrophic lateral sclerosis               |
| AMP          | Adenosine monophosphate                     |
| AMPK         | AMP-activated protein kinase                |
| APP          | Amyloid precursor protein                   |
| ATP          | Adenosine triphosphate                      |
| BBB          | Blood-brain barrier                         |
| BDNF         | Brain-derived neurotrophic factor           |
| BMI          | Body-mass index                             |
| CaMK         | Calmodulin-dependent kinase                 |
| CNS          | Central nervous system                      |
| CK1          | Casein kinase 1                             |
| CSTB         | Cathepsin B                                 |
| CTF          | C-terminal fragment                         |
| ERR $\alpha$ | Estrogen-related receptor $\alpha$          |
| FF           | Fast-fatigable fibers                       |
| FFA          | Free fatty acid                             |
| FR           | Fast, fatigue-resistant fibers              |
| GDNF         | Glial cell line-derived neurotrophic factor |

|          |  |
|----------|--|
| GLUT4    | Glucose transporter 4  |
| HCR      | High capacity runner   |
| HERITAGE | The HEalth, RIsk factors, exercise Training, And GENetics Family Study |
| HIF      | Hypoxia-inducible factor   |
| HRT      | High responders to training  |
| HRTT     | High responders to training-trained                                    |
| HSA      | Human skeletal alpha-actin   |
| IGF1     | Insulin-like growth factor 1   |
| IL-6     | Interleukin 6  |
| IL-13    | Interleukin 13   |
| IRS      | Insulin receptor substrate   |
| LCM-seq  | Laser capture microdissection-sequencing                               |
| LCR      | Low capacity runner  |
| LRT      | Low responders to training   |
| LRTT     | Low responders to training-trained                                     |
| MAPK     | Mitogen-activated protein kinase                                       |
| MN       | Motor neuron   |
| mRNA     | Messenger ribonucleic acid   |
| MSTN     | Myostatin  |
| MCK      | Muscle creatine kinase   |
| NMJ      | Neuromuscular junction   |
| NRF      | Nuclear respiratory factors  |
| NRTN     | Neurturin  |

|       |  |
|-------|--|
| PGC-1 | Peroxisome proliferator-activated receptor gamma coactivator 1 |
| PI3K  | Phosphoinositide 3-kinase                                      |
| PKB   | Protein kinase B   |
| PS1   | Presenilin-1   |
| S     | Slow twitch, fatigue resistant fibers                          |
| SCI   | Spinal cord injury   |
| WHO   | World Health Organization                                      |



# 1 INTRODUCTION

Living longer and better has been a centuries-old goal for humankind. As much as the human imagination allowed, this goal rested on the shoulder of myths and plant-based potions, being rather unattainable until the 19th century. The advent of sanitation and healthcare practice allowed historic extensions of life expectancy for the majority of the population. This stride, accompanied by technological advances, brought new challenges associated with pushing the boundaries of human longevity. According to the World Health Organisation (WHO), the European median age is the highest in the world; with the proportion of people aged over 65 is forecast to double in 2050. As we live longer, the chances of spending this time in good health are decreasing in parallel. Increasingly sedentary lifestyles and unhealthy diet have led to a high incidence of obesity, metabolic dysfunction and associated complications. Worldwide, type 2 diabetes alone claims more than 3 million lives annually. Alongside, the prevalence of major neurological disorders is on the rise with cerebrovascular diseases being the leading cause of death among them and dementia the second (1, 2). In addition, age-related muscle atrophy impairs physical activity leading to additional co-morbidities. These challenges expose the limits of our current therapeutic toolbox and necessitate novel approaches to mitigate disease and increase the quality of life.

It is well known that physical exercise improves health and reduces the risk of premature mortality (3, 4). Regular exercise stimulates a multi-organ cross-talk that benefits a variety of systems leading to an improvement in health. Arguably, exercise is one of the most effective strategies to attenuate metabolic dysfunction and age-related health decline. Understanding the mechanisms and systemic factors that mediate exercise benefits and how they are regulated could provide clues to develop new approaches to fight disease.

From anatomical analysis by Vesalius to cell level by Virchow and molecular resolution by Sterling & Bayliss, our approach to understanding biology has been an effort to zoom in. Advances in technology carried this evolution further and enabled an unprecedented level of resolution with tools like the single-cell sequencing and expansion microscopy. Still, zooming in doesn't always lead to clear views and may confine our perception. Equally important is to keep in sight and evaluate systemic connections, to zoom out.

Here, I present my scientific efforts to take a broad overlook at the molecular mechanisms underlying the decline in neuronal and muscle function and position exercise-induced myokines as potential players to overcome these.



## **2 SYSTEMIC EFFECTS OF AGING AND PHYSICAL INACTIVITY**

The modern world has become an increasingly convenient place that revolutionized our lifestyle. Progressive innovation reshaped what it means “to work” as machine power replaced manpower and the digital world emulated the physical world to the extent of replacing it in certain aspects. The traditional approach to social life, transport and labor are increasingly replaced with contemporary ones that involve electronic devices and fiberoptic cables. As a result, human behavior has been, and continues to be, modified tremendously. Even though the technological means allowed us to develop more resilience against threats from predators and spared us from starving due to limited food availability, the drastic changes in modern life led to a more sedentary lifestyle. This, in turn, led to increased incidence of chronic diseases including cardiovascular diseases, metabolic disorders (e.g diabetes, obesity, metabolic syndrome) and neurological disorders such as Alzheimer’s disease (5). Today, diabetes is among the leading causes of death in the world and excess weight and obesity kills more people than malnourishment(6). The global burden of neurological disorders also keeps growing and around 50 million people live with a form of dementia, a number set to triple by 2050(7).

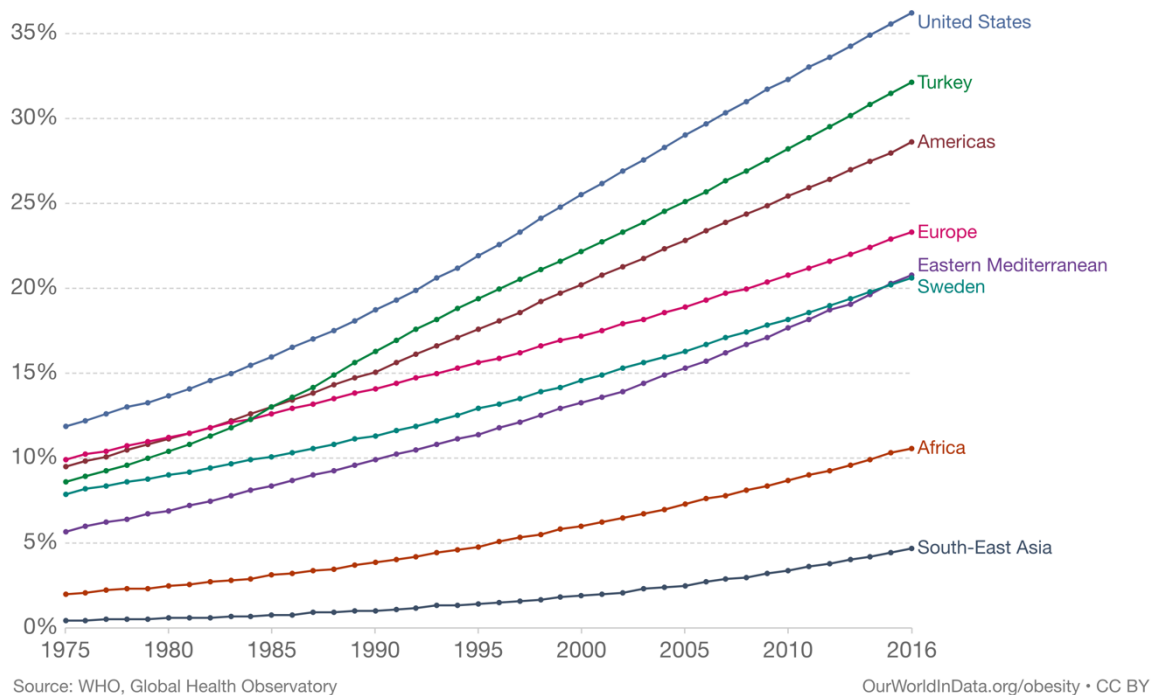
Physical inactivity is a major risk factor for many chronic pathologies, including those mentioned before, and has also been shown to exacerbate the morbidity and mortality rates (3, 4, 8). In addition, a growing body of evidence links sedentary overindulgent lifestyles and resulting metabolic alterations to accelerated brain aging and neurodegeneration (9, 10).

The pathological mechanisms triggered by physical inactivity and aging are subjects of extensive research and systemic factors likely play a considerable part in linking inactivity and chronic disease.

### **2.1 METABOLIC DYSFUNCTION**

In the modern world, prevalent physical inactivity combined with almost infinite caloric supply leads to a rise in the incidence of metabolic diseases at a tremendous pace. Today, obesity and associated diseases pose a real threat to public health as in the past few decades, the prevalence of obesity almost tripled in Europe alone and continues to increase worldwide at an alarming rate. Based on the latest estimates by WHO, in 2016, around 1.9 billion adults were overweight and among these 650 million people were obese, corresponding to 13% of the entire human population (Figure 1). The prevalence among children and adolescents is

even more alarming. As of 2019, the number of overweight or obese children under the age of 5 is reported as 38 million. Developed countries seem to have the largest share in the global obesity epidemic. In the USA alone, the prevalence of obesity is around 30%, tailed by the European union with roughly 20% of people obese. This is closely reflected by the Swedish population with 22% of the adult population considered obese in the 2016 WHO report.



**Figure 1:** Share of adults that are obese from 1975 to 2016 (11). Individuals with body Mass Index (BMI) 25 or higher is considered overweight while BMI greater than 30 is defined as obesity.

Obesity commonly co-exists with type-2 diabetes, dyslipidemia, and hypertension (12). Additionally, it is associated with a wide range of pathologies from fatty liver to cancer and Alzheimer's disease. From an evolutionary perspective, humans transitioned from the environment with limited food availability to infinite access to food in a relatively short time. Therefore, biological adaptations to scarcity in the form of storing excess energy now may have been working against the overabundant and sedentary lives of our times. In essence, obesity is a result of energy imbalance due to excess food intake that far exceeds the energetic demands of the body. This extra energy is primarily stored in white adipose tissue depots throughout the body in the form of lipids. If the excess energy intake continues and fills the adipose tissue capacity, it spills over to other tissues. This ectopic fat accumulation is linked to additional pathologies such as mitochondrial dysfunction and systemic inflammation (13).

### **2.1.1 Type-2 Diabetes**

There is broad consensus on the strong association between obesity, insulin resistance and type 2 diabetes. The WHO estimates point to obesity and excess weight (BMI over 25 kg/m<sup>2</sup>) as the underlying factor for approximately 70% of new diagnoses for type 2 diabetes. Thus, in parallel with obesity, the prevalence of type 2 diabetes is also increasing at alarming rates. Based on the most recent epidemiological data, as much as 463 million adults are estimated to have diabetes worldwide and half of them go undiagnosed (14). If the current trends continue, this number is projected to reach 700 million by 2045. Moreover, diabetes prevalence seems to increase with age, as the 65-99-year-old group represent 20% of people with diabetes. With a rapidly aging population, the world will have to face the inevitable challenges in many fronts including economic and public health consequences.

The defining feature of diabetes is impaired glucose homeostasis. Therefore, to fully understand type 2 diabetes, it is crucial to have an insight into the glucose metabolism.

#### **Glucose metabolism**

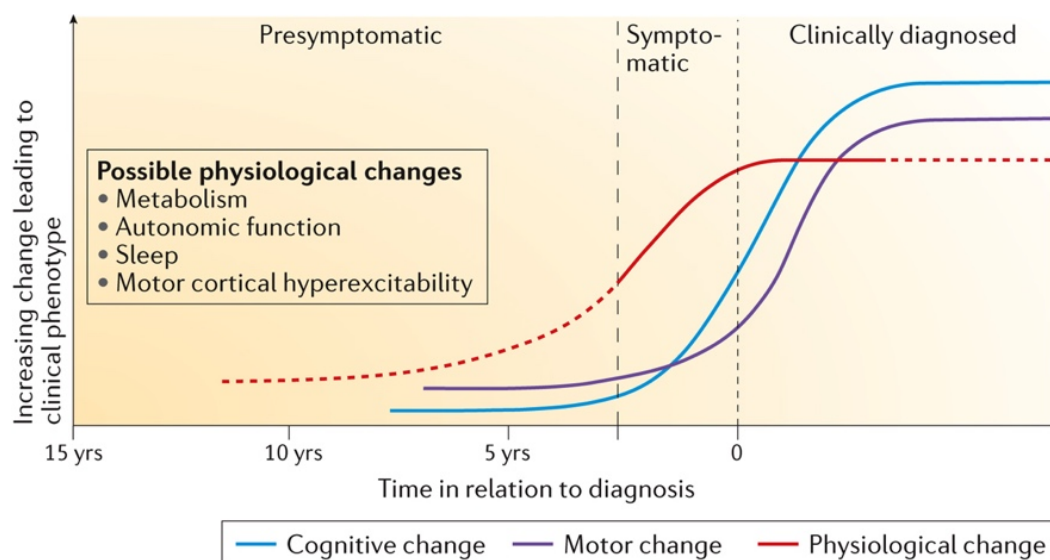
Blood glucose increases after food consumption and triggers the signals to stimulate glucose uptake and storage or utilization. One of these signals is insulin which targets liver, skeletal muscle and adipose tissue but it can affect a variety of cells and tissues (15). The control of blood glucose levels by insulin serves to ensure efficient energy storage and regulation of energy metabolism in multiple tissues. To achieve this, insulin signals through a complex network with multiple nodes and controls several cellular processes. From a very simplistic point of view, upon insulin binding, the insulin receptor activates insulin receptor substrates (IRS) which then relays the signal to multiple downstream effectors. The main components of this network are the phosphoinositide 3 - kinase (PI3K)/protein kinase B (PKB/AKT) pathway and the Ras-mitogen-activated protein kinase (MAPK) pathway (16). Once activated, the PI3K-AKT pathway orchestrates most of the metabolic response and enables cells to properly handle the elevated glucose levels in circulation (this ability is often termed glucose tolerance). For example, increased glucose uptake through promoting translocation of glucose transporter (GLUT) to the cell membrane and storage in the form of triglycerides in adipose tissue or glycogen in skeletal muscle and liver.

Failure to trigger the response to elevated glucose levels (hyperglycemia) results in metabolic dysfunction and in time builds up glucose intolerance, together with increased insulin levels (hyperinsulinemia)(16). In such conditions, insulin sensitivity of target tissues progressively declines, eventually building up insulin resistance. If not managed properly, hyperglycemia

and insulin resistance can lead to life-threatening complications linked to metabolic dysfunction, such as cardiovascular disorders and may even lead to neurodegeneration.

## 2.2 NEURODEGENERATION

Physical inactivity and increased energy intake are proposed to reduce the ability of the adaptiveness of the brain to energetic challenges and result in failure to keep optimal brain function and render it vulnerable to disease (17–20). During aging, together with other systems, our brain function also declines in multiple ways including impairments in cognition, motor coordination and neuroplasticity (18, 21, 22). Although cell-intrinsic changes may explain the decline in brain function to a certain extent, accumulating evidence indicates that systemic, metabolic changes likely play an important role in the initiation and progression of the functional decline of the brain (18).



**Figure 2:** Progression of physiological, cognitive and motor changes in neurodegenerative disorders, from Ahmed, R. M. et al. (2018), reprinted with permission (23).

Pathological protein aggregation, mitochondrial dysfunction, gliosis, vascular damage and autophagic dysfunction are among the mechanistic similarities of several predominant neurodegenerative diseases (including Parkinson’s disease, AD and other types of dementia, and ALS) (23–26). Clinical and epidemiological data suggests that physiological changes, including metabolic changes, predate the motor and cognitive dysfunction by about 5 years in several prevalent neurodegenerative diseases (Figure 2) (23). Early studies on the brains of dementia patients suggested impaired glucose and oxidative metabolism (27). Likewise, longitudinal studies indicate that metabolic disorders obesity, diabetes and atherosclerosis are predisposing factors for dementia (28–30). Furthermore, cerebrovascular changes have been associated with developing dementia, with supporting evidence showing blood-brain

barrier (BBB) breakdown in AD brains (31–34). In line with this, non-invasive brain imaging suggests that age is negatively correlated with cerebral blood flow (35) as well as BBB integrity (36) in humans. Reduced blood flow can lead to impaired energy metabolism in the brain due to reduced delivery of nutrients as well as other circulatory factors that might be neuroprotective. Supporting evidence obtained using animal models shows reduced glucose transporters in the aged brain (37) and remarkably, old animals display a reversal of the age-related cerebrovascular and cognitive dysfunction when exposed to factors in young blood (38). Additionally, compromised BBB may promote penetration of aggregate-prone proteins and inflammatory agents from circulation (39–41).

Taken together, these and other findings mount a compelling case for the involvement of systemic factors related to energy metabolism or inter-organ communication at the onset or progression of neurodegeneration (41, 42). Evaluating neurodegenerative diseases as systemic multifactorial syndromes and understanding the physiological changes during the disease continuum could help finding early windows of opportunity for intervention before the functional deterioration.

### **2.2.1 Dementia and Alzheimer's Disease**

Dementia is one of the greatest burdens of neurodegeneration and it is characterized by progressive loss of memory and cognitive abilities. Although the major risk factor for dementia is aging, it is not an inevitable consequence of aging, rather a pathological syndrome that poses a real threat to public health with serious societal and economic implications (43). As the world population is rapidly aging, the incidence of dementia has also been on the rise. Currently, WHO estimates indicate that worldwide 50 million people live with dementia and project a rise to 152 million people by 2050.

The most prevalent form of dementia is Alzheimer's Disease (AD), representing around 60–70% of cases. Commonly, sporadic AD patients develop a late-onset disease, past age 65, while the rare familial form of the disease can manifest itself at as early as 30 years old and progress rapidly (44, 45). Patients exhibit a variety of symptoms and because there is no reliable diagnostic tool yet, physicians rely on these progressive symptoms for clinical diagnosis (45, 46). Initial stages of the disease typically involve memory problems, confusion and difficulties in concentration, and later on progress to severe memory deficits, motor dysfunction, mood swings and other cognitive and behavioral impairments. Late stages of the disease place a heavy burden on the caregivers to aid the patients in physical activities of daily life as well as to manage mental health (47). The caregiving and medical costs are part

of the challenge the society has to tackle considering the increasing prevalence of AD worldwide.

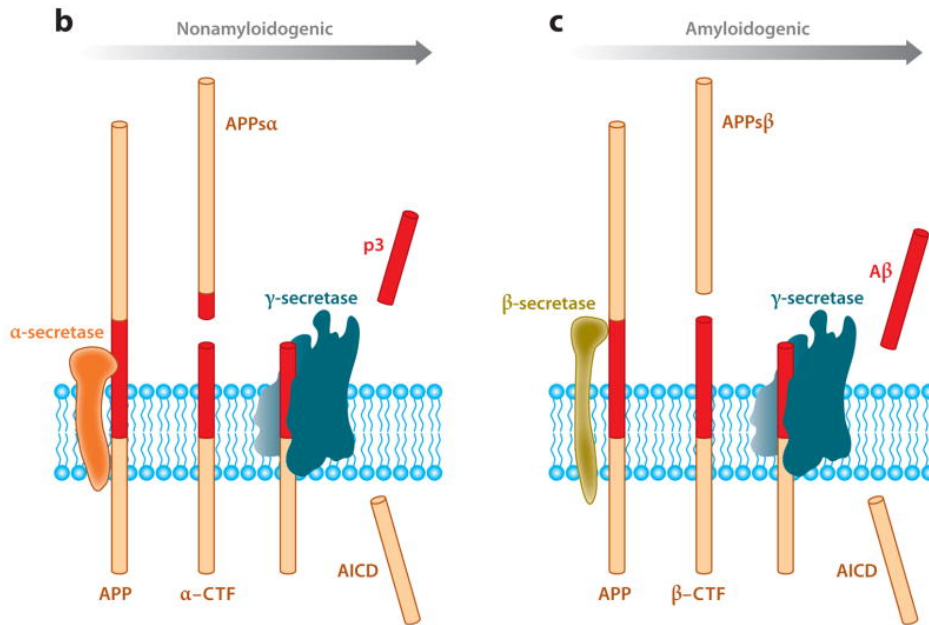
Given the lack of an effective treatment and the dire predictions for the spread of the disease, the need for an improved understanding of the disease pathology is evident.

### **2.2.2 Pathophysiology of Alzheimer's Disease**

Characteristically, AD brains contain aggregated amyloid plaques composed of amyloid  $\beta$  ( $A\beta$ ) peptide and the neurofibrillary tangles formed by tau protein (45, 48). Accompanying these, vascular damage, neuronal loss, gliosis, oxidative damage, neuroinflammation, mitochondrial and autophagic dysfunction are among the other hallmarks AD pathology (45). There are different theories about what triggers the disease and one of them, the amyloid cascade hypothesis, places  $A\beta$  pathology upstream of other pathologies (48, 49). Other prominent theories propose alternatives such as neurofibrillary tangles, vascular damage, inflammatory cues, or a combination of concomitant factors (50–52). While the discussion is ongoing, the consensus point around this issue seems to be that AD is a multifactorial disease involving a wide range of pathologies.

Genetic mutations that cause increased  $A\beta$  production, plaque burden, neuronal dysfunction and memory impairments form the basis for the ongoing investigation into amyloid metabolism. Compelling evidence indicate that the progression of AD mirrors other neurodegenerative diseases which involve physiological changes before the first clinical symptoms appear (23, 50). Accordingly, in the context of this thesis, the focus points will be some of the events that are upstream of the  $A\beta$  plaque formation.

Much attention has been directed at the mechanism of  $A\beta$  production through APP (Amyloid precursor protein) processing (Figure 3). As a substrate, APP processing leads to toxic  $A\beta$  production through amyloidogenic pathway (53). The initial cleavage is executed by  $\beta$ -secretase enzyme leading to a soluble form of APP and  $\beta$ -CTF. Next,  $\beta$ -CTF is further cleaved by  $\gamma$ -secretase to generate the toxic  $A\beta$  peptides. These insoluble  $A\beta$  monomers accumulate and form neurotoxic  $A\beta$  fibrils which are deposited as extracellular plaques. Some evidence suggests that intraneuronal  $A\beta$  accumulation precedes the formation of the plaques in the extracellular space and may be a tipping point in the disease progression (54).



**Figure 3:** APP processing: The nonamyloidogenic processing starts with  $\alpha$ -secretase cleavage which creates a soluble APP $\alpha$  peptide and a C-terminal fragment attached to the membrane ( $\alpha$  CTF) and ends with final  $\gamma$ -secretase cleavage of  $\alpha$ CTF into smaller and non-toxic fragments P3 and AICD. On the contrary, amyloidogenic processing contains sequential cleavage by  $\beta$ -secretase and  $\gamma$ -secretase generating aggregate-prone A $\beta$  monomers and AICD. (From O’Brian, Wong, Annual Rev. Neuro., 2011, reprinted with permission)(55).

The multiprotein enzyme complex  $\gamma$ -secretase has a central role in APP processing and familial early-onset AD pathology (44, 56–59). Different mutations in its catalytic subunit presenilin 1 (PS1), its isoform presenilin 2 (PS2), and substrate amyloid precursor protein (APP) has been shown to lead to early pathological A $\beta$  plaque load and cognitive dysfunction due to toxic A $\beta$  peptide accumulation. To date, population genetics studies identified nearly 300 mutations in PS1 alone, affecting the pathology to varying degrees and some of these mutations served as indispensable tools to generate research models to study (60).

Currently, there is no available therapy for AD and despite the long history of the disease, drug development remains nascent with some symptomatic relief. Therefore, there is considerable attention to find new ways and identify new factors that could affect pre-onset pathological changes. Among the emerging areas, this thesis will explore the involvement of autophagic dysfunction and impaired glucose metabolism.

### Autophagic dysfunction in Alzheimer’s Disease

Many neurodegenerative diseases that affect humans are associated with accumulation and aggregation of proteins in neurons and a number of them with mitochondrial dysfunction (26, 61). Autophagy plays an essential role in cellular homeostasis through the turnover of old or dysfunctional peptides, proteins, protein-aggregates and even organelles (62). In healthy

neurons, autophagy is constitutively active and works quite efficiently (63). Thus, the role of autophagy in neuronal dysfunction and neurodegeneration has been studied extensively in the past decades (64–68). Of these, several studies suggested that autophagy and endolysosomal pathway may play a role in A $\beta$  metabolism and APP trafficking (69–74). Cataldo et al. were the first to show accumulated autolysosomes in post-mortem brain samples from AD patients using electron microscopy (71). Later on, more evidence demonstrated that immature autophagic vesicles accumulate in the degenerating neurons of AD patients (63, 75–79). These observations suggest that autophagy is involved in disease progression and subsequent A $\beta$  aggregation. In support, reduction in autophagic activity correlates with aging, where aggregate-prone proteins similar to A $\beta$  can cause dementia and cognitive dysfunction (80).

### **Impaired Glucose Metabolism in AD**

Epidemiological studies indicate that type 2 diabetes is a risk factor for AD as diabetes patients have doubled chance of developing AD (81, 82). Longitudinal studies also provide additional evidence for this strong association and also suggest that diabetic patients show a worse disease progression than age-matched healthy people (83–86). In addition, neuroimaging of diabetic patients revealed structural changes similar to AD patients, in particular brain atrophy in the same regions (87–89). Perhaps more interestingly, diabetes patients display an impaired amyloid metabolism in pancreas (90). Furthermore, synaptic, mitochondrial and autophagic dysfunction are also among the pathologies common to AD and diabetes (91). Based on the data showing defective brain insulin signaling and glucose uptake in AD (92, 93), some researchers even proposed that AD is a type of diabetes (94). Although the jury is still out on this particular issue, a considerable amount of data suggest that hyperglycemia and insulin resistance could have detrimental effects in the brain, such as synapse loss, autophagic malfunction and neuronal death (93, 95, 96). Ultimately, these may trigger further dysfunction in A $\beta$  metabolism and lead to AD pathology. More insight into the ways to fine-tune cellular and systemic metabolic function could provide new strategies to alleviate the burden of neurodegeneration and metabolic dysfunction.

### **3 EXERCISE AS AN INTERVENTION**

“Eat less. Move more.” This simple mantra for health dates as far back as to Hippocrates, built on the long history of physical activity from Ancient Greeks and Romans. This seemingly rudimentary approach remains valid, perhaps today more than ever, with a pandemic blanketing all areas of our lives, lest our mobility. Naturally, the body adapts itself to physical inactivity but with serious consequences such as lower bone mineral density, loss of muscle mass and elevated blood pressure. Over time this leads to a lower functional capacity and a shorter lifespan. Indeed, physical inactivity contributes to a myriad of chronic diseases, including cardiovascular diseases, diabetes, obesity and even certain types of cancer and dementia (4, 5, 97). In addition, low exercise capacity is a predictive factor for premature mortality (3, 4). The other side of the coin is regular exercise which can act as a preventive factor for over 30 chronic diseases with its diverse health benefits (5). Moreover, besides being a preventive factor, exercise is prescribed as a therapy for many diseases (98, 99).

#### **3.1 EXERCISE CAPACITY AND RESPONSE**

Exercise is viewed as both primary prevention strategy and a disease intervention for chronic diseases (98). There is considerable focus on the value of exercise as an intervention after the disease onset where the aim is to prevent further decline in functional capacity, if not restore. An example of this is cardiac rehabilitation following a myocardial infarction which benefits the patient's health but cannot reverse the anatomic pathology (100). As a prevention strategy, exercise fosters general well-being which decreases the possibility of developing chronic diseases and thereby reduces overall mortality rates (3, 4). Perhaps, this is the most effective way to spread the benefits of exercise far and wide. However, not all people have the ability to perform the daily required amount of exercise due to lack of access or debilitating diseases (101, 102). Patients suffering from neuromuscular disorders, morbidly obese or bed-ridden lack the conditions to meet the daily exercise requirements. Some people, even though they can exercise, cannot gain as much from it because of the inherent variability in exercise capacity (103–105).

The outcomes of exercise training in terms of physiological adaptation are highly heterogeneous even in homogeneous groups with similar physiological traits such as weight, age, gender and fitness level. Underlying reasons for this are multi-layered and linked to the complexity of exercise capacity as a physiological trait. Both genetic and environmental factors contribute to exercise capacity and these factors can separately explain up to 50% of

the variance in humans (106, 107). Moreover, the inheritable factors at play can be classified as intrinsic which operates at sedentary state, and adaptive which is acquired through training and can be described as exercise response. The HEalth, RIsk factors, exercise Training, And GENetics (HERITAGE) Family Study, designed to dissect the effects of genetic and environmental factors on exercise capacity, provided evidence that there is strong familial inheritance in response to aerobic exercise in humans (103, 105, 106, 108). The response rate in terms of gains in maximal oxygen consumption ranged from 0 to 100% with nearly 20% of participants showing little to no change. Although it is evident that this high variance entails a strong genetic component, the underlying factors to responsiveness to aerobic exercise remain largely unknown.

The efforts to unravel the mechanisms that can improve our understanding of the link between exercise capacity and disease risk lacked accurate modelling until recently (109–111). Chemical-based models are often artificial interventions and gene-targeted models of inbred animals may not fully reflect polygenic, complex diseases including cardiovascular disease, diabetes and neurodegenerative diseases (112). In the early 2000s, new models emerged to study the intrinsic and response phenotypes and research into dissecting their roles and underlying molecular mechanisms have gained more momentum (109, 111, 113–115).

Increasing exercise capacity with exercise training is strongly associated with lower mortality rates from all causes(3). Given the variability in responsiveness to exercise and considering the limitations to exercising, finding ways to recapitulate the exercise effect with pharmaceutical intervention attracts considerable interest. In reality, mimicking exercise is no simple feat. Not least considering the variety of adaptations most of which depend on the type, duration and intensity of the exercise (116, 117). Plus, the health benefit of exercise in its entirety is too complex to be mimicked in a drug. Nonetheless, individual aspects of it can be harnessed to develop ways to combat the adverse effects of physical inactivity. Even a fraction of the adaptations might be of help in improving health, such as improving metabolism in diabetic patients or maintaining muscle mass in cancer patients. In this context, understanding the mechanisms governing these adaptations is crucial to develop therapeutic strategies.

### **3.2 SKELETAL MUSCLE ADAPTATIONS TO EXERCISE**

Skeletal muscle constitutes 40% of human body mass and contributes to the adaptation to changes in energy demand as well as whole-body energy homeostasis. Muscle contractions

contribute largely to our posture, breathing, and movement. As the primary tissue that enables physical activity, skeletal muscle often receives considerable attention in the context of exercise physiology.

Muscle response to exercise involves numerous changes in cellular processes including contractility to generate force and movement, mitochondrial content and function to meet with energy demands, tissue remodeling to repair muscle fiber damage, and transcriptional regulation to orchestrate it all (118–120). These processes are a culmination of a progressive change in mRNA and protein content which would then translate to optimal performance to maintain metabolic homeostasis in the face of an acute energetic challenge(121). Although this wave of changes stimulates a response, this transient response starts to fade away within hours and diminishes in a matter of days. Only when repeated, these acute pulses accumulate to long-term changes that facilitate maximal contractile function, substrate utilization and eventually improved muscle metabolism and resistance to fatigue (122). Consequently, while acute exercise triggers temporary changes, the majority of chronic adaptations to exercise results from the cumulative effect of regular exercise. These adaptations vary depending on a range of factors, including the exercise type, duration and intensity as well as the individual resting state, age, and the genetic background (123–126).

Different exercise types elicit different physiological changes leading to a training-specific outcome. Simplistically, these are improved aerobic capacity for endurance exercise and increased muscle mass and strength for resistance exercise. Recent reviews cover the subject of exercise-induced hypertrophy and its regulation in great detail (127, 128). In the context of this thesis, the focus will be on the adaptations to endurance exercise.

### **3.2.1 Energy metabolism**

Early adaptations to an acute bout of exercise include transcriptional changes in genes that mediate muscle function (129, 130). Moreover, around 1000 phosphorylation sites on over 550 proteins are reported to change following acute exercise demonstrating the vast network of pathways modified by exercise (131, 132). Many of these acute changes are related to energy metabolism.

Muscle contraction requires energy to fuel cellular mechanisms that contribute to force generation. These mechanisms depend on ATP and include maintaining an excitable sarcolemma,  $\text{Ca}^{2+}$  signaling through sarcoplasmic reticulum and the actin-myosin machinery (120). Metabolic factors such as enzymatic capacity, fuel and oxygen delivery influence the capacity to meet with the energy demands during exercise (116, 133). In addition, the nature

of the exercise, as in exercise intensity, duration, and mode, have a substantial effect on fuel utilization and source (126, 134). While the intensity of the exercise determines the fuel source and stimulates mobilization of extra fuel from energy stores if necessary, the energy expenditure and the ATP demand are tied up to the required power (122). For low-intensity exercise, the main energy source is glucose from circulation or derived from liver glycogen and free fatty acids (FFAs) from fat stores. When the sub-maximal exercise is kept at same intensity but prolonged, the contribution from lipid oxidation increases with the duration of exercise (122, 135, 136). During prolonged low to moderate-intensity exercise, the majority of skeletal muscle fibers engaged are of a type characterized by slow contraction and high oxidative capacity. Moderate to high-intensity exercise, on the other hand, shifts away from lipid oxidation and is more reliant on glucose derived from muscle glycogen (134). For example, during a short maximal capacity exercise, such as a sprint, due to the magnitude of energy required the regular ATP turnover raises almost 100-fold (137, 138). To exert maximal effort, more fast-fibers are recruited which primarily rely on glucose as a fuel. Therefore, through oxidative phosphorylation of intramuscular fuel storage, mainly glycogen and creatine-phosphate breakdown, the pathways for ATP synthesis get ramped up (139, 140). After the glycogen stores depleted, additional glucose from the liver glycogen stores and more fatty acids from adipose tissue are mobilized to be oxidized (122).

Conversely, adaptations to long-term regular exercise training are mainly within the oxidative metabolism pathways (141). In response to such training programs, skeletal muscle boosts transcriptional and enzymatic machinery to maximize its functional work capacity (142). To achieve that, fatty acid oxidation, substrate and oxygen uptake increase to feed into the oxidative phosphorylation.

In that sense, muscle metabolism is coupled to the vascular system to promote delivery and uptake of oxygen and fuel. In exercised muscle, the vascular system undergoes remodeling including an increase in capillary density (142, 143). Alongside, a higher mitochondrial content provides the necessary expansion in the capacity of the muscle to oxidize the upcoming substrate and generate the ATP required for force and contraction(144).

Macromolecule and organelle turnover through proteasome system and autophagy is another cellular process that participates in the adaptation to the challenges in cellular energy homeostasis including exercise (145, 146). Restriction in energy supply stimulates a compensatory protein and mitochondria turnover via autophagy to provide components for protein synthesis and to maintain homeostasis (146, 147). In an early study, Salminen and Vihko demonstrated that prolonged aerobic exercise increases autophagic flux to recycle

cellular components for regenerating muscle fibers (148). Particularly, they spotted damaged mitochondria in autophagosomes suggesting that in addition to its role in keeping basal homeostasis, autophagy serves to mitigate exercise induced disruptions including oxidative damage due to dysfunctional mitochondria. Subsequent studies confirmed the essential role of autophagy in exercise adaptation both in rodents (149–152) and humans (153–156). Blocking formation of autophagosomes diminishes training-induced increase in mitochondrial proteins, capillarization and improvement in aerobic capacity and results in altered glucose metabolism (150, 152).

### **3.2.2 Neuromuscular function**

For skeletal muscle function, muscle fibers rely on the input from axonal branches of motor neurons (MNs) in the spinal cord. Another way exercise promotes an increase in functional capacity is through improving this communication at the synapse site called neuromuscular junction (NMJ) (157). This coupling plays an essential role in almost every voluntary movement throughout life.

Proper NMJ development and function is critical for the skeletal muscle function. With aging or neuromuscular diseases, a progressive decline occurs in functional NMJs which is associated with loss of motor coordination, muscle weakness and atrophy (158, 159). NMJ dysfunction has been implicated as early-onset symptom and progression of sarcopenia and also observed in early stages of neuromuscular diseases and ALS (159–162). Lifelong activity has been reported to halt the progressive decline by supporting the maintenance of functional NMJs, with elite runners keeping their functional motor units into old age (163). Additionally, there is evidence showing that an exercise intervention also halts the age-related decline in NMJ function and morphology (164–166).

Physiologically and morphologically, NMJs are highly receptive and adaptive to exercise-induced changes which can occur at both the nerve (presynaptic) and the muscle (postsynaptic) compartment. In an early seminal work which analyzed NMJ electrophysiological properties of rat skeletal muscle, it became evident that endurance exercise induces an increase in stimulus-induced neurotransmitter (mainly Acetylcholine, ACh) release and raises resistance to fatigue during sustained stimuli (167). These adaptations serve to ensure effective communication from neuron to muscle during prolonged neuromuscular activity. Since effective NMJ function is a prerequisite for whole muscle function, these effects contribute to delayed muscle fatigue as well.

#### **Pre-synaptic changes**

Morphologically, exercise leads to increased presynaptic nerve terminal branching both in number and total length despite a maintained average branch length (162, 166, 168). In addition, nerve terminals contain more ACh vesicles in agreement with the aforementioned increased neurotransmitter release.

### **Post-synaptic changes**

As the nerve terminal is remodeled, postsynaptic endplate also undergoes an architectural change upon exercise. Together with a greater number of ACh receptors (AChR), the total area occupied by ACh receptors have been reported to increase (166, 169, 170). Remarkably, the release sites at the presynaptic terminals and receptor area on the postsynaptic membranes display matching exercise-induced changes. This demonstrates the tight coupling between the motor neuron and the muscle fiber to ensure efficient neurotransmission, even when NMJ is under significant reconfiguration. In fact, seminal work from the early 90s revealed that the training induced changes in the presynaptic terminals exhibit specificity based on the properties of the muscle fibers they innervate(171).

### **Muscle fiber – motor neuron coupling**

According to their physiological properties motor units are classified into three groups with speed of contraction as the main determinant: slow twitch, resistant to fatigue (S), fast-resistant (FR) and fast-fatigable (FF) (172, 173). In a similar manner to muscle fiber types, the functional diversity of motor units enables specification of the muscle fiber recruitment order and sustains prolonged activity. Metabolic and firing properties of the motor neuron and its motor units are coupled and it has been demonstrated that muscle fibers can adapt their contractility to the changes in synaptic input. For example, when fast muscle fibers are innervated by a slow motor neuron, they transform to acquire slow twitch fiber properties, and vice versa (173). While exercise-induced fiber type changes have been reported before, the jury is still out to determine whether exercise can stimulate such a shift. Similarly, our understanding of exercise-induced motor neuron plasticity is still limited. Only in recent years there has been a stride to reveal molecular signatures of MN identity (174–178) and examine the retrograde influence of muscle fiber adaptations on motor neurons and NMJ function (178). This topic still remains ripe with opportunity for additional research especially because we have just started to understand the detailed effects of exercise training on the elegant neuromuscular system and the plasticity of the NMJ.

### 3.2.3 Regulatory mechanisms of skeletal muscle adaptations

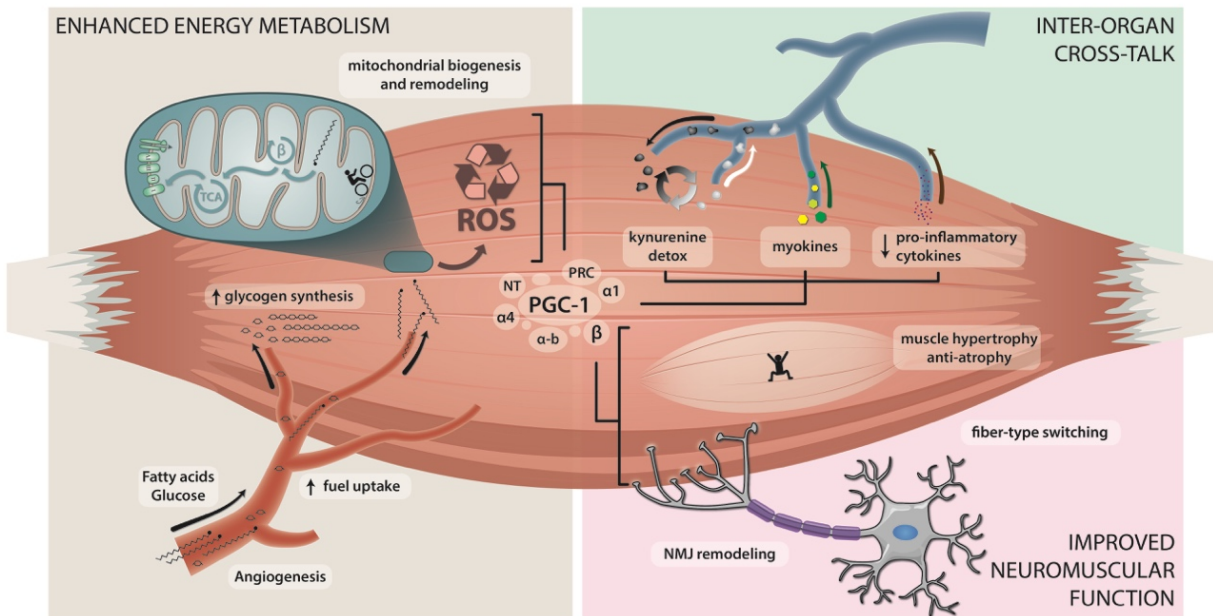
Many of the skeletal muscle adaptations are mediated by the coordinated action of a number of signaling and transcriptional regulatory mechanisms for exercise-induced gene expression and protein synthesis (120, 122). include  $\text{Ca}^{2+}$ /Calmodulin-dependent protein kinase (CaMK), mitogen-activated protein kinases (ERK1/2 and p38 MAPK) and AMP-activated protein kinase (AMPK). Subsequent cascades of reactions result in activation of multiple transcription factors and their coregulators (activators and repressors).

From a molecular perspective, the peroxisome proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) is a central regulator of several mechanisms that couple cellular energy demand and supply(179). Almost all members of PGC-1 family of coactivators have an effect on cellular energetics. In addition, depending on the tissue, PGC-1 coactivators can interact with a variety of transcription factors to mediate tissue-specific transcriptional programs.

Following its identification as a transcriptional regulator of genes involved in cold-induced thermogenesis in brown adipose tissue (180, 181), an extensive body of work established PGC-1 $\alpha$ 1 as an important regulator of skeletal muscle adaptation to exercise (179, 182–184). Skeletal muscle PGC-1 $\alpha$ 1 expression levels, both mRNA and protein, rapidly increase in response to acute exercise (184, 185), with the highest abundance observed in the first couple of hours after the exercise (121).

PGC-1 $\alpha$ 1 coactivates transcription of hundreds of genes encoding for proteins involved in mitochondrial biology through the nuclear respiratory factor and estrogen-receptor-related receptor- $\alpha$  (ERR $\alpha$ ) while simultaneously promoting mitochondrial-DNA replication and transcription (181, 186, 187). Along with promoting consumption of fuel and oxygen in mitochondria, PGC-1 $\alpha$ 1 also facilitates an enhanced fuel and oxygen uptake by inducing angiogenesis through increased VEGF expression independent of its traditional regulator hypoxia-inducible factor 1 (HIF-1). The PGC-1 $\alpha$ 1-induced angiogenic gene program is not necessary for basal tissue vascularization but it is required for exercise-induced angiogenesis (188, 189). Furthermore, overexpression of PGC-1 $\alpha$ 1 in skeletal muscle increases glucose uptake through upregulation of the insulin-sensitive glucose transporter 4 (GLUT4) (190). Finally, PGC-1 $\alpha$ 1 elevates basal autophagic flux and also increases mitochondrial turnover in trained muscle (191).

Known as the master regulator of mitochondrial biogenesis, high levels of PGC-1 $\alpha$ 1 bring skeletal muscle many advantages including enhanced fuel uptake, oxidative capacity and neuromuscular function (179) (Figure 4). Indeed, transgenic animals overexpressing PGC-1 $\alpha$ 1 specifically in skeletal muscle (MCK-PGC-1 $\alpha$ 1) are protected from muscle atrophy, age-related sarcopenia and insulin resistance (182). Remarkably, the same animals also display an extraordinary endurance capacity without training and are resistant to contraction-induced fatigue.



**Figure 4:** PGC-1 mediated exercise adaptations in skeletal muscle (Correia et al 2015, reprinted with permission)

Interestingly, it has been shown that diabetic mice(192) and humans (193) suggest a loss of PGC-1 $\alpha$ 1 function in skeletal muscle, which could contribute to the mitochondrial dysfunction and impaired glucose metabolism observed in these diseases. Considering that nearly 85% of the insulin-stimulated glucose uptake happens in skeletal muscle, factors regulating this process influence whole-body glucose metabolism. Moreover, mice lacking PGC-1 $\alpha$  in whole body have defective autophagy machinery and impaired mitophagy suggesting that PGC-1 $\alpha$ 1 is necessary for exercise-induced autophagy (194).

Since any skeletal muscle function is dependent on neuromuscular function, it is also not surprising that PGC-1 $\alpha$ 1 is driving some of the exercise induced changes in NMJ structure and function (178, 195–197). Strong evidence highlights that PGC-1 $\alpha$ 1 coactivates the transcription of a variety of genes that are involved in improving post-synaptic NMJ morphology and function (195, 196). Its absence retards AchR clustering and reduced levels of critical genes for NMJ function such as AchRs, muscle-specific kinase and utrophin,

suggesting that PGC-1 $\alpha$ 1 plays an essential role in NMJ maturation and maintenance (195). Its utility has been put to use in a mouse model of muscle dystrophy where skeletal muscle PGC-1 $\alpha$ 1 expression ameliorated muscle function and in a genetic model of ALS carrying SOD1G37R mutation where muscle PGC-1 $\alpha$ 1 increased AchR clustering and helped retain muscle mass and function (195, 198).

The effects of PGC-1 $\alpha$  on NMJ are not limited to the postsynaptic level and expand into the presynaptic compartment as well (178, 196, 197). Transgenic mice overexpressing PGC-1 $\alpha$  in the skeletal muscle exhibit longer branches and more synaptic vesicles at the nerve terminals. In addition, it appears that these animals have a high frequency of slow motor units where fast fibers were innervated by slow motor neurons (196). This confirmed the initial findings reported by Chakkalakal et al. suggesting that muscle PGC-1 $\alpha$ 1 can have an influence on the coupling between motor neuron and muscle fiber identities(178). Taking the oxidative and fatigue resistant phenotype induced by muscle overexpression of PGC-1 $\alpha$ 1 into consideration, the shift towards slower identity is plausible and it fits the physiological output. A similar shift happens when PGC-1 $\alpha$ 1 is delivered postnatally, after synapse elimination at the NMJ is completed. It is important to note that muscle-driven plasticity of motor neuron identity has long been sidelined and understudied. The specific signals that might act retrogradely to induce this effect remain to be determined and offer new possibilities for diseases that involve neuromuscular dysfunction. In this regard, using a microfluidics system to model muscle-MN communication and NMJ formation, our group recently identified a neurotrophic factor called Neurturin (NRTN) that acts downstream of PGC-1 $\alpha$ 1 and mediates its effects on pre-synaptic remodeling by promoting motor neuron recruitment as well as NMJ formation (197).

### **3.3 WHOLE-BODY ADAPTATIONS TO EXERCISE**

Although the muscle plays a big part, both acute and regular voluntary exercise orchestrates numerous adjustments well beyond the skeletal muscle (116). As the motor cortex drives the locomotion through spinal cord and muscles, the brain also signals to other systems to ensure metabolic demands are met and the whole-body homeostasis is maintained.

Accumulating evidence shows that exercise improves brain function in multiple ways both in rodents and humans (199–201). Improvements in cognition, motor coordination and balance, memory and learning are to name a few. Accordingly, the pathology of a variety of neurological diseases such as stroke, traumatic brain injury, anxiety and depression are alleviated by physical exercise. Some of the beneficial effects of exercise have been attributed

to direct changes in intramuscular mechanisms regulating potentially detrimental mechanisms. For example, exercise offers protection from stress-induced depression through PGC-1 $\alpha$ 1 mediated kynurenine detoxification (202, 203).

Cardiovascular and respiratory systems are critical for the whole-body adaptations to exercise. Increased cardiac function together with improved vascular system contributes to an enhanced aerobic capacity which is protective against cardiovascular diseases (204). The respiratory system increases ventilation proportional to the exercise intensity and facilitates sufficient oxygenation (116). In addition, sympathetic and autonomous systems monitor and adjust critical parameters such as heart rate, blood pressure among others.

Exercise-driven effects in energy metabolism observed in skeletal muscle are also reflected to adipose tissue. Among these are elevated mitochondria content, increased free fatty acid mobilization, beta-oxidation and lipolysis (205, 206). In parallel, liver metabolism drastically changes in response to energetic demands of exercise(207). As a nutrient sensor, the liver is in a critical position to maintain whole body energy homeostasis during fasting or high energy demand situations where glucose is in short supply. As skeletal muscle glycogen stores get depleted, glucose release to bloodstream from liver increases, first through hepatic glycogen breakdown (glycogenolysis) and then from non-carbohydrate substrates (gluconeogenesis). In addition, secreted proteins from the liver, such as the insulin-like growth factor 1 (IGF1), are involved in glucose metabolism.

Under low glucose conditions like prolonged intense exercise, the liver produces ketone bodies as an alternative energy source for oxidative phosphorylation (208). Of these,  $\beta$ -hydroxybutyrate which can cross the BBB, has been reported to increase in circulation after voluntary wheel running in mice and promote exercise-induced plasticity in the brain through neurotrophic factors (209).

Exercise also effects immunity although in a complex and context dependent manner (210–212). Regular exercise is known to reduce systemic inflammation levels (213). Conversely, some studies claim prolonged intense exercise triggers systemic inflammation and increases infection risk (213–215). Furthermore, muscle repair and regeneration requires a precisely orchestrated immune cell activation (211, 212). The detailed mechanisms of the exercise-immune system interplay still remain enigmatic and may involve fine tuning of inflammatory cues from multiple sources. Interleukin-13 (IL-13), for example, is released by resident immune cells in the exercised muscles and enhance endurance by increasing metabolic flexibility in mice (216).

## **4 INTER-ORGAN COMMUNICATION**

At any given time, an intricate network of communication coordinates a multitude of basal mechanisms to keep homeostasis which results from collaborative effort by multiple systems and organs. During a challenge induced by an external stress, such as exercise, each member of this network triggers an adaptive response and activates another channel for crosstalk so the system as whole can ensure proper function.

In this realm, there has been much effort to expand our knowledge about mechanisms involved in the inter-organ communication, both in health and disease. Systemic factors involved in this process can originate from multiple tissues and cell types, go through a complex journey spanning various organs and stimulate different downstream signals. So far, we only scratched the surface of this journey, albeit only for specific factors and conditions. Inquiries into the circulating factors employed a range of strategies from the century-old parabiosis (38, 217, 218) to plasma transfer (219–221), advanced large-scale proteomics (222–224) and bioinformatic tools (225).

In the context of exercise and skeletal muscle, although the clinical benefits of exercise have been known for a long time, the bulk of what we know about the systemic mediators of these effects are relatively recent discoveries. Future investigations into muscle-derived factors that enact exercise-related changes in distant parts of the body could provide useful tools to our arsenal in fighting with effects of inactivity, aging and associated disorders.

### **4.1 MUSCLE AS A SECRETORY ORGAN**

More than half a century ago, Goldstein hypothesized that skeletal muscle released a humoral factor in response to the elevated energy demands during physical activity (226). The early view was that the contracting skeletal muscle induces a physiological and metabolic response in other organs and this may be independent of the nerve input. This hypothesis was strengthened with the finding that electrically-stimulated muscles lacking both efferent and afferent nerve impulses due to spinal cord injury induce nearly same physiological changes as the uninjured muscles of healthy patients (227–229). So, the quest to decipher the language of the skeletal muscle began.

It was during the search for a link between changes in the immune system and muscle contractions when Pedersen and colleagues turned to cytokines as potential messengers. In a study with marathon runners, they observed a robust increase in Interleukin-6 (IL-6) levels in the post exercise plasma and at first considered it as a result of immune response to

prolonged exercise (230, 231). Instead, Steensberg *et al.* revealed that the skeletal muscle-derived IL-6 accounts for the elevated levels in circulation (232). Further studies confirmed the metabolic effects of muscle-derived IL-6, mainly as an energy sensor mediating glucose turnover during exercise (232–235). Altogether, these studies and others accumulated into the hypothesis that muscle-derived cytokines may be involved in exercise-induced changes and long-term metabolic adaptations.

Identification of muscle as a secretory organ opened up new avenues of research. In a way, it paralleled the concept of adipose tissue as an endocrine organ, which was initially regarded as fat storage and later on revealed as a major secretory organ thanks to the work of the Spiegelman group (236) towards the end of the 1980s, the Friedman group in the 1990s (237) and the following discoveries of adipokines (reviewed in 182,213).

## **Myokines**

Echoing the term adipokine, in 2008 Pedersen and Febbraio coined a term for the “muscle cytokines” and called for “cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert either autocrine, paracrine, or endocrine effects” to be classified as myokines (239).

By now, the research into myokines revealed that several hundred peptides are secreted from skeletal muscle (240) and novel myokines continue to expand our understanding of how skeletal muscle communicates with the rest of the body.

## **4.2 MYOKINES WITH AUTOCRINE AND PARACRINE EFFECTS**

The adaptive response to exercise likely reflects the combined action of myokines and other factors to induce profound changes in skeletal muscle transcriptional machinery as discussed above and overall metabolism. Different myokines elicit different and sometimes redundant effects on muscle physiology, which indicates that despite having overlapping functions, the outcome may be regulated by their abundance, receptor availability and other spatially delimited cues.

### **4.2.1 On muscle metabolism**

The first muscle-derived factor to be named as myokine, IL-6, was discovered due to its effect on muscle glucose metabolism in an autocrine fashion. Known primarily as a pro-inflammatory cytokine, IL-6 has been implicated in insulin resistance in obesity and type 2 diabetes (239, 241). At first, IL-6 involvement in the metabolic response to exercise appear

counterintuitive due to its pro-inflammatory characteristics and the anti-inflammatory nature of exercise. However, there is evidence that exercise-induced IL-6 release from the skeletal muscle is highly proportional to the duration and intensity of the exercise and has beneficial effects on muscle metabolism (212, 242). Systemic administration of IL-6 to mimic the exercise effect in humans sensitizes skeletal muscle to insulin and promotes GLUT4 translocation to the plasma membrane (233). Moreover, in vitro and in vivo evidence suggest that this action is mediated by AMPK. Contrary to what might have been expected, preclinical studies with IL-6-deficient mice showed no difference in glucose uptake or AMPK activity after treadmill running or at resting state (243, 244) or restricted to a specific muscle type (245). In addition, energy metabolism appeared to be unaltered in mice with embryonic IL-6 deletion in muscle (246). Although these data raise questions on the role of IL-6 in skeletal muscle glucose uptake, additional investigations using inducible tissue-specific conditional knockout models should allow a more careful dissection of pleiotropic nature of IL-6 actions.

Muscle contraction and exercise also promote expression of brain-derived neurotrophic factor (BDNF). Although BDNF is mainly known for its actions on neurons (247, 248), for muscle fibers it can also act as a metabolic regulator in response to exercise (249, 250). Human muscles ramp up BDNF expression following exercise and in turn BDNF enhances lipid oxidation in an AMPK-dependent manner, thus BDNF contributes to fuel selection and energy expenditure in skeletal muscle (251). In a similar way but through different mechanism, another exercise-induced myokine called Musclin influences energy expenditure by promoting mitochondrial biogenesis through PGC-1 $\alpha$ 1 (252, 253).

#### **4.2.2 On muscle growth**

Besides metabolic improvement, another exercise-induced change that can be locally influenced by myokines is muscle growth. Myostatin (MSTN), one of the oldest myokines that fulfils the criteria outlined by Pedersen & Febbraio (239), is known to negatively regulate muscle growth (254, 255). Famously, genetic ablation of MSTN results in a massive muscle hypertrophy phenotype in mice (255), dogs (256) cattle (254, 257) and humans (258). With such a distinctive knockout phenotype, the regulation and function of MSTN received a lot of attention with hopes of utilizing this information to boost muscle mass in a variety of species, including humans. Therapeutically, control of muscle mass by MSTN have been explored in a number of muscle-wasting conditions (259). One of these is complete spinal cord injury (SCI), which in rodent models led to an increased sensitivity to MSTN (260). Blocking MSTN throughout 8 weeks-post-SCI recovered some of the body mass but mostly

in the upper limbs and not in the paralysed muscles indicating that MSTN inhibition alone is not sufficient to prevent muscle loss after denervation-induced muscle wasting. MSTN has also been associated with the pathogenesis of muscle wasting in cancer cachexia and sarcopenia. (261–264). The effects and the clinical premise of MSTN for these muscle wasting conditions still need to be explored taking the progressive and complex nature of muscle loss into account. Similar to MSTN, myotube-produced IL-6 is also reported to regulate satellite cell-mediated myogenesis where the genetic deletion of IL-6 led to muscle hypertrophy (265). Yet the complexity of IL-6 biology also calls for a matching multifaceted approach to dissect this connection to muscle size. Another recently identified myokine, apelin, is proposed as an autocrine agent that induces muscle hypertrophy (266) and interestingly there is some data showing an age-related progressive decline in skeletal muscle apelin expression suggesting a potential contribution to age-related sarcopenia(267).

#### **4.2.3 On neuromuscular function**

Myokines can mediate muscle function through remodelling of NMJs. As a factor closely associated with motor neuron survival and function, it comes as no surprise that BDNF has effects on NMJ morphology and function. In a recent study, Delezie et al. demonstrated that skeletal muscle-specific BDNF knockout reduces endplate volume, promotes glycolytic muscle fiber phenotype and enhances endurance performance (250). Increasing evidence places BDNF as an emerging myokine with effects on skeletal muscle metabolism and function.

Finally, covering all the myokines with autocrine or paracrine effects which are reviewed elsewhere would go beyond the scope of this thesis (268, 269).

### **4.3 MYOKINES WITH ENDOCRINE EFFECTS**

#### **4.3.1 On neurological functions**

Over the course of a millenia, philosophers argued a version of the latin phrase “Mens sana in corpore sano” meaning a healthy mind in a healthy body. Evolutionary, epidemiological and clinical evidence have demonstrated the neurobiological benefits of exercise suggesting a link between skeletal muscle and central nervous system (CNS). Although the existence of such a link has been speculated for a long time, the underlying mechanisms and systemic mediators of this connection have remained largely unexplored until recently. Even though many myokines have autocrine or paracrine functions as discussed above, some are released into the bloodstream and affect other organs. The ever-expanding research into muscle

secretome and myokines may, at least in part, provide insight into the long-suspected connection between skeletal muscle and CNS.

CNS adaptations to exercise are broad but perhaps the most prominent are the changes in hippocampus and the memory driven by BDNF. In addition to its role in the benefits of physical activity in the brain, multiple reports suggest that BDNF may also take part in metabolic and behavioral responses to exercise, including glucose metabolism (10, 270). Indeed, evidence from human studies show that exercise-induced BDNF expression is not confined to brain, it is also induced in the skeletal muscle (271). However, the findings on whether the muscle-derived BDNF is released to circulation after exercise have been inconclusive. Some studies report an increase and others no change or a decrease in the peripheral levels of BDNF (199, 271). Several factors could contribute to this inconsistency including exercise intensity and duration, type of exercise, and measurement method to name a few. Of note, these studies often do not aim to distinguish the source of BDNF and just determine the serum or plasma level upon exercise.

While the debate whether muscle-derived BDNF is released into circulation remains inconclusive, FNDC5/Irisin, a myokine primarily known for its role in exercise-induced browning of adipose tissue has also been shown to induce BDNF expression in the hippocampus after endurance exercise (272). Blocking FNDC5 specifically in the brain results in impaired hippocampal plasticity and memory while boosting the FNDC5/Irisin levels rescues this phenotype (273). Furthermore, these effects seem to be translated to peripheral administration of FNDC5/Irisin, which offers protection against A $\beta$ -induced memory impairment. Myokines mediating beneficial effects of exercise are often altered in age-related neurodegenerative diseases where cognitive impairment is accompanied by aggravated muscle loss and physical inactivity. In line with this, Lourenco et al observed that levels of FNDC5/Irisin were markedly reduced in the cerebrospinal fluid and the hippocampus of AD patients (273). Although cleavage and secretion of FNDC5/Irisin is still subject of a debate, systemic elimination from circulation with a neutralizing antibody seem to abolish the effects of exercise on cognitive function in an AD mouse model (273).

Some recent studies pinned a role to another myokine cathepsin B (CTSB) in exercise-induced brain plasticity. Treadmill running elevates CTSB levels in gastrocnemius muscle and plasma and CTSB levels in human plasma positively correlates with the fitness level as well as spatial memory. In addition, genetic ablation of CTSB hampers the memory improvement and adult neurogenesis associated with voluntary running in mice (274).

However, CTSB function in the CNS has been a controversial topic due to its pathogenic effect promoting cell death following several brain injury models (275, 276).

#### **4.3.2 On energy metabolism**

Given that adipose tissue is the largest site of energy storage in the body, muscle and adipose tissue are functionally connected coordinators of energy homeostasis. Increased energy demand during exercise is met by fatty acid mobilization from the adipose tissue to skeletal muscle where an internal machinery to enhance fatty acid oxidation is also triggered by exercise. Myokines are involved in this axis have effects on lipolysis and adipose tissue metabolism. Exercise induced IL-6 secretion serves to feed this energy loop where skeletal muscle release of IL-6 stimulates lipolysis and FA mobilization to provide necessary fuel for the sustained physical activity (277). Besides being an energy storage, adipose tissue, depending on its location and type, can also serve as a source of heat to protect against cold exposure(278) This thermogenic regulation is another way myokines affect adipose tissue metabolism and it is achieved by promoting a brown adipose tissue phenotype with increased energy expenditure.

FNDC5/Irisin is one of these myokines promoting a brown fat phenotype in response to exercise (279). In mice, acute treatment with recombinant FNDC5/Irisin is sufficient to induce browning and insulin sensitivity while in humans there is still ongoing debate around this topic. Similarly, another myokine called meteorin-like (Metrnl) has been identified as downstream effector of PGC-1 $\alpha$  isoform 4 with effects on thermogenesis and browning and its role in human adipose tissue remains elusive (280).

## 5 AIMS

Overarching aim of this thesis is to investigate mechanisms involved in the systemic effects of physical inactivity and provide new perspectives for future therapeutic interventions.

Specific aims:

**Paper I:** To understand the mechanisms that regulate Presenilin-1 function on Alzheimer's disease pathogenesis

**Paper II:** To investigate the molecular signatures of inherent and response components of aerobic exercise capacity in the skeletal muscle and utilize them to predict muscle-derived factors that can have systemic effects

**Paper III:** To delineate the role of the neurotrophic factor Neurturin in the skeletal muscle adaptations to exercise and muscle fiber-motor neuron coupling



## 6 RESULTS AND DISCUSSION

### 6.1 PAPER I: ANTI-AMYLOIDOGENIC FUNCTION OF PRESENILIN 1

One of the hallmarks of AD is A $\beta$ -associated toxicity, including the senile plaques that are often used as a post-mortem diagnostic marker. The  $\gamma$ -secretase complex is an essential enzyme for APP processing, and its catalytic subunit Presenilin 1 (PS1) is mainly responsible for A $\beta$  generation in neurons. Despite its pivotal role, the precise mechanisms that govern PS1 function have remained enigmatic. In **Paper I**, we investigated potential mechanisms that can regulate PS1 function and uncovered a unique role for PS1 in A $\beta$  metabolism (Figure 5). We showed that PS1 can reduce A $\beta$  production by directing  $\beta$ -CTF for autophagic clearance and provided mechanistic evidence for PS1-phosphorylation at serine 367 (S367) as a regulator of this activity.

Studies of PS1 phosphorylation indicate that it does not affect  $\gamma$ -secretase activity, while some specific residues are suggested to potentially influence its stability or conformation (281). In addition, cryo-electron microscopy revealed that human  $\gamma$ -secretase displays a closed conformational structure associated with its pathogenic activity (282). Detailed analysis of PS1 phosphorylation pattern pointed at potential sites that seem critical for this closed pathogenic conformation (283). S367 was among these sites and it seemed to have a critical role in mediating PS1 activity. Our investigation identified its role as a signal to promote degradation of  $\beta$ -CTF before it is further cleaved to produce A $\beta$ .

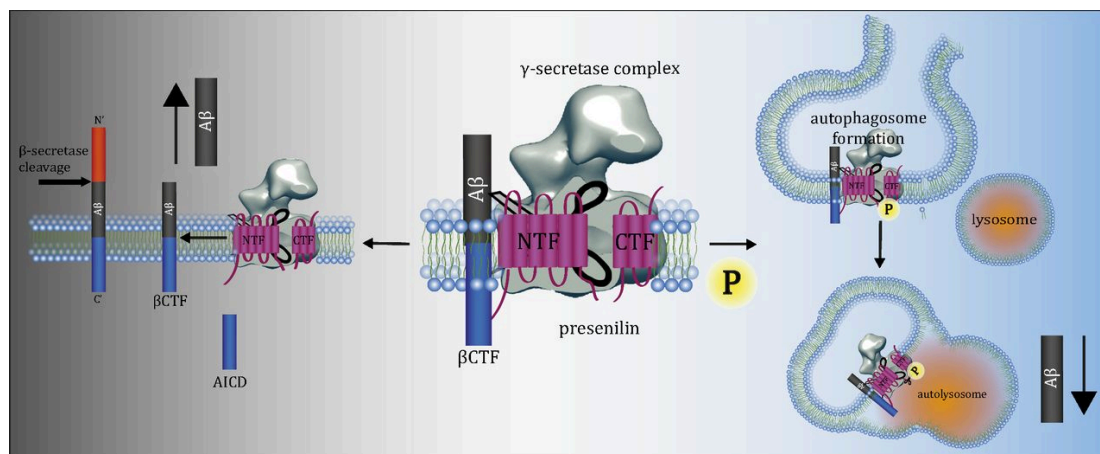
To study protein phosphorylation, performing amino acid substitutions to alter the chemical characteristics is a commonly used approach. For example, substitution of the serine (Ser) residue by alanine (Ala) serves to prevent phosphorylation at that particular residue as Ala is not chemically receptive to phosphate groups. Using this approach, we developed an in vivo substitution model where S367 is replaced by Ala in J20, a murine model of AD (here in J20-S367A animals are referred as S367A). In agreement with the findings of Maesako et al, this substitution did not interfere with  $\gamma$ -secretase activity demonstrating phosphorylation of S367 does not influence assembly or function of  $\gamma$ -secretase (283). Interestingly, both soluble and insoluble A $\beta$  levels increased in the brains of S367A animals and they displayed a heavier plaque burden in the absence of S367 phosphorylation.

For a positive control, we wanted to model S367 phosphorylation in the same way as S367A and replaced the S367 with aspartic acid residue which chemically resembles the phosphorylated Serine (S367D). However, these animals did not show the autophagy-driven

protective effect with similar levels of A $\beta$  and plaque deposition as S367A. There can be multiple reasons for this, starting with the challenge of achieving a true phosphomimetic substitution of Serine with Aspartic acid. Others have reported similar failures to mimic the actual phosphorylation effect (284–287). Structure and charge of the phosphate group could be necessary for this particular activity, or the structural properties of replaced residue might influence the outcome.

We also set out to identify the kinase responsible for phosphorylating this residue. Using motif-based prediction tools, we identified the casein kinase 1 (CK1) family as a candidate. This was in line with previous data pointing at the potential involvement of CK1 in  $\gamma$ -secretase regulation (288). A systematic in vitro knockdown of known isoforms and treatment with isoform specific inhibitors revealed CK1 $\gamma$  isoform phosphorylates PS1 at S367. Based on our findings, CK1 $\gamma$  activators can be explored as potential therapeutic agents for AD.

Autophagy and APP processing has been linked in some studies (289–291). Based on our results, both in vitro and in vivo evidence points to impaired autophagic flux in the absence of PS1 S367 phosphorylation. In a parallel study, our group further detailed this mechanism showing that PS1 phosphorylation at S367 facilitates autophagosome-lysosome fusion (292).



**Figure 5:** Working model for the dual role of PS1 in A $\beta$  metabolism (293).

Taken together, these results suggest that PS1 phosphorylation at S367 is crucial for  $\beta$ CTF degradation through autophagy. The implications of this study are broad as they open a new avenue in PS1 biology and A $\beta$  metabolism. If broad or neuron-specific autophagic impairment drives the pathology of AD, advancing our understanding of autophagy-promoting mechanisms such as exercise and their specific mediators could potentially be exploited as preventive measures for neurodegeneration.

## **6.2 PAPER II: DISSECTING INTRINSIC AND ADAPTIVE COMPONENTS OF EXERCISE CAPACITY**

There are numerous benefits to increasing exercise capacity, including reduced mortality and morbidity rates. Conversely, low exercise capacity is linked to a higher risk of developing complex diseases. In this study, we revealed muscle transcriptional signatures of intrinsic aerobic capacity and response to aerobic training in rats and humans.

Exercise capacity is a complex trait and is linked to chronic metabolic disorders. We took advantage of a novel approach to study the intrinsic and acquired exercise capacity in skeletal muscle. In the early 2000s, Koch and Britton developed low and high aerobic capacity rat models using a two-way selective breeding strategy based on untrained endurance running performance (113, 115). Similar to humans, exercise capacity also involves an inheritable component for these rats, therefore selective breeding led to the divergence of low and high capacity runner rats (LCR and HCR). A similar breeding strategy for response to training led to the low and high responder rat lines (LRT and HRT), which have the same baseline capacity but divergent responses to aerobic exercise training.

Using these models, our muscle transcriptome analysis identified potential molecular pathways and genes that might influence aerobic exercise capacity and response to aerobic exercise. Since there is no “wild type” control for these models, comparisons are done between the extremes, creating a duality in the results. For instance, if a differentially regulated gene is induced in HCR, it is reduced in LCR. The muscle transcriptome of HCRs was enriched for genes related to angiogenesis and oxygenation, well-known muscle adaptations to exercise (294–298). Conversely, the LCR transcriptome profile was associated with biological functions concerning inflammation and cardiovascular disease. This is in line with the reports documenting the poor health condition of LCR rats and their risk of developing complex diseases (299–302). Altogether, these findings suggested that the transcriptional profiles can be useful for investigating mediators of exercise capacity.

Our analysis for potential upstream regulators pointed at DNA methylases as activated in LCRs and reduced in HCRs. An extensive body of knowledge links DNA methylation and genetic inheritance and regulation of gene expression. Although the exact mechanisms remain elusive, there is some evidence suggesting that exercise induces DNA hypomethylation in the human skeletal muscles (303, 304). Considering the inheritability of exercise capacity, this could be a potential mechanism to pass it on to the offspring.

An obstacle to fully harness the therapeutic potential of exercise programs is the variability in individual response to training. Therefore, understanding what factors govern the adaptive capacity holds a great value to devise future exercise-based therapies. Skeletal muscle transcriptome of HRT-trained (HRTT) and LRT-trained (LRTT) rats did not show obvious signatures compared to the HCR-LCR rats. Skeletal muscle has been shown to be a major contributor to the HCR phenotype with increased local oxygenation, vascularization and enhanced fuel handling (294, 296, 297, 305). Exercise training response, on the other hand, might involve other organs and mechanisms. When trained, low responders regulated a higher number of genes compared to high responders. This raises the possibility that inherent inhibitory systems may be acting to prevent exercise adaptations, for example, inflammatory cues (306, 307).

Secreted factors (secretome) could execute wide-reaching effects and could provide new avenues to harness the health benefits of exercise. We employed a bioinformatic pipeline to predict muscle secretomes of exercise capacity and response. One striking result is the high number of circulatory factors predicted to be enriched in the high capacity muscles. Inherent high exercise capacity may rely on high levels of such factors involved in energy metabolism. Conversely, responders are predicted to have more factors secreted to the extracellular matrix. Interestingly, these were upregulated in low responders. Failure to cope with the repeated stress from the exercise training could induce a signal for repair and remodeling in LRTT muscles. This is consistent with previous data on exercise-induced pathways in both rats and humans (306).

Lastly, we compared muscle transcriptome signatures of exercise capacity and response profiles of rats and humans (123). Correlation of mitochondrial respiration levels with gene expression at the untrained state revealed 8 genes shared between rats and humans with high aerobic capacity. Interestingly, expression of a known epigenetic modulator, PKD1, positively correlated with aerobic capacity in humans. There is active research into epigenetic modifications and inheritance of exercise adaptations and diet-induced changes (308). Possibly, PKD1-mediated histone modifications might be involved in determining the intrinsic exercise capacity. Additional investigation into validating this correlation and detailing potential links to exercise responsive genes could further this hypothesis.

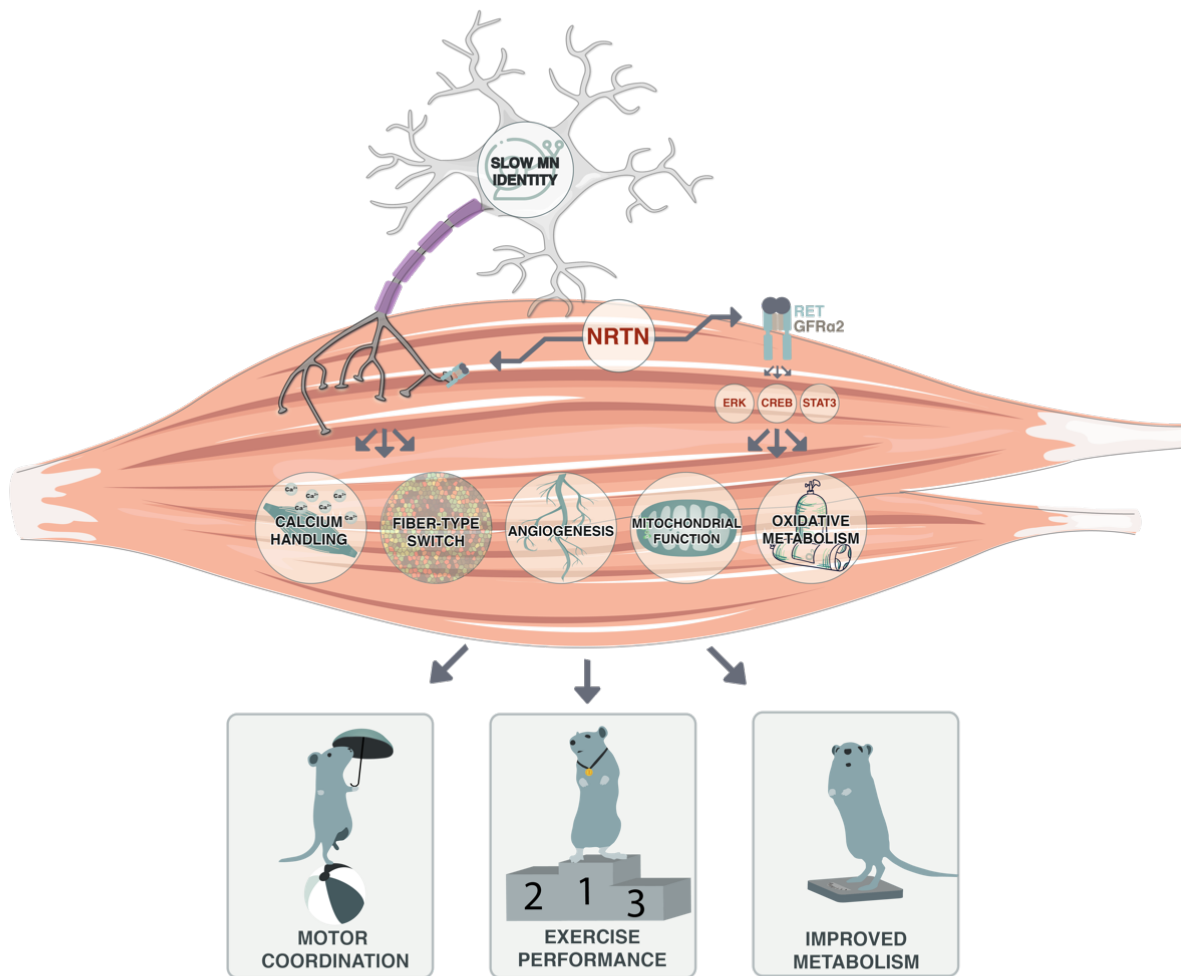
Overall, this study lays the foundation for dissecting the molecular signatures of exercise capacity and response in skeletal muscle. Understanding the drivers and mechanisms of these components could have a big impact on the ongoing efforts to design personalized exercise programs as a prevention and therapeutic intervention strategy.

### **6.3 PAPER III: EXERCISE-INDUCED MYOKINE NEURTURIN PROMOTES SLOW MOTOR NEURON IDENTITY, ENHANCES OXIDATIVE METABOLISM, ENDURANCE AND MOTOR COORDINATION**

It is widely appreciated that the transcriptional coactivator PGC-1 $\alpha$  mediates many of the known exercise benefits, including skeletal muscle adaptations. Pharmaceutical targeting of skeletal muscle PGC-1 $\alpha$  could induce some of the exercise adaptations and expand the possible treatment options for many muscular, metabolic, and neurological disorders. However, efforts to activate or mimic PGC-1 $\alpha$  have been largely unfruitful (309), mainly because transcriptional coactivators are quite challenging therapeutic targets and systemic activation of PGC-1 $\alpha$  could have unwanted effects (310). For this reason, much effort is directed at identifying factors acting downstream of PGC-1 $\alpha$  which would circumvent these hurdles and still deliver some of the PGC-1 $\alpha$  mediated effects.

In this study, we explored one of the myokines known to be induced by PGC-1 $\alpha$ , NRTN, and its effects on muscle metabolism and function (Figure 6). Among the known PGC-1 $\alpha$ -induced myokines, which mainly act systemically as endocrine agents, NRTN stands out with its autocrine effects on muscle metabolism and retrograde influence on motor neurons at the same time. Our results indicate that NRTN holds a therapeutic value both from the metabolic and neuromuscular perspectives. To explore the effects of muscle-derived NRTN, we generated a transgenic mouse expressing NRTN under the human skeletal muscle actin promoter (HSA-NRTN).

Metabolically, HSA-NRTN animals closely resemble the phenotype of PGC-1 $\alpha$  muscle transgenic animals (MCK-PGC1 $\alpha$ ) (182). They exhibit a similar switch in metabolic characteristics of muscle fibers, smaller and more oxidative fibers with enhanced mitochondrial function and increased vascularization. At the same time, NRTN seems to reprogram the muscle transcriptome to optimize energy metabolism, in particular fuel handling. Expression of genes involved in fatty acid uptake, intracellular transport and oxidation were markedly induced while glycolytic genes were repressed in skeletal muscles of HSA-NRTN mice. Although these animals are leaner and have improved glucose metabolism, there was no change in energy expenditure or adipose tissue-driven thermogenesis, representing a divergence point from other PGC1 $\alpha$ -driven myokines.



**Figure 6:** Summary of paper III results. Myokine Neurturin enhances calcium handling, vascularization and oxidative metabolism in the skeletal muscle. Alongside, Neurturin promotes a slow motor neuron identity. Functional outcomes of muscle-derived NRTN are better motor coordination, endurance performance and improved metabolism.

Our data demonstrate a NRTN-driven shift towards slow MN characteristics. HSA-NRTN animals display smaller NMJs with less complexity and reduced branching at nerve terminals, which morphologically describe slow MNs (311). This is another point where HSA-NRTN phenotype does not fully overlap with that of MCK-PGC-1 $\alpha$ 1 (312, 313). Similarly, NRTN did not activate a post-synaptic gene program in the muscle fibers, unlike PGC-1 $\alpha$ 1. Together, our data indicates that NRTN-induced NMJ remodeling results from its retrograde signaling to MNs. Indeed, transcriptomic analysis of spinal MNs of HSA-NRTN mice showed transcriptional changes with reduced expression of fast MNs markers (*Chodl*, *Calca*) and a trend towards increased slow MN markers (*Sv2a*, *Esrrb*). Although not planned as an experimental output, genotype-blind laser capture microdissection of MNs indicated a shift towards smaller soma size in these animals which is consistent with slow MN identity. Overall, these results demonstrate an extraordinary role for muscle fiber-derived NRTN in remodeling NMJ and retrogradely mediating MN identity.

Other studies also demonstrated some muscle-secreted neurotrophic factors acting on NMJs (178, 250, 314). However, it seems that neurotrophic factors induce different changes on NMJ and muscle function even though they all promote neuronal survival and growth. From the same family, muscle-derived glial cell line-derived neurotrophic factor (GDNF), induces poly-innervated NMJs with larger motor units (314, 315). Even though they share a co-receptor and act as trophic factors for motor neurons (197), based on our and others data, NRTN does not induce the same effect as GDNF (316). Similarly, a recent study showed that muscle-derived BDNF promotes a shift towards glycolytic fiber features and a smaller NMJ endplate volume, acting exactly opposite to NRTN (250). Consequently, the adaptation to exercise probably results from the combinatorial response from different neurotrophic factors and the spatiotemporal changes in their abundance could affect the final outcome. This could explain the relative low amounts of NRTN in human skeletal muscles as function of neurotrophic factors could depend on spatiotemporal cues.

Functionally, NRTN transgenic animals display impressive motor coordination and improved performance on the treadmill. This goes in line with the shift towards slow motor units which provide higher fatigue resistance compared to FF units. We demonstrated that systemic delivery of NRTN has an influence on skeletal muscle and leads to an improvement in motor coordination and glucose metabolism, raising hopes for its potential utilization for metabolic or neuromuscular disorders.



## 7 CONCLUSIONS AND FUTURE PERSPECTIVES

One of our best weapons against chronic diseases such as neurodegeneration and metabolic dysfunction is physical exercise. However, our understanding of these three and their underpinning mechanisms is still incomplete and requires more research to facilitate the development of novel health-promoting strategies. In that sense, the studies that make up this thesis served to extend our understanding of neurodegeneration, explored the mechanisms underlying exercise capacity and function and laid out biological function of a potential agent that can act on metabolism and neuromuscular function.

In the AD physiology, autophagy has been documented as a “double-edged sword”. With some studies showing reduced A $\beta$  pathology upon its stimulation and others pointing at autophagosomes as an A $\beta$  production compartment (77, 290, 317–319). It would be interesting to investigate if any autophagy-modulating small molecules could activate this PS1 S367 favored-degradation of A $\beta$ . Also, further research is required to determine whether specific variants of the  $\gamma$ -secretase complex and PS1 determine the balance between its contrasting actions.

Alternatively, exercise has been shown to induce autophagy both in the periphery and the brains of AD mouse models (150). This is further supported by human studies showing that regular exercise improves cognition and ameliorates A $\beta$  pathology in AD patients (320–322). Enhanced synaptic plasticity, improved memory and cognition, and resistance to stress have been among the known exercise adaptations. Likely, exercise-induced factors may hold some answers to reversing hallmarks of aging. Exploring the circulating factors such as myokines could carve new paths that can lead to novel targets to circumvent neurodegeneration and metabolic dysfunction.

To achieve this, accurate models to study chronic diseases are essential. However, predominant models of complex metabolic diseases may not fully address the underlying complexity. Many of the current models might reflect the response to injury or the reorganization of biological mechanisms due to the loss of a particular gene. While in reality, complex diseases result from the combination of genetic variants and responses to environmental factors. Adding to this, chronic diseases emerge not as discrete events, but as a group of pathologies, such as metabolic syndrome and neurodegeneration. From this perspective, exercise capacity and response models take a more holistic approach to modelling complex metabolic diseases. They promise great scientific value as discovery tools.

One avenue could be developing a better understanding of the systemic factors which determine these phenotypes, thus broadening our perspective for new therapeutic targets. Recent large-scale multi-omics analyses provide evidence for genomic regulation of plasma proteome and suggest that circulating protein levels might be regulated at multiple levels, including transcriptional and post-transcriptional changes (222–224). High throughput multi-omics analyses, including with large cohorts of the exercise capacity and response models can provide the perfect landscape and pave the way for further investigations to map out the detailed mechanisms and identify new targets, including novel myokines. Unfortunately, development of tools for unbiased and high-throughput proteomics still lags behind transcriptomic technologies. Yet, emerging technologies for labelling and sequencing proteins might soon evolve and list a catalogue of the whole-body secretome (323–325).

Our understanding of myokines has been expanding. The recent discovery of extracellular vesicles (EV) as transport mediums for secreted/circulatory factors has widened the perspective to secretome and the scope of potential myokines with endocrine effects (326). Further investigations could capture the EV-mediated secreted factors that might contribute to the intrinsic exercise capacity and/or response phenotypes.

As a myokine, NRTN orchestrates a remarkable portion of skeletal muscle adaptations to exercise. In addition, NRTN retrogradely influences MNs and promotes a slow MN identity which has been associated with resistance to neurodegeneration (172). Clinical trials for Parkinson's Disease aimed at utilizing central and intracranial administration of NRTN as a neuroprotective agent failed to report effective outcomes. In the periphery, the effects of muscle-derived NRTN seems to originate from its autocrine function. Given its heparan sulfate binding properties, possibly NRTN has a limited range of activity once secreted from the muscle (327, 328). Potentially, structural modifications in NRTN can relieve its heparan-sulfate affinity and increase its target range and exposure. Recent strides to unravel the crystal structure together with its receptor provide the necessary groundwork for this and future research could explore the modified versions of NRTN for treatment of metabolic disorders or neuromuscular diseases such as ALS (327–329).





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