From Molecule to Motion: Understanding Skeletal Muscle Insulin Resistance

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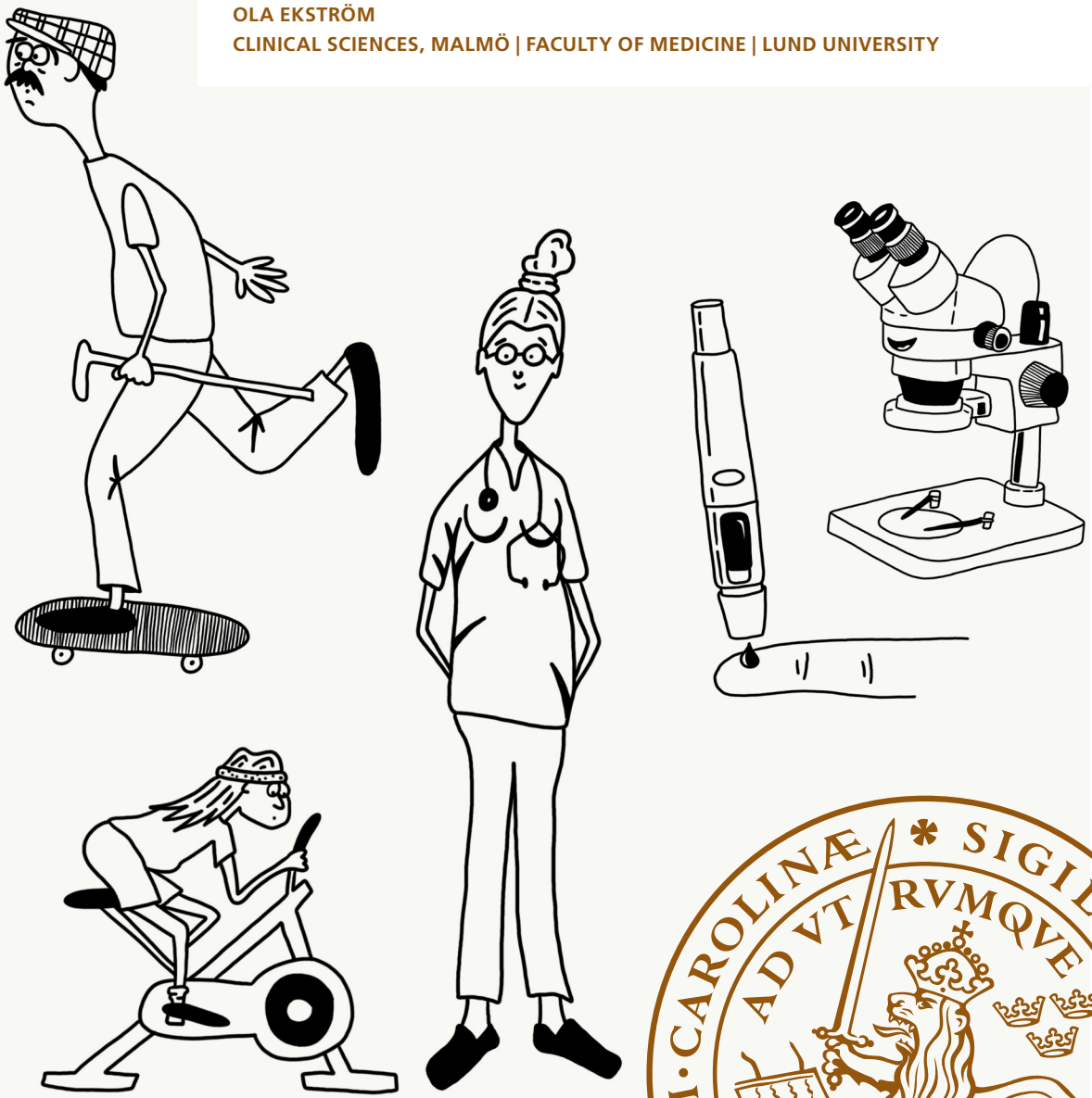

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From Molecule to Motion

Understanding Skeletal Muscle Insulin Resistance

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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be defended on Friday 24th of November at 09.00 in Medelhavet, Wallenberg Lab, Skåne University Hospital, Malmö

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Abstract: Diabetes, particularly type 2 diabetes, remains a significant health concern, intricately linked to muscle function and exercise responses. Grasping the underpinnings of this relationship can provide invaluable insights into preventive and therapeutic strategies. This thesis offers a series of explorations into the nuanced dynamics between muscle properties, physical exertion, and diabetes development.

In Paper I, we explored the role of Tenascin C (TNC), an essential extracellular matrix glycoprotein, during short anaerobic efforts. Through assessing the changes in TNC serum levels post an anaerobic exercise test in healthy males, we began to appreciate the nuanced relationship between muscle stress and systemic TNC release. This opened the door for a deeper understanding of muscle tissue responses and adaptations to exercise.

Paper II introduced a novel methodology to estimate skeletal muscle fiber type distributions. Given the limitations of traditional techniques, our approach utilized single-nuclei RNA sequencing to offer a fresh perspective on muscle fiber analysis. This innovative method has the potential to streamline our understanding of muscle fiber types and their relationship with overall health, especially in the context of exercise responses.

In Paper III, the spotlight was on insulin resistance and its connections to skeletal muscle properties. By relating skeletal muscle gene expressions in non-diabetic males to measures of insulin sensitivity, we uncovered potential markers that could influence exercise-induced insulin responses. This reinforced the idea of muscle health being a critical component in the broader landscape of metabolic health.

Finally, Paper IV investigates the long-term associations between early adulthood fitness metrics and subsequent diabetes subtype development. Utilizing extensive health datasets, our findings hinted at the possibility that early-life exercise and fitness levels might have implications for diabetes risks in later stages of life.

This thesis underscores the interplay between skeletal muscle function, exercise-induced responses, and diabetes subtype development. The findings illuminate the possible role of early-life physical fitness and muscle health in predicting and potentially mitigating diabetes risks later in life, emphasizing the need for future research and targeted interventions in these domains.

Key words: Type 2 Diabetes, Skeletal Muscle, Exercise, Tenascin C, Insulin Resistance

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In loving memory of Per Ekström
- forever and always a source of inspiration

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Ekström O, Strom K, Mir BA, Laurila E, Wessman Y, Lehtovirta M, et al. Increasing circulating levels of Tenascin C in response to the Wingate anaerobic test. *Clinical Physiology Functional Imaging*. 2023;43(4):271-7.

Paper II

Oskolkov N, Santel M, Parikh HM, **Ekström O**, Camp GJ, Miyamoto-Mikami E, et al. High-throughput muscle fiber typing from RNA sequencing data. Skeletal Muscle. 2022;12(1):16.

Paper III

Parikh HM, Elgzyri T, Alibegovic A, Hiscock N, **Ekström O**, Eriksson KF, et al. Relationship between insulin sensitivity and gene expression in human skeletal muscle. *BMC Endocr Disord*. 2021;21(1):32.

Paper IV

Ekström O, Jansåker F, Eriksson K-F, Thangam M, Sundquist K, Ahlqvist E, Hansson O. The Impact of Diabetes Subtypes on Early Adulthood Strength, Endurance & BMI: A Cohort Study of 4417 Men. *Manuscript* (2023)

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Jansaker F, **Ekström O**, Memon AA, Hansson O, Johansson SE, Sundquist K. Examining the causal effect of type 2 diabetes on ischemic heart disease - A longitudinal study with four measurements (1980-2017). *Diabetes Res Clin Pract*. 2023;198:110595.

Strom K, Morales-Alamo D, Ottosson F, Edlund A, Hjort L, Jorgensen SW, **Ekström O** et al. N(1)-methylnicotinamide is a signalling molecule produced in skeletal muscle coordinating energy metabolism. *Sci Rep*. 2018;8(1):3016.

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Ström K, Oskolkov N, Karaderi T, Kalamajski S, **Ekström O**, Miyamoto-Mikami E, et al. Genetic variation at RAB3GAP2 and its role in exercise-related adaptation and recovery.*Manuscript in review* 2023.

Ekström O, Dwibedi C, Brandt J, Adiels M, Franzén S, Abrahamsson B, Rosengren A. Response to semaglutide and dapagliflozin in patients with type 2 diabetes of different pathophysiology. *Manuscript in review*. 2023

Abbreviations

T2D	Type 2 Diabetes
GLUT4	Glucose Transporter Type 4
SIRD	Severe Insulin-Resistant Diabetes
SIDD	Severe Insulin-Deficient Diabetes
MOD	Mild Obesity-related Diabetes
MARD	Mild Age-related Diabetes
SAID	Severe Autoimmune Diabetes
GADA	Glutamic Acid Decarboxylase Antibody
T1D	Type 1 Diabetes
LADA	Latent Autoimmune Diabetes in Adults
BMI	Body Mass Index
GWAS	Genome-wide association studies
SLC2A4	Solute Carrier Family 2 Member 4
FFAs	Free Fatty Acids
C:F	Capillary-to-fiber ratio
RAB3GAP2	RAB3 GTPase Activating Non-Catalytic Protein Subunit 2
ATP	Adenosine Triphosphate
MCTs	Monocarboxylate Transporters
EPO	Erythropoietin
TNC	Tenascin C
VO2max	Maximal Oxygen Consumption
MSAT	Muscle SATellite cell cohort
ANDIS	All New Diabetics in Scania
MRS	Magnetic Resonance Spectroscopy
totRNAseq	Total RNA sequencing
snRNAseq	Single-nuclei RNA sequencing
QPCR	Quantitative Real-time PCR (Polymerase Chain Reaction)
ANOVA	Analysis of Variance
RCT	Randomized Clinical Trial

Preface

I've always been fascinated by the human body, especially how it reacts to exercise. In medical school, I was initially drawn to orthopaedics because I thought it would combine my love for exercise and medicine. But I quickly realized that many orthopaedic surgeons, who I thought would share my interests, were better at bone procedures than really understanding muscle function and exercise physiology. The numerous internet jokes about their limited interest in human physiology might not be entirely unfounded.

As the first years medical school progressed, my attention soon shifted. I found myself increasingly captivated by metabolic diseases, seeing in them the connection between exercise and health that I had been seeking. This led to my thesis on the potential of high-intensity training to combat metabolic syndrome.

It was during this phase that I met Karl Fredrik Eriksson. Starting as an opponent during a defence, Karl Fredrik became a significant mentor, a mentorship that would shape both my research and future clinical aspirations. Karl Fredrik, with his deep knowledge, kindness, and dedication, introduced me to Ola Hansson. Ola H and I shared a mutual excitement for scientific discovery, not really for praise, but for genuine understanding. This collaboration, initially, took me deeper into lab work, where I learned to culture skeletal muscle cells, particularly from Cook Myosite, a name now hard to forget. Professor Leif Groop's introduction added another dimension. A towering figure in diabetes research, he was both an inspiration and, in a lighter vein, a benchmark I hoped to impress.

Within Ola Hansson's group, I've come to appreciate the important value of joy as a driving factor in science. Research has underscored that enjoyment is not just a bonus, but an essential component for sustained scientific research and new ideas(1). The collective enthusiasm for science in this milieu is palpable, continuously reignited by an environment free from inhibitory hierarchies.

With Ola’s guidance, inspired by the stimulating environment around me, we began designing my PhD project. Instead of following a preset out-of-the-box solution. We designed the project's foundation from scratch. While the initial blueprint underwent several changes over the years, thanks to unforeseen challenges – from financial constraints, clinical commitments, to even global pandemics – the underlying drive, driven by joy and curiosity, never waned.

Alongside my research journey, I took my initial clinical steps. Right out of medical school, I registered as a PhD student and engaged in a "Forskar AT", which provided the bandwidth to kick off my project. This period was crucial, marked by the demanding data collection processes of the MSAT study, culminating in the first three papers of my thesis.

After earning my license to practice medicine, my clinical trajectory took me to family medicine, driven by a passion for general health and preventive care. However, juggling the intricacies of family medicine with rigorous research proved challenging, especially during personal life challenges. Luckily, Karl Fredrik's support emerged once more, guiding me towards Internal Medicine at Malmö Hospital. This change placed me closer, both in terms of location and clinical perspective, to my research work. This change gave me a renewed energy, helping me to move forward and finish my PhD project.

Looking back over these years, it's evident that my journey is both shaped and fuelled by invaluable mentorships, clinical experiences, and an underlying curiosity about exercise physiology and metabolic disease. My approach to scientific research has been that of a generalist—drawing from a breadth of knowledge and attempting to integrate these diverse insights into a comprehensive clinical perspective. This journey has been as much about the people and experiences as it has been about the science. This introduction not only sets the stage for my research but also captures the essence of a journey marked by learning, hurdles, and an enduring passion for understanding and care.

Diabetes: From Basics to Subtypes

Diabetes is a significant global health concern, with its prevalence, economic burden, and social impact escalating over recent decades. The International Diabetes Federation's Diabetes Atlas reported that in 2021, there were 537 million adults living with diabetes worldwide. This dramatic rise contrasts starkly with the 108 million adults estimated by the World Health Organization to have diabetes in the 1980s. Projections suggest this number can increase to 700 million by 2045(2, 3).

Economically, the global expenditure on diabetes care is expected to surpass $760 billion USD by 2045(4), with costs related to management and associated loss of productivity. In the United States, the economic burden reached over $327 billion in 2017(5).

In Sweden, the situation remains critical. Here we have observed a consistent rise in diabetes prevalence, with recent estimates suggesting that approximately 5-6% of the adult population is affected(6). A Swedish study from 2016 highlights the substantial economic impact of type 2 diabetes complications. Hospital-based care for these complications amounted to €919 per person, with 74.7% directly attributed to diabetes. Moreover, work absences due to diabetes complications posed an even greater cost, reaching €1317 per person, underscoring the broader societal implications of the disease(7).

The economic impact in Sweden aligns with global trends, placing a notable strain on its healthcare budget and necessitating rigorous research into understanding and managing the disease more effectively.

The Type 2 Diabetes Puzzle

Type 2 diabetes (T2D) is a multifactorial disease with a complex interplay of genetic and environmental factors contributing to its development and progression. The pathophysiological mechanisms underlying the disease are diverse and interconnected, involving insulin resistance, impaired insulin secretion, alterations in glucose and lipid metabolism and even inflammation(8, 9). This complex diversity has led researchers to continuously search for new tools and approaches to better understand and treat the individual factors contributing to T2D(10).

Insulin resistance at the cellular level results from impaired insulin signalling, reducing glucose uptake in tissues like skeletal muscle, adipose tissue, and the liver. With skeletal muscle being a crucial site for whole body glucose disposal, any disruptions can yield significant consequences on the entire body(11, 12).

Insulin resistance can eventually lead to hyperglycaemia and the overproduction of insulin by pancreatic beta cells to compensate for the reduced insulin sensitivity (13). Chronic hyperglycaemia resulting in glucotoxicity can also further impair beta cell function, eventually leading to insufficient insulin secretion(14). Additionally, alterations in lipid metabolism, such as increased free fatty acid levels, can contribute to insulin resistance and beta cell dysfunction through a process known as lipotoxicity(14).

The Impact of Lifestyle

Lifestyle factors play a significant role in the development of T2D. Obesity, physical inactivity, and poor diet are well-established risk factors for the disease (15). These factors can contribute to insulin resistance, systemic inflammation, and altered metabolism, further exacerbating the disease's pathophysiological mechanisms(16, 17). A comprehensive understanding of these mechanisms and their clinical implications is crucial for tailoring individualized treatments and management strategies.

Historical Milestones

History of diabetes research dates back to ancient times, when the symptoms of the disease were first documented(18). However, it was not until the early 20th century when Sir Frederick Banting and Charles Best discovered the role of insulin in regulating blood glucose levels, marking a major milestone in diabetes research(19). Since then, our understanding of the disease has significantly advanced, leading to

the development of the concept around multiple subtypes of diabetes and the development of various pharmacological and non-pharmacological interventions to manage the condition(20-22).

Beyond Blood Sugar

With advances in diabetes treatment, T2D is increasingly being viewed as a risk factor for future end stage organ disease, such as cardiovascular disease, nephropathy, neuropathy, retinopathy rather than a disease causing significant mortality and morbidity itself due to hyperglycaemia(23). Modern pharmacological interventions have considerably improved glycaemic control, reducing the risk of acute complications such as diabetic ketoacidosis and the hyperosmolar hyperglycaemic state(24-26). However, long-term exposure to diabetic factors still contributes to the development of end-organ damage and associated complications, emphasizing the importance of early identification and individual management of people at risk for T2D(27-29). Particularly, complications stemming from prolonged insulin resistance, such as liver disease, chronic kidney disease and cardiovascular disease demonstrate the critical nature of targeting and understanding this condition(30-33).

Exercise and improved physical fitness are associated with increased insulin sensitivity, glucose uptake, and glycogen storage capacity in skeletal muscles(34). These improvements are largely due to enhanced muscle insulin signalling and glucose transporter 4 (GLUT4) expression, which facilitate glucose uptake and utilization in muscle cells(35, 36). Besides physical exercise and/or weight loss, there is a scarcity of interventions, such as drugs, that target muscle insulin resistance(37, 38). The understanding of muscle insulin resistance is hence not only important for its role in diabetes but also its broader implications in metabolic health and associated complications.

Diabetes Subtypes

Delving deeper into diabetes heterogeneity, Ahlqvist et al. (2018) identified five distinct subtypes of diabetes in the ANDIS study, providing a refined classification system for a better understanding of the disease. These subtypes are Severe Insulin-Resistant Diabetes (SIRD), Severe Insulin-Deficient Diabetes (SIDD), Mild Obesity-related Diabetes (MOD), Mild Age-related Diabetes (MARD), and Severe Autoimmune Diabetes (SAID).

Each subtype has unique pathophysiological mechanisms and clinical implications, briefly described below.

Severe Insulin-Resistant Diabetes (SIRD):

Characteristics: Characterized by insulin resistance.

Associated Risks: Increased risk of diabetic kidney disease and fatty liver disease

Severe Insulin-Deficient Diabetes (SIDD):

Characteristics: Patients display insulin deficiency.

Associated Risks: Higher risk of diabetic retinopathy.

Mild Obesity-related Diabetes (MOD):

Characteristics: Associated with obesity and early onset.

Associated Risks: Mild metabolic disturbances compared to other subtypes.

Mild Age-related Diabetes (MARD):

Characteristics: Characterized by older age at onset.

Associated Risks: Generally lower risk of complications than SIRD and SIDD.

Severe Autoimmune Diabetes (SAID):

Characteristics: Characterized by the presence of GADA and early onset. This encompasses individuals typically labelled as T1D and LADA.

Associated Risks: Poor metabolic control. High prevalence of ketoacidosis

Each subtype offers insights into different diabetes causes and outcomes. Importantly, among these subtypes, the Severe Insulin-Resistant Diabetes (SIRD) directly underscores the centrality of insulin resistance in its pathophysiology. Using these classifications, notably SIRD, as a foundation, this thesis aims to explore muscle insulin resistance's wider effects.

Early-life determinants, epigenetics, and heterogeneity in diabetes

Diabetes, a complex metabolic disorder, presents with significant heterogeneity in its subtypes and underlying etiological factors. In Paper IV of this thesis, we explored the associations between early-adulthood physical fitness metrics, BMI, and the subsequent development of distinct diabetes subtypes. Using Swedish registry data, our research revealed that specific diabetes subtypes, notably SIRD

and MOD, showed variations in early adulthood physical parameters. These findings underscore the importance of early-life determinants in diabetes heterogeneity. Our work also emphasizes the translational potential of melding register data with genetics, possibly leading to molecular insights behind observed phenomena and the value of proactive early detection and targeted intervention. The integration of genetic perspectives, such as Polygenic Risk Scores, offers a comprehensive view of the intricate interplay of genes and environment in diabetes manifestation.

	Maximal Aerobic Workload (Wmax)		
	(1)	(2)	(3)
MARD	-22.675***	-8.051***	-5.795***
	(1.797)	(1.635)	(1.591)
MOD	3.078**	1.361	-9.950***
	(1.564)	(1.422)	(1.387)
SAID	-2.114	-4.114	-6.123*
	(3.552)	(3.229)	(3.141)
SIDD	-10.666***	-6.054***	-8.993***
	(1.929)	(1.754)	(1.706)
SIRD	-22.793***	-10.501***	-15.178***
	(2.345)	(2.133)	(2.075)
Method change		45.645***	43.777***
		(0.217)	(0.211)
BMI			3.990***
			(0.036)
Constant	266.799***	249.121***	163.315***
	(0.117)	(0.135)	(0.794)
Observations	211,452	211,452	211,452
R2	0.001	0.175	0.219
Adjusted R2	0.001	0.175	0.219
Note:			*p<0.1; **p<0.05; ***p<0.01

Table 1 Regression analysis on the differences in maximal aerobic workload (measured in watts), During military conscription testing, among diabetes subtypes (MARD, MOD, SAID, SIDD, SIRD) compared to controls. Three models were used: unadjusted (Model 1), adjusted for a known method change (Model 2), and further adjusted for BMI (Model 3). Regression coefficients and standard errors are shown for each subtype, with significance denoted as *p<0.1; **p<0.05; ***p<0.01.

Besides physical fitness metrics in early adulthood, other early-life experiences are important in shaping the health trajectories of individuals, influencing the risk of metabolic conditions. One such determinant in relation to metabolic diseases is birth weight. The relationship between birth weight and the subsequent risk of developing metabolic diseases in adulthood has been well-documented (39). Specifically, low birth weight, possibly indicative of intrauterine growth restriction, is associated with

an increased risk of insulin resistance, T2D, and cardiovascular diseases later in life (40, 41). The connection is theorized to stem from adaptive changes in fetal physiology due to a nutrient-limited environment, anchored in the fetal programming concept(42). While fetal programming offers one perspective, it's also possible that risks of metabolic diseases originate from maternal metabolically unfavourable predispositions. Such biases could result in diminished placental efficiency leading to low birth weight, decreased muscle mass, and a decline in pancreatic β-cell mass(43).

Adding to this, recent epigenetic studies show that early-life environmental exposures might cause changes in DNA methylation patterns and other molecular markers(44). These can adjust gene activity without altering the DNA itself, potentially increasing metabolic disease risk.

By recognizing these early-life determinants, the medical and science community can try to craft tailored prevention strategies, emphasizing the importance of a comprehensive understanding of diabetes.

In the coming sections, we will look closely at the role of skeletal muscle in how our body uses energy. We'll learn about how exercise affects our bodies and why physical activity is important from an evolutionary point of view. By studying the details, from taking muscle samples to analysing genes and study epidemiological connections between performance and diabetes subtypes I hope to understand better how muscle function and exercise relate to insulin sensitivity. Overall, the aim is to learn more about muscle insulin resistance, which is important not just for diabetes but also for our general health.

Skeletal Muscle in Human Metabolism

Anatomy and Function of Skeletal muscle

Skeletal muscle is the most abundant type of muscle in the human body, constituting about 35% of total body weight, containing more than 50% of all body proteins and it is responsible for a wide range of functions, including locomotion, posture, and metabolic regulation(45). It accounts for around 80% of the post prandial glucose disposal in humans. Having a fully functioning insulin response is crucial to maintain a non-pathological blood glucose levels(11).

In general, muscle mass is affected by the equilibrium of protein synthesis and breakdown, which respond to known factors like nutrition, hormone levels, physical activity, ageing and certain diseases, including cancer(46-48).

At the cellular level, skeletal muscle is composed of elongated, multinucleated cells termed muscle fibers, which contain myofibrils, the functional units responsible for muscle contraction. These myofibrils contain the contractile proteins actin and myosin, which generate force through filament interaction.

While the evidence for muscle contraction's molecular basis was present as early as the mid-1800s, it wasn't until the 1950s that researchers, including two unrelated Huxleys, recognized that striated muscle sarcomeres consist of overlapping filament sets that slide past each other during muscle shortening(49, 50). This sliding filament theory, initially criticized, is now fundamental to our understanding of muscle function, underscoring the consistent principles governing all muscle types(51).

Lund University has also, historically, played a role in advancing our understanding of muscle function. Notably, KAP Edman's 1979 work offered valuable insights into the dynamics of muscle fiber shortening, further refining our knowledge of the sliding filament theory(52). On a related note, KAP Edman's dedication to muscle research was truly remarkable. Edman, who spent a considerable part of his academic career at Lund University, continued to contribute to the field even in his later years, notably publishing a research paper as the sole author at the age of 88(53).

The Power of Exercise

Regular exercise during diet-induced weight loss in individuals with obesity and prediabetes has been shown to yield a significantly greater improvement in whole-body insulin sensitivity than diet alone. This enhanced insulin sensitivity correlates with an increase in muscle gene expression associated with mitochondrial biogenesis, energy metabolism, and angiogenesis. Moreover, while diet alone does enhance multi-organ insulin sensitivity, incorporating exercise delivers remarkable additional benefits on insulin kinetics, underscoring the vital role of physical activity in augmenting the therapeutic effects of weight loss(54).

Recent genome-wide association studies (GWAS) have begun to unveil crucial genetic links between skeletal muscle and insulin resistance. Notably, a focus on dynamic, post-glucose-challenge measures identified loci related to GLUT4 regulation in skeletal muscle(55). One standout discovery was the SLC2A4 locus, which encodes the GLUT4 transporter. Variations in this gene have been shown to impact its role in postprandial glucose uptake in muscle cells, emphasizing the value of understanding genetic influences on skeletal muscle's metabolic functions.

Given the distinct metabolic properties of these muscle fiber types, their role in glucose metabolism and insulin sensitivity becomes evident. Exercise interventions that promote a shift towards a more oxidative profile can potentially improve glucose metabolism and insulin sensitivity, especially in individuals with T2D or those at risk(38).

Moreover, delving deeper into the molecular mechanisms underlying the relationship between muscle fiber type composition and glucose metabolism might unveil novel therapeutic targets for preventing and managing T2D.

Skeletal Muscle Fiber Diversity

Understanding the characteristics of skeletal muscle fibers is key for insights into human metabolism and muscle function. Traditionally, human skeletal muscle fibers been classified into distinct types based on their metabolic and contractile properties.

Type I fibers, or slow-twitch fibers, are characterized by their high mitochondrial content and oxidative capacity, making them more resistant to fatigue and suitable for endurance activities(56, 57).

While sedentary lifestyles can lead to increased lipid content in these fibers with negative metabolic consequences, the 'athlete's paradox' presents an intriguing observation: endurance athletes, despite high levels of physical activity, also

accumulate lipids in their muscles like metabolically challenged individuals. However, in athletes, these lipids serve as a readily accessible energy source during prolonged exercise, contrasting sedentary individuals where lipid accumulation can disrupt insulin signalling and lead to insulin resistance(58, 59).

In contrast, Type II fibers, often referred to as fast-twitch fibers, are equipped with a higher glycolytic capacity, enabling them to produce energy through anaerobic metabolism. These fibers contain a larger number of glycolytic enzymes and exhibit faster calcium cycling, facilitating quick bursts of strength or speed. However, due to their reliance on anaerobic energy production and reduced mitochondrial density compared to Type I fibers, they fatigue more rapidly(60).

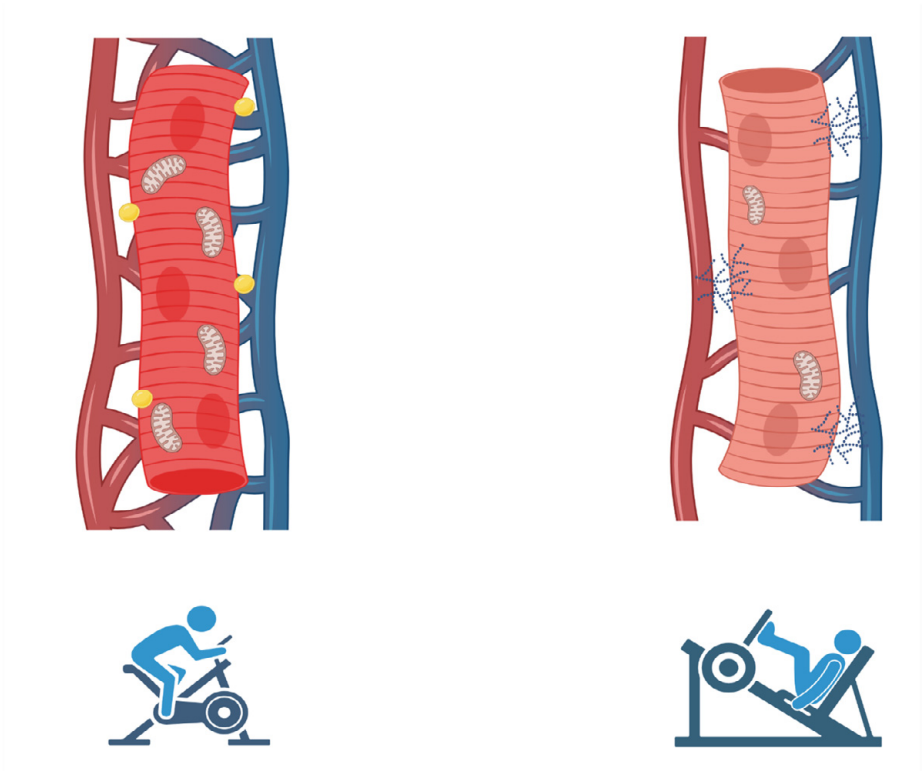

Figure 1. Illustrative Overview of Human Type 1 and Type 2 Muscle Fibers.
Type 1 fibers (dark red): Abundant in mitochondria and myoglobin, these fibers support aerobic metabolism and prolonged activities, symbolized by the ergometer bike. The rich color indicates high myoglobin, while intramuscular lipid droplets reflect fat-based energy sources. Surrounding dense capillary network ensure continuous oxygen supply. Type 2 muscle fibers (light color): Optimized for anaerobic metabolism and short-duration high-intensity actions, represented by the leg press machine. These fibers have fewer mitochondria; their pale shade shows reduced myoglobin. Internal glycogen granules fuel quick energy spurts. Created with Biorender.com

In type I fibers, there's a higher presence of proteins that help with glucose uptake but a lower presence of those related to insulin regulation, compared to Type II fibers(61). This indicates that type I fibers are more efficient at using sugar but respond to insulin similarly as type 2 fibers. Moreover, people with obesity or T2D tend to have fewer type I fibers, resulting in less efficient energy metabolism(62, 63)

Historically, human Type II fibers have been subdivided into Type IIa and Type IIx. However, the distinction between these subtypes is currently debated, with newer research pointing towards just two primary clusters based on their metabolic and contractile characteristics(64).

In our research (Paper II), we've introduced a method to estimate the Type I versus Type II fiber distribution(65). This high-throughput, minimal-invasive method aligns with the emerging perspective that focuses less on the distinction between Type IIa and IIx fibers and more on the broader dichotomy between slow and fast fibers.

Rodent models introduce additional complexity with their own set fiber types like Type IIa, IIb, and IIx(60, 66). This emphasizes the importance of species-specific considerations in muscle research and highlights the need for human-specific studies when translating findings to human normal, and pathophysiology.

The preferential atrophy of Type II fibers characterizes conditions like sarcopenia, affecting not only muscle strength but also metabolic health, given the role of muscle fiber types in influencing metabolic outcomes like insulin resistance(67, 68).

Muscle Capillarization

The skeletal muscle microvasculature, with its vast network, plays an essential role in supporting muscle function. The primary sites for these exchanges in skeletal muscles are the capillaries. Their degree of proliferation and strategic positioning, often organized as both longitudinal and transverse capillaries that envelop the muscle fibers, can directly influence parameters such as mean transit time, capillary surface area, and diffusion distance. These factors, in turn, can have implications on muscle function, athletic performance, and metabolic health(69).

Importance of Capillary Density in Muscle Physiology

For optimal skeletal muscle performance, the availability of oxygen and energy substrates is paramount. While it is understood, due in part to the foundational work of Bengt Saltin and his contemporaries in the 1980s, that cardiac output is a determinant for oxygen delivery to muscles, the internal diffusion process, especially from the capillaries to the myofibrils, cannot be overlooked(70, 71). The nuances in this diffusion process, including the oxygen gradient, area for diffusion, mean transit time, and diffusion distance, are all modulated by the capillary network. Furthermore, the proliferation and organization of these capillaries, as seen in endurance athletes, offer insights into the criticality of maintaining a dense capillary network for improved muscle function(72, 73).

Structure of the Capillary Network

Traditionally, the capillary network within the skeletal muscle have been looked at as a series of mostly parallel-flowing vessels stemming from terminal arterioles and culminating in venules. This perspective has lately been refined when the concept of capillary fascicles was presented(74). These fascicles, which extend along muscle fibers, indicate an intricate organization aligned with the skeletal muscle's fascicle structure. Such architectural insights reiterate the importance of understanding capillary structure and function to fully understand all aspects of muscle physiology.

The Role of Capillarization in Glucose Uptake

Beyond serving as pipelines for oxygen delivery, capillaries are gatekeepers for metabolic substrate exchange, a relationship that's intricately tied to the muscle's functional demands. A growing area of interest is its potential influence on glucose uptake, a key process for understanding metabolic diseases and, particularly, skeletal muscle insulin resistance. Evidence suggests that capillaries impact muscle glucose uptake by mediating both glucose and insulin transport(75). Conversely, conditions like insulin resistance seem to feature diminished capillary density, limiting optimal substrate delivery and thus hampering muscle function(76, 77). In animal models, decreased capillary density is linked with diminished peripheral glucose uptake(78), underscoring the need for human studies to delineate this relationship further.

Conclusion and Future Implications: The Role of RAB3GAP2 in Muscle Capillarization

The complex network of capillaries within skeletal muscles offers more than just oxygen and nutrient delivery—it serves as a foundational component of metabolic health. Moreover, the adaptability of our body to alterations in blood flow is remarkable. Whether it's the intricate capillary adaptations in muscles to support increased activity or nutrient flux, the cerebral vasculature adjusting to cognitive demands(79), or the uterine response to hormonal shifts influencing its vascular dynamics(80), the body's ability to regulate and adapt to blood flow changes spans a vast array of organs and functions, underscoring its remarkable adoptability.

Muscle capillarization plays an important role in determining the efficiency of glucose uptake, with reduced capillarization being a hallmark of conditions like insulin resistance. Beyond the capillaries' structural and functional dynamics, the genetic underpinnings that drive these dynamics are also crucial.

An extensive multidisciplinary research initiative from our group has culminate in a comprehensive examination of the gene RAB3GAP2 and its association with skeletal muscle capillary-to-fiber ratio (C:F) (Ström K. et al., 2023, under review). This project has identified a variant, rs115660502, linked to both variations in the C:F ratio and differential expression of RAB3GAP2 in skeletal muscles. This work is the result of an extensive collaboration between different research groups with diverse expertise, integrating a myriad of methodological approaches from GWAS genetic analysis, clinical datasets, to in vitro experiments, and leveraging insights from the MSAT cohort as detailed in Paper I of this thesis. Remarkably, those harbouring the G allele of this variant showed heightened capillarization, suggesting

a potential genetic predisposition for some individuals toward enhanced muscle vascularization.

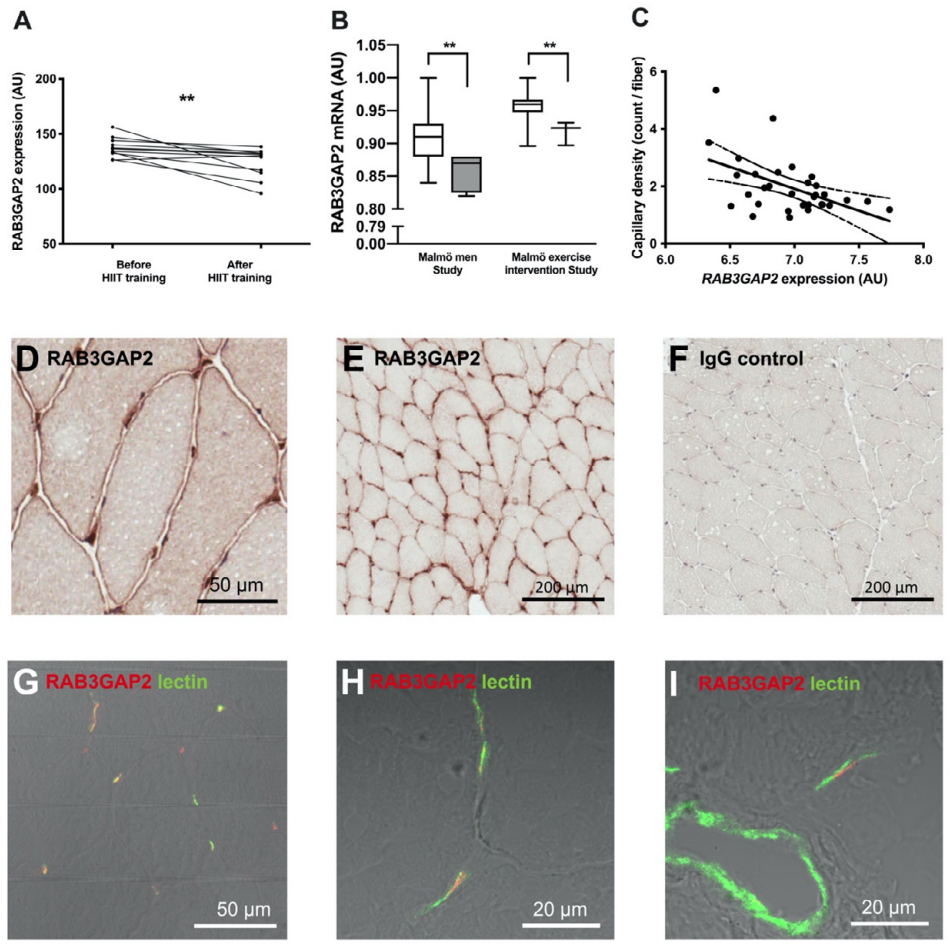

Figure 2. (**a**) Expression of *RAB3GAP2* mRNA in skeletal muscle after 6-weeks high-intensity intermittent training(167). $p_{Wilcoxon}$ = 0.002, n = 11. (**b**) Expression of RAB3GAP2 mRNA across rs115660502 genotypes in the MM (n = 33 vs 5) and MEI studies (n = 36 vs 3), $p_{Meta\text{-}FDR}$ = 0.007. (**c**) Plot of *RAB3GAP2* mRNA expression versus capillary-to-fiber ratio in the Malmö Men cohort. r = 0.38, $p_{Spearman}$ = 0.03, n = 32. (**d**) Lower and (**e**) higher magnification of RAB3GAP2 protein localization (brown) in human skeletal muscle. (**f**) Negative control using non-immune IgG with same concentration as in (**e**). Nuclei stained with hematoxylin (blue) in **d**-**f**. (**g**) Lower and (**h**) higher magnification of confocal immunofluorescence images of RAB3GAP2 (red) and the endothelial marker lectin (green) of human skeletal muscle, demonstrating RAB3GAP2 localization to the endothelium (green). (**i**) Confocal immunofluorescence of human skeletal muscle stained for RAB3GAP2 (red) and lectin (green), demonstrating RAB3GAP2 localization to capillaries, but not to large vessels. ** $p < 0.01$

Beyond just exercise adaptation, RAB3GAP2 seems to regulate several key pathways. By modulating the release of proteins like TNC and CD70, it influences both immune responses and angiogenesis. Given the shared inflammatory markers between exercise-induced responses and metabolic diseases such as T2D(81), the regulation of these pathways by RAB3GAP2 may provide a mechanistic bridge between exercise adaptation and metabolic health. Additionally, the potential for certain genetic profiles, such as those with the G allele of rs115660502, to influence susceptibility to stress-induced endothelial and muscle damage hints at broader implications for understanding individualized responses to different stressors, be it through exercise or infectious agents.

These revelations bring into focus the intertwined nature of genetics, capillarization, and metabolic health. It underscores the imperative to study capillarization not in isolation but as part of a complex system influenced by genetics and environmental factors. Moving forward, understanding these interactions may help paving the way for more personalized exercise and metabolic interventions.

Metabolic Pathways in Muscle

Glucose Metabolism

Skeletal muscle is important for whole-body glucose metabolism due to its significant role in insulin-stimulated glucose uptake and utilization(82). During exercise, as energy demand surges, skeletal muscle amplifies glucose uptake through both insulin-dependent and insulin-independent pathways(83). The former involves the translocation of GLUT4 to the cell membrane, facilitating glucose influx. On the other hand, exercise-induced muscle contractions activate the insulin-independent pathways, resulting in enhanced intracellular signalling that also boosts GLUT4 translocation.

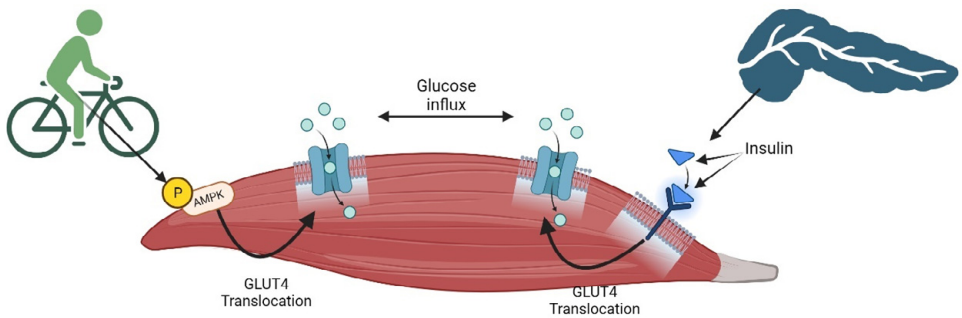

Figure 3. Dual Mechanisms Facilitating GLUT4 Translocation in Skeletal Muscle.
Exercise/Contraction-Dependent Pathway (left): Amplifies glucose uptake through activation of intracellular signals, notably AMPK phosphorylation, responding to increased muscle energy demands during physical activity. Insulin-Dependent Pathway (right): Initiated by insulin secretion from the pancreas, this pathway promotes GLUT4's movement to the cell membrane, ensuring efficient glucose influx in response to rising demand. *Created with Biorender.com*

It's important to note that insulin resistance in skeletal muscle can diminish glucose uptake, leading to hyperglycaemia(11). Those with T2D often display a diminished oxidative capacity in skeletal muscles, possibly driven by fiber type disparities. This deficiency can compound the effects of insulin resistance(84).

Although insulin resistance in skeletal muscle can impair glucose uptake, research is clear that regular physical activity benefits insulin sensitivity in skeletal muscle

in both healthy and individuals with diabetes(85-87). This improvement is associated with increased mitochondrial biogenesis and oxidative capacity(88, 89).

Lipid Metabolism

Skeletal muscle is a vital player in lipid metabolism, efficiently utilizing free fatty acids (FFAs) as an energy source during low-intensity exercise or prolonged fasting(90). Mitochondria in skeletal muscle are crucial for this process, converting FFAs into ATP through beta-oxidation. However, disruptions in these pathways are linked to insulin resistance and the onset of T2D(91).

In skeletal muscle of individuals with obesity and T2D, there is a decreased ability to convert fatty acids to fatty acyl-CoAs, suggesting disruptions in these metabolic pathways. Interestingly, exercise training has been demonstrated to counteract these alterations and enhance insulin responsiveness(92).

Lipotoxicity emerges when lipid accumulation in muscles surpasses the muscle's storage or oxidation capacity. This overaccumulation gives rise to lipid intermediates such as diacylglycerols and ceramides, which are known to interfere with insulin signalling, thus potentially exacerbating insulin resistance(93).

On the molecular front, as highlighted in Paper III of this thesis(94), genes involved in lipid metabolism hold significant influence over muscle insulin resistance. Such genes could modulate the muscle's oxidative capacity, further impacting its ability to handle glucose and, by extension, its role in T2D progression.

Importantly, lipid accumulation in non-adipose tissues like skeletal muscle is linked to a heightened cardiovascular disease risk, irrespective of total body fat(95). Intriguingly, there's a distinct association between increased intramuscular fat deposition and a higher risk of heart failure, especially in cases typified by a reduced ejection fraction(96). Even after adjusting for other cardiometabolic risk factors, this connection underscores the profound effect of lipid metabolism within skeletal muscles on cardiovascular, as well as metabolic, health.

Amino Acid Metabolism

In addition to glucose and lipid metabolism, skeletal muscle is also a key player in amino acid metabolism. Muscle proteins are continuously synthesized and degraded, maintaining an equilibrium between protein synthesis and breakdown, influenced by periods of muscle activity and rest(97, 98). Amino acids serve as important substrates for energy production in skeletal muscle, especially during exercise or prolonged energy restriction(99-101). Abnormalities in amino acid

metabolism have been associated with insulin resistance and T2D, although the exact mechanisms are not fully understood(102, 103).

Lactate Metabolism

Skeletal muscle lactate metabolism is another important aspect of energy homeostasis during exercise. Lactate, a byproduct of anaerobic glycolysis, was once considered as merely a waste product; however, recent research has shown that lactate has more complex and significant roles in metabolism. In fact, it serves as a critical energy source and a signalling molecule under various physiological conditions(104, 105).

During intense exercise, the increased reliance on anaerobic glycolysis leads to the production and accumulation of lactate in the skeletal muscle(106). As the lactate concentration rises, it is transported from the muscle cells into the bloodstream through monocarboxylate transporters (MCTs), which are essential for lactate transport across cell membranes(107). The lactate produced in the muscles can be utilized by other tissues, such as the heart and the liver(108). The heart can take up and oxidize lactate as an energy source, especially during intense exercise when oxygen availability is limited(109). In the liver, lactate is utilized as a substrate for gluconeogenesis, culminating in the conversion of lactate to glucose, constituting the Cori cycle(110).

Furthermore, lactate has been shown to act as a signalling molecule involved in various processes such as angiogenesis, immune response modulation, and cell proliferation(111-113). This highlights the multifaceted role of lactate in skeletal muscle metabolism and the human body's adaptive responses to exercise.

In summary, skeletal muscle plays a vital role in human metabolism, particularly glucose homeostasis, lipid metabolism and lactate metabolism. The interplay between different energy substrates, metabolic pathways, and the adaptive responses of skeletal muscle during exercise and rest periods highlights the complexity and importance of skeletal muscle in maintaining overall metabolic health.

Exercise Physiology

The Nordic Legacy

Exercise physiology is a field of research with a long and illustrious history in Scandinavia, a region that has made significant contributions to the understanding of human performance, health, and well-being. The Nordic countries have a strong tradition in exercise physiology, with pioneers such as Per-Olof Åstrand and Bengt Saltin, who made groundbreaking discoveries in the 1960s and 1970s. Their work helped establish the foundation for our understanding of how the human body responds to and adapts to physical activity.

Per-Olof Åstrand's seminal studies on the effects of physical fitness on work capacity and the influence of exercise intensity on oxygen uptake set the stage for decades of research in this field(114, 115). Bengt Saltin further advanced our knowledge of muscle metabolism, mitochondrial function, and the impact of training on aerobic capacity(116, 117). These early achievements in exercise physiology research have had a lasting impact on our understanding of the importance of physical activity for human health.

Erythropoietin (EPO) research in the 1990s, led by Björn Ekblom and colleagues, significantly impacted our understanding of endurance performance(118). While their findings stirred debate and highlighted the complexities of studying doping in sports, they underscored the importance and challenges of scientific exploration into elite human performance(119).

As we strive to address the burden of T2D and associated complications, it is crucial to continue building upon this rich Scandinavian research tradition. Advances in our understanding of the molecular and cellular mechanisms underlying the beneficial effects of exercise have the potential to inform the development of more effective, individualized prevention and treatment strategies for people with or at risk of developing T2D. Moreover, it is maybe even more essential that we consider the broader societal context and advocate for public policies that facilitate healthier, more active lifestyles.

Types of Exercise: Aerobic vs Anaerobic

There are two primary types of exercise: aerobic and anaerobic(120). Aerobic exercise involves low to moderate intensity activities sustained over an extended period of time, such as running, cycling, or swimming. This type of exercise relies on oxygen to produce energy through oxidative phosphorylation in the mitochondria(121, 122).

Anaerobic exercise, on the other hand, involves high-intensity, short-duration activities like sprinting or weightlifting. In anaerobic exercise, energy is produced primarily through glycolysis without the reliance on oxygen(123).

Body Responses: Acute and Chronic Adaptations

Exercise induces both acute and chronic adaptations in the human body. Acute adaptations encompass short-lived changes that transpire during or immediately post-exercise, including an elevated heart rate, amplified ventilation, augmented blood flow, and skeletal muscle metabolic alterations(124).

Acute adaptations to exercise also include molecular shifts. In Paper I, we discovered that serum levels of the extracellular matrix glycoprotein Tenascin C (TNC) surged notably after an anaerobic exertion. This rise was associated with performance measures such as peak power and power drop, hinting at a linkage to mechanical strain and enhanced microvascular blood flow(125). Furthermore, TNC has been pinpointed to stimulate the proliferation of muscle stem cells, emphasizing its potential significance in muscle tissue remodelling and regeneration(126).

Chronic adaptations are enduring modifications resulting from prolonged exercise training, such as enhanced aerobic capacity, improved insulin sensitivity, and shifts in skeletal muscle fiber type distribution and metabolism(124).

These adaptations aids exercise performance and contributes to health. One early insight from Malmö and Lund University, a study in Diabetologia by Eriksson and Lindgärde in 1991, highlighted how physical exercise can prevent T2D(127).

Exercise and Physical Activity for Diabetes Prevention and Management

Considering the significant impact of exercise on glucose metabolism and overall health, incorporating regular physical activity into the management plan for individuals with T2D is crucial. Current recommendations suggest that individuals with T2D should engage in at least 150 minutes of moderate to vigorous aerobic exercise per week, spread over at least three days(128, 129). Resistance training, performed at least twice a week, is also recommended to promote muscle strength.

It is worth noting that these recommendations are quite general, and there is growing evidence supporting the need for more individualized exercise prescriptions to maximize training response, adherence, and compliance(130, 131). Research indicates that individuals with a family history of T2D may respond differently to exercise interventions than those without such a history. Specifically, individuals with family history might experience a less pronounced response to exercise, requiring higher volume and/or intensity to achieve similar results(132). By tailoring exercise recommendations to suit individual needs, preferences, and capacities, patients may be more likely to sustain their engagement in physical activity and in the long run take advantage of associated health benefits.

In addition to individualized exercise prescriptions, changes at the societal and political level are necessary to encourage physical activity and create environments that support healthy behaviours. This includes for example investments in infrastructure like bike lanes and parks(133, 134). By creating an environment conducive to physical activity, individuals will be better positioned to adopt and maintain active lifestyles, reducing the risk of developing T2D and improving overall public health. By promoting infrastructure changes such as improved bike lanes, parks, and urban design, we can create environments that encourage physical activity and make it more accessible for everyone, ultimately contributing to the prevention and management of diabetes on a population level(135, 136).

The Evolutionary perspective

The evolutionary perspective on physical activity and skeletal muscle metabolism offers valuable insights into contemporary health challenges, especially T2D. Today's sedentary lifestyles starkly contrast with those of our ancestors, who thrived in environments demanding consistent physical activity. This discord might play a role in current chronic disease trends, including the surge in T2D(137, 138).

Evolutionary Adaptations for Endurance

Throughout evolutionary history, humans were physically active, from hunting and gathering to evading predators. Key adaptations, such as elongated limbs, large gluteal muscles, and relative hairlessness, enabled long-distance running and efficient thermoregulation(139). The adoption of bipedalism provided advantages in locomotion and environment perception. This superior endurance capacity and effective thermoregulation became essential for human survival. Moreover, endurance behaviours, potentially like persistence hunting, might have driven the evolution of larger human brains by enabling access to high-quality food sources(140, 141).

Fiber Differences: Humans vs. Primates

A distinction in muscle fiber distribution between humans and non-human primates further underscores our evolutionary trajectory. Humans typically have a higher proportion of Type I fibers, which are adept at sustained, endurance activities. In contrast, many non-human primates possess a more significant share of Type II fibers, suited for rapid, powerful actions(57).

Recognizing these evolutionary underpinnings deepens our understanding of the importance of physical activity in maintaining health and warding off diseases like T2D.

Human Metabolic Evolution and Skeletal Muscle

Advancements in metabolomics have illuminated evolutionary metabolic shifts among species. A important study by Bozek et al. examined the metabolomes of humans, chimpanzees, macaque monkeys, and mice across different tissues, including the brain and skeletal muscle(142). Their findings underscored an accelerated evolution in human prefrontal cortex and skeletal muscle metabolomes, particularly affecting neural and energy metabolism pathways. This suggests an intricate link between human brain and skeletal muscle evolution, potentially bearing implications for metabolic diseases, including T2D.

Human evolution seems to have favoured endurance, as indicated by observed adaptations(143). In contrast, chimpanzees, surpass human muscle in maximum dynamic force and power output. not necessarily due to superior isometric force or maximum shortening velocities, but largely owing to a higher proportion of Type II fibers. This difference suggests that over the course of human evolution, there was a shift towards repetitive, low-cost contractile behaviour, which may have reduced maximum dynamic force and power outputs. In essence, while our human lineage evolved to prioritize endurance and repetitive muscular tasks, our closest primate relatives retained greater explosive power(144). In 1962, James Neel postulated the "thrifty genes" concept, suggesting genes evolved to optimize fuel storage during periods of food scarcity(145). While the concept has been both supported and challenged over the years, it's not seen as the sole explanation for modern metabolic issues like obesity and T2D(146, 147). Yet, the idea has emphasized the significance of evolutionary perspectives in understanding these conditions, highlighting the intricate dance between genetics, environment, and behaviour.

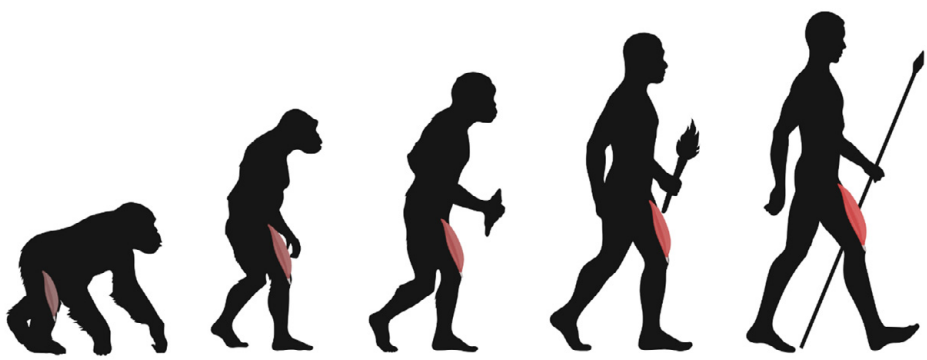

Figure 4: Evolutionary progression of human locomotion and muscle adaptation.
The intensifying red hue in the muscle region symbolizes the increasing proportion of Type 1 fibers, reflecting the evolutionary shift towards sustained physical activity and endurance capacities. Created with BioRender.com

By examining the evolutionary history of human physical activity and metabolism, researchers can gain a deeper understanding of the factors that contribute to the development of T2D and other metabolic disorders. This knowledge can inform the development of targeted interventions that leverage our evolutionary heritage to promote healthier lifestyles, optimize skeletal muscle metabolism, and prevent or manage diabetes.

Rationale and Aims

The rise in metabolic diseases, particularly diabetes, has made it crucial to better understand the factors at play. Given skeletal muscle's essential role in glucose metabolism, its function in insulin resistance becomes central. The approach of this thesis employs various scientific methods to gain a clearer understanding of muscle insulin resistance and its complications. The intent is to offer valuable insights to guide future research.

Paper I: Our objective was to understand the factors influencing skeletal muscle's reaction to high-intensity exercise. We focused on the release patterns during the Wingate test, considering aspects such as power output, VO2max, fasting glucose, and characteristics of isolated muscle cells. While we initially looked at responses in healthy individuals, this knowledge can lay the groundwork for exploring responses in those with metabolic disorders.

Paper II: This paper introduces tools useful for expansive research into metabolic diseases. Specifically, we aim to harness these tools for in-depth exploration of muscle insulin resistance, paving the way for potential treatments.

Paper III: Here, we delve into fundamental components like mitochondrial function and its potential connection to insulin resistance. Grasping these base mechanisms is vital as they may influence the onset of muscle insulin resistance and subsequent treatment paths.

Paper IV: Our goal was to explore connections between different diabetes subtypes and early adulthood attributes like BMI, strength, and aerobic endurance. By using fitness data from Swedish military conscripts who later developed diabetes, we aimed to discern if there were distinct fitness patterns in their younger years that correlated with specific diabetes subtypes.

Further, with a broader lens:

Understanding Muscle Insulin Resistance: Throughout the papers, we strive to chart the progression of muscle insulin resistance, looking at everything from cell-level pathways to early markers.

Potential Treatment Pathways: Drawing from insights in Papers 1 and 3, we aim to identify possible therapeutic targets, bridging the gap from molecular understanding to mechanistic approaches.

Early Identification and Intervention: Building on the findings of Paper 4, the goal is to detect early indicators of muscle insulin resistance, which can inform and guide early intervention strategies.

A Comprehensive Perspective: The aim of this thesis is to offer a broad understanding of muscle insulin resistance by connecting detailed scientific findings with their clinical relevance, with the hope of directing future research paths.

Methodology

In this thesis, we employ a diverse set of methods, indicative of an interdisciplinary approach. These methods were chosen to address our research questions from different perspectives, emphasizing a broad understanding over focusing narrowly on one specific technique. The goal has been to provide a multifaceted view of the topic in a clinical perspective.

Study Populations

The choice of study populations is instrumental in shaping the insights derived from our research. We relied on two primary cohorts:

- MSAT (Muscle SATellite cell cohort): Comprised of 39 healthy men aged between 20 and 55 years, participants were recruited via social media and local cycling clubs. The study involved three distinct visits: the initial visit included a medical examination, blood sampling, and an Åstrand test for equipment familiarization. The second visit featured a Wingate test, followed by a muscle biopsy. Blood samples were taken before and after the Wingate and VO2max tests, performed during the second and third visits, respectively. This design facilitated an understanding of muscle activity under varying conditions and physical strain.
- ANDIS (All New Diabetics in Scania): As previously outlined, The ANDIS cohort (n=~27000, still recruiting) is a well-characterized cohort of individuals with newly diagnosed diabetes from Scania, Sweden. It's been instrumental in pinpointing five distinct diabetes subtypes. ANDIS merges genetic, clinical, and phenotypic data to provide a multidimensional view of diabetes.
- INSARK (Inskrivningsarkivregistret): As presented in Paper IV, the register contains data from standardized testing of individuals, primarily men, undergoing military conscription between 1969 and 2018. The register encompasses digital records for approximately 2 million individuals, with about 90% coverage for men born between 1951 and 1987. Conscripts underwent assessments that included verbal, spatial, logical, and technical ability tests, along with medical, physical, and psychological

evaluations(148). We have ethical approval for analysing data from INSARK on the individuals included in ANDIS – creating opportunity for further analysis than those presented in Paper IV.

Ethical Approvals

All studies carried out ensured adherence to ethical guidelines, with approvals obtained from both the ethical board, and later on the national ethical authorities. Ensuring the welfare, autonomy, and rights of the participants was of paramount importance throughout the research process. Specific diary numbers are stated specifically in the papers.

Muscle Biopsy Collection and Processing

Muscle biopsies have long been the gold standard for direct assessment of muscle tissue. Historically, the procedure relied on the Bergström needle, an approach pioneered by Dr. Jonas Bergström in the 1960s(149). The procedure, although highly effective, is invasive and may lead to participant discomfort. It's performed under local anaesthesia, involving a small incision in both the skin and muscle fascia to access the muscle.

However, with technological advancements and the pursuit of participant comfort, the spotlight has turned to micro biopsies. These are less invasive and considerably mitigate discomfort, marking a small, but significant, stride in the biopsy collection methodology(150).

Isolation and Culture of Muscle Cells

Human primary muscle cells are an important in vitro model, providing an accurate representation of human muscle physiology. A section of the biopsy is used to isolate muscle satellite cells. The biopsy samples undergo mincing and are subjected to a digestion process at 37°C. The resulting cells are strained and centrifuged, with the pellet then suspended in a growth medium. The initial plating phase allows fibroblasts to adhere to the dish, while the suspended cells are cultured on a matrigel-coated flask. This growth medium is replaced periodically.

When cells approach confluence, the medium undergoes sequential changes, facilitating cell differentiation over 8 days. To control the growth of proliferating cells, such as fibroblasts, Cytarabine is introduced. Selection for further analysis is

based on clear gene expression indicators of a successful myoblast-myotube transition.

Culturing human muscle cells, as opposed to using animal cells or established cell lines, provides an authentic representation of human muscle physiology(151, 152).

Determination of Muscle Fiber Type Distribution

In this project, both traditional and novel methodologies were applied to evaluate muscle fiber type distribution. Immunohistochemistry, a widely accepted method, was employed due to its robust and validated capability to identify and visualize different muscle proteins(153, 154). Nonetheless, this technique demands quite large tissue samples and is labour-intensive.

In contrast, the method described in Paper 3 leverages transcriptomic analysis to study muscle fiber types using smaller biopsies, offering efficiency in both sample size and time.

Other methods for fiber typing range from invasive ones like electrophoretic separation(155) to non-invasive techniques like magnetic resonance spectroscopy (MRS)(156). While each technique has its merits, the selection often depends on the specific goals and constraints of the study.

Exercise Testing: VO2max and Wingate Assessments

This project employed two classical tests in exercise physiology to gain insights into aerobic and anaerobic performance: the Wingate Test and the VO2max test.

Wingate Anaerobic Test

Originating from the Wingate Institute in Israel during the 1970s, the Wingate Test serves as a straightforward method to gauge anaerobic power and capacity. Conducted on a cycle ergometer, participants are asked to cycle at their maximum effort for a duration of 30 seconds. From this, it's possible to derive anaerobic capacity and maximal power output, relevant for activities demanding short, intense bursts of energy but could also be used as a surrogate measure for muscle properties like fiber type distribution(157, 158).

VO2max testing

For a clearer understanding of aerobic capacity, the VO2max test is commonly used. Often done on an ergometer bike, participants start with a manageable intensity, which gradually intensifies until they reach the point of exhaustion. The peak oxygen consumption during this period is what is referred to as VO2max(159).

It's recognized that VO2max is truly reached when there's no increase in oxygen consumption despite the intensification of the exercise, the heart rate is near its predicted maximum, and the respiratory exchange ratio goes beyond 1.1(160).

RNA Sequencing, Bioinformatics, and Data Analysis

To deeper investigate the molecular complexities of skeletal muscle, a combination of modern RNA sequencing methodologies and traditional validation techniques was employed.

- Global skeletal muscle gene expression profiling: We used oligonucleotide microarrays to profile muscle gene expression, establishing a connection between expression profile and insulin sensitivity. Microarrays provide a high-throughput platform to measure the expression levels of thousands of genes simultaneously(161, 162).
- Total RNA sequencing (totRNAseq): An all-encompassing method that sequences all types of RNA present in a sample, offering a holistic perspective on transcriptional activity(163).
- Single-nuclei RNA sequencing (snRNAseq): Targets RNA from individual nuclei, enabling detailed exploration of cell-type-specific gene expression, especially in tissues where cell dissociation is challenging. Using snRNAseq as a benchmark, cluster expression signatures from specific gene markers assist in interpreting muscle fiber nuclei type through linear matrix decomposition(164).
- Quantitative Real-time PCR (QPCR): To corroborate key findings, QPCR was employed. This technique calculates and normalizes expression levels, offering precision and validation for the results derived from RNA sequencing. (165)

All the derived sequencing data were subjected to advanced bioinformatics tools, described in detail in each paper, ensuring rigid data analysis.

Statistics

The application of rigorous statistical methods is important in ensuring the validity of data interpretations. In this thesis, a range of statistical techniques were implemented to analyse and interpret the multifaceted data(166):

- Descriptive Statistics: Furnishes basic insights into datasets by summarizing their primary features. Typical measures include means, standard deviations, and frequency distributions.
- Inferential Statistics: Used to draw conclusions from data that might not be immediately obvious. Methods such as t-tests, ANOVA, and chi-square tests were utilized to discern differences between groups or associations.
- Regression Analysis: Facilitates the understanding of the strength and character of the relationship between a dependent variable and one or multiple independent variables.
- Multivariate Analysis: Employed when examining more than two variables concurrently. It's instrumental in deciphering complex datasets and discerning interactions between numerous variables.

Proper application of these statistical methods is crucial for drawing meaningful conclusions and understanding the significance and implications of the findings.

Results

Conclusions and future directions

The work presented in this thesis provide insights into the complexities of diabetes, focusing on skeletal muscle insulin resistance. By adopting a broad approach to studying these phenomena and related pathophysiology, we reveal clinically valuable patterns and connections, creating a solid foundation for continued diabetes research. Our findings on the fiber type dichotomy, comparing with rodent fiber types, and in the light of discussed evolutionary perspective we highlight the need for using human-specific models to better understand our metabolic processes and related diseases.

Conclusions

Tenascin C Response to Exercise: Paper I showed a significant increase in Tenascin C (TNC) levels in blood after intensive exercise. Combined with in vitro findings, we suggest TNC has a role in muscle remodelling post-exertion.

Muscle Fiber Typing via Genetic Analysis: In Paper II we introduce a novel method for assessing muscle fiber type distribution. This cost-effective and accurate technique holds the potential to significantly advance our understanding of muscle fiber dynamics in relation to health and disease.

Insulin Resistance and Gene Expression: Paper III highlights a connection between 180 genes and insulin sensitivity. Showing the complexity of insulin resistance in skeletal muscle. Genes like SIRT2 and FBXW5 point to the importance of lipid metabolism and mTOR signalling.

Early-life Physical Fitness and Diabetes Subtypes: Paper IV's findings are pivotal. The observed association between early-life physical fitness and specific diabetes subtypes later in life accentuates the profound influence of early-life health determinants. This underscores the importance of understanding events preceding a diabetes diagnosis and how these differ across diabetes subtypes.

Future Directions

There's still a lot to learn about insulin resistance in skeletal muscle. Studying it from different angles can give a more comprehensive understanding of the disease's pathophysiological pathways. This can lead to enhanced treatment strategies, whether through lifestyle adjustments or pharmacological means.

A forthcoming study is set to compare muscle fiber type distribution among the SIDD and SIRD clusters, using the fiber typing method introduced Paper II. This kind of detailed look at skeletal muscle in people with T2D hasn't been done before. It can add to the ANDIS project and help researchers understand T2D better.

In light of Paper IV, Sweden's extensive and unique register data presents a great opportunity. We can study other factors or events that come before a diabetes diagnosis among the different diabetes types. For example, we could investigate common infections or other health issues that might be linked to certain subtypes.

Building upon the insights of this thesis, particularly from Papers I and IV, there's potential to undertake more extensive interventional studies. Envisioning a tailored exercise program optimized for skeletal muscle metabolic benefits, like increased capillary density, and implementing a randomized clinical trial (RCT) for individuals with newly diagnosed diabetes of different subtypes could be important. The primary end point of such a trial would be the prevention or delay of diabetes complications.

Expected results from these studies could offer practical clinical advice. This might include immediate exercise suggestions for patients and, in the long term, treatments using medication to influence muscle properties.

Popular Science Summary (In Swedish)

Typ 2-diabetes (T2D) är en av världens snabbast växande hälsoutmaningar. Bara i Sverige har över 400,000 personer diagnostiserats med T2D, och globalt beräknas siffran vara över 300 miljoner. Trots det stora antalet drabbade individer och den allvarliga belastningen som sjukdomen lägger på individer och sjukvårdssystem, är dagens diagnostik och behandlingsmetoder inte alltid tillräckliga.

T2D ökar risken för komplikationer som hjärt-kärlsjukdomar, ögonproblem, njurproblem och känselbortfall, särskilt i fötterna. Dessa komplikationer kan ofta vara närvarande redan vid diagnos. Eftersom tidiga symtom på T2D är svåra att identifiera, kan sjukdomen ofta gå oupptäckt under flera år. För att minska risken för komplikationer är det viktigt att försöka identifiera diabetes så tidigt som möjligt.

Traditionellt definieras T2D av ett högt blodsocker, men vi inser nu att sjukdomen är långt mer komplex och multifaktoriell där ett högt socker endast är slutprodukten. Även vid utvecklad sjukdom har olika individer olika kliniska egenskaper, sjukdomsprogression, läkemedelsrespons och risk för komplikationer.

I T2D, till skillnad från Typ 1 Diabetes där de insulinproducerande cellerna i bukspottkörteln slås ut, genererar bukspottkörteln oftast fortfarande insulin under en lång period. Men kroppens respons på detta insulin är nedsatt, vilket resulterar i att cellerna inte tar upp socker som de borde. Därför samlas sockret i blodet och höjer blodsockernivåerna – detta fenomen kallas insulinresistens. Insulinresistens drabbar primärt lever, fett och muskel.

En betydande kunskapslucka gäller vår förståelse av muskelns roll i insulinresistens och diabetes. Muskel har identifierats som en nyckelfaktor i insulinresistens och därmed också utvecklingen av T2D. Trots denna kunskap saknas det riktad och/eller individanpassad behandling på området. Förutom träning och kost finns det inte många behandlingsmetoder, som mediciner, som fokuserar på insulinresistens i musklerna. Inte heller finns det någon lättillgänglig och kostnadseffektiv diagnostik, såsom blodprover, som fångar insulinresistens i muskel.

Vi behöver därför forska mer för att få en detaljerad förståelse kring ämnet. Hur påverkar fysisk aktivitet T2D? Vilka faktorer, genetiska och miljömässiga, bidrar

till insulinresistens och dess utveckling? Kunskap som förhoppningsvis, i förlängningen, kan bidra till nya förebyggande åtgärder och behandlingar.

Detta projekt syftar till att, till viss mån, försöka fylla dessa viktiga kunskapsluckor. Genom en bred vetenskaplig ansats med fokus på klinisk relevans har mitt avhandlingsarbete tagit sig an frågan ur ett flertal aspekter.

I den första studien fokuserade vi på proteinet Tenascin C (TNC), ett protein som finns i kroppens extracellulära matrix och där nylig forskning visat att TNC kan rekrytera så kallade muskelstamceller vid exempelvis muskelskada. I vår studie fick 39 friska män genomgå en serie utförliga tester och provtagning för att kartlägga bland annat muskelfibersammansättning via muskelbiopsier och maximal aerob/anaerob kapacitet på en testcykel. Från muskelbiopsierna isolerades muskelstamceller som förädlades till muskelceller i odlingsskålar för senare cellförsök. I studien visar vi att mängden TNC i blodet ökade i genomsnitt med 23% efter en 30 sekunder lång ”all out” ansträngning på cykel. Mängden frisatt TNC i blodet verkade öka med maximal uppnådd effekt i Watt. Vi kunde också visa att genuttrycket av TNC ökar vid högre mognadsgrad hos muskelceller.

Detta antyder att TNC spelar en roll vid muskelns svar på ansträngning, sannolikt som en del av återhämtning och kompensation efter träning.

Den andra studien handlar om en nyutvecklad metod för att bedöma muskelfibertyp via genetisk analys. Vi har skapat en metod som, baserad på RNA-sekvensering, kan skatta muskelfibersammansättningen, det vill säga förhållandet mellan andelen snabba och långsamma muskelfibrer. Jämfört med traditionella, mer arbetskrävande, metoder fann vi en mycket god korrelation. Vår nya metod ger en möjlighet att analysera tusentals prover på ett mer automatiserat sätt än tidigare vilket ger en betydande kostnadseffektivitet jämfört med sedvanliga metoder. Den nya metoden kräver också, i genomsnitt, mycket mindre vävnad. Detta möjliggör användandet av betydligt mindre biopsinålar som tolereras bättre av individen.

I den tredje studien utforskade vi genetiken bakom insulinresistens i muskelvävnad. Genom att jämföra genuttryck i muskelprover från 38 män, utan diabetessjukdom, fann vi 180 gener som korrelerade med insulinresistens. Särskilt noterade vi att gener som SIRT2, som är involverad i fettmetabolism, och FBXW5, som reglerar mTOR-signalering, var starkt associerade med insulinkänslighet. mTOR är en känd faktor vid muskeltillväxt och träningssvar.

Den fjärde studien undersöker vilken roll spelar fysisk kapacitet, styrka och/eller kondition, i ung ålder för hur framtida diabetessjukdom ter sig. Det är sedan tidigare känt att hög fitness i ung ålder minskar risken för typ 2 diabetes. Det är däremot inte särskilt välbeforskat huruvida fitness i ung ålder påverkar sjukdomsförloppet för de som ändå drabbas av sjukdomen.

Med ANDIS-registret, som omfattar Alla Nya Diabetiker I Skåne, har kollegor på Lunds universitet med hjälp av datormodellering kunnat identifiera fem distinkta

diabetesundergrupper med unika sjukdomskarakteristika vid insjuknandet. Dessa subgrupper öppnar upp för en mer riktad och precis forskning kring olika drivande faktorer vid diabetessjukdom.

Subgrupperna är uppdelade på följande vis:

•**Grupp 1, SAID** (allvarlig autoimmun diabetes): liknar i stort sett typ 1 diabetes samt LADA (latent autoimmun diabetes hos vuxna).

•**Grupp 2, SIDD** (allvarlig insulinbristande diabetes): innefattar individer som har högt HbA1C, nedsatt insulinproduktion och en medelmåttig insulinresistens.

•**Grupp 3, SIRD** (allvarlig insulinresistent diabetes): Definieras av övervikt och en hög grad av insulinresistens.

•**Grupp 4, MOD** (måttlig fetma-relaterad diabetes): innefattar individer med kraftig övervikt som insjuknar vid en relativt ung ålder.

•**Grupp 5, MARD** (måttlig åldersrelaterad diabetes): representerar den mest omfattande gruppen (cirka 40%) och består huvudsakligen av de äldre patienterna.

Genom att koppla ANDIS till det svenska Inskrivningsarkivregistret (INSARK) har vi kunnat jämföra validerade mätningar av kondition och muskelstyrka vid mönstring (vid 18 års ålder) bland 4,417 män som alla utvecklat diabetes senare i livet.

I vår analys såg vi att individer diagnostiserade med vissa subtyper av diabetes (som MOD och SIRD) hade minskad knästyrka, greppstyrka och kondition jämfört med andra undergrupper, som MARD. Mycket av effekterna verkar drivas av ett högre BMI hos individerna med dessa subtyper. Vid justering vid BMI kvarstod intressant nog en nedsatt kondition hos SIRD-gruppen. Detta alltså ca 40 år innan de får sin diabetesdiagnos. Resultaten ger insikter i potentiella tidiga livsfaktorer bidragande till diabetes. Detta öppnar för framtida forskning för att mer i detalj undersöka bakomliggande mekanismer.

Insulinresistens, särskilt i muskulatur, är en underforskad del i T2D. Genom den forskning som presenteras här tar vi förhoppningsvis ett litet steg närmare att förstå muskelns centrala roll i diabetesutveckling. Projektet har givit en bas för fortsatt forskning på området och vi rekryterar just nu för ny studie där vi, genom vår nyutvecklade metod från studie 2, planerar att jämföra muskelfiber-sammansättningen hos diabetessubgrupperna SIDD och SIRD.

Med ökad insikt kan vi hoppas på att skapa effektiva och tillgängliga förebyggande strategier och behandlingar, vilket kan förbättra livskvaliteten för miljontals drabbade individer.

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