From Department of Physiology and Pharmacology
Karolinska Institutet, Stockholm, Sweden

ENVIRONMENTAL REGULATION OF METABOLISM: FROM TRANSGENERATIONAL EFFECTS OF NUTRITION TO ACUTE EFFECTS OF EXERCISE

Petter Alm

Karolinska Institutet

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Environmental regulation of metabolism: From transgenerational effects of nutrition to acute effects of exercise

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Petter Alm

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Andreas Vesalius, Berzelius väg 3, Solna

Principal Supervisor:
Prof Juleen R. Zierath
Karolinska Institutet
Department of Molecular Medicine and Surgery
Division of Integrative Physiology

Co-supervisor(s):
Prof Anna Krook
Karolinska Institutet
Department of Physiology and Pharmacology
Division of Integrative Physiology

Opponent:
Prof Susan Ozanne
University of Cambridge

Examination Board:
Prof Eva Blomstrand
Gymnastik- och idrottshögskolan

Prof Ulf Hedin
Karolinska Institutet
Department of Molecular Medicine and Surgery

Prof Birgitte Holst
University of Copenhagen
Department of Biomedical Sciences

“Only scared people run”

- H.K.

ABSTRACT

The incidence of metabolic disease has risen rapidly in the last half-century leading to both individual suffering and a burden to society. The importance of physical activity for metabolic health is well known. Furthermore, there is increasing evidence that parental food choice and obesity can affect the metabolic health of future generations. Although we do not fully understand through which mechanisms these effects are occurring. Further insight into these processes can prove to be crucial in the fight against metabolic disease. Therefore, the overall aim of this thesis is to study mechanisms through which environmental interventions affect metabolic health.

In **study I**, we investigate the skeletal muscle transcriptomic response of middle-aged men, either healthy or diagnosed with type 2 diabetes, after one bout of aerobic exercise. The two groups had a similar response to exercise just after the exercise bout. In contrast, three hours after the exercised bout skeletal muscle from diabetic subjects had an increased RNA and protein content of inflammatory markers. Thus, we describe that in skeletal muscle from subjects with type 2 diabetes there is an exacerbated inflammatory response. Whether this response is adaptive or mal-adaptive remains to be determined.

In **study II**, we exposed male rats to a high fat diet for 10 weeks before they were bred with chow-fed females. Male F1 rats, fed a chow diet, were used to generate the F2 generation. Some of the F1 and F2 offspring were further challenged with a high fat diet. When evaluating the metabolic health of the offspring, we found that females with a high fat-fed ancestry did not gain as much body weight when they were exposed to a high fat diet themselves, but were even more glucose intolerant than their cage-mates with chow-fed ancestry. We found *Let-7c* to be a possible epigenetic carrier of the ancestral high fat diet. In conclusion, we provide evidence that transgenerational inheritance can cause metabolic phenotype through *Let-7c*.

In **study III and IV**, we evaluated tissue-specific differences in of the transcriptome using skeletal muscle and liver from the animals in **study II** respectively. We used an initial transcriptomic approach and investigated the tissues with gene arrays. We found that the unfolded protein response was activated, possibly through the ATF-6 branch, in skeletal muscle from female rats with high fat fed grandfathers compared to females with a grandfather fed a chow diet, when subjugated to a high fat diet themselves. In the liver, we found that a grandpaternal high fat diet altered the TNF-α signaling pathway, independent of the offspring's own diet. These two studies show transgenerational inheritance in specific tissues.

Together, the work in this thesis highlights that effects of diet show transgenerational inheritance leading to both whole-body and tissue-specific metabolic changes. On the other hand, we show that exercise has a different response in the context of type 2 diabetes. Thus, the studies in this theses show how environmental factors can affect your metabolic health both indirectly and directly.

LIST OF SCIENTIFIC PAPERS

I. **Alm PS**, Pillon N, Arner E, Fritz T, Olsson T, Carninci P, Caidahl K, Wallberg-Henriksson H, Krook A, Zierath JR. *Acute exercise reveals an elevated and selective inflammatory response in skeletal muscle of individuals with type 2 diabetes.* Unpublished, in manuscript.

II. de Castro Barbosa T, Ingerslev LR, **Alm PS**, Versteyhe S, Massart J, Rasmussen M, Donkin I, Sjögren R, Mudry JM, Vetterli L, Gupta S, Krook A, Zierath JR, Barrès R. *High fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring.* Mol Metab. 2015 Dec 25;5(3):184-197.

III. **Alm PS**, de Castro Barbosa T, Barrès R, Krook A, Zierath JR. *Grandpaternal-induced transgenerational dietary reprogramming of the unfolded protein response in skeletal muscle*. Mol Metab. 2017 May 22;6(7):621-630.

IV. Castro Barbosa T, **Alm PS**, Krook A, Barrès R, Zierath JR. *Paternal high fat diet transgenerationally impacts the hepatic inflammatory response two generations later.* Unpublished, in manuscript.

CONTENTS

LIST OF ABBREVIATIONS

A2M	Alpha 2-Macroglobulin
ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
Avy	agouti viable yellow
BMI	Body mass index
C. elegans	Caenorhabditis elegans
cATF6	cleaved activating transcription factor 6
CCL2	Chemokine (C-C motif) ligand 2
CCL21	Chemokine (C-C motif) ligand 21
cDNA	Complementary DNA
CHOP	C/EBP homologous protein
CRP	C-reactive protein
CX3CL1	Chemokine (C-X3-C motif) ligand 1
CXCL1	Chemokine (C-X-C motif) ligand 1
CXCL10	Chemokine (C-X-C motif) ligand 10
CXCL12	C-X-C motif chemokine 12
CXCL16	C-X-C motif chemokine 16
CXCL2	C-X-C motif ligand 2
CXCL9	Chemokine (C-X-C motif) ligand 9
D. melanogaster	Drosophila melanogaster
DMR	differentially methylated region
EDL	Extensor Digitorum Longus
EGR1	early growth response protein 1
EGR2	early growth response protein 2
eIF2α	Eukaryotic translation initiation factor 2 subunit 1
ER	endoplasmic reticulum
GLUT4	Glucose transporter 4
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GpatCD-CD	Grandpaternal-Chow on Chow
GpatCD-HF	Grandpaternal-Chow on HFD
GpatHF-CD	Grandpaternal-HFD on Chow
GpatHF-HF	Grandpaternal-HFD on HFD
GSEA	gene set enrichment analysis
Hp	Haptoglobin
IFN-γ	Interferon gamma
IKK	IκB kinase
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-13	Interleukin 13
IL-16	Interleukin-16

IL-1α	Interleukin 1 alpha
IL-1β	Interleukin 1 beta
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IR	Insulin receptor
IRE-1	inositol-requiring enzyme 1 α
IRS	Insulin receptor substrate
IUGR	intrauterine growth restriction
JAK	Janus kinase
JNK	c-Jun N-terminal kinase
KEGG	Kyoto Encyclopedia of Genes and Genomes
miRNA	micoRNAs
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
PatCD-CD	Paternal-Chow on Chow
PatCD-HF	Paternal-Chow on HFD
PatHF-CD	Paternal-HFD on Chow
PatHF-HF	Paternal-HFD on HFD
PCA	principal component analysis
PERK	protein kinase R (PKR)-like endoplasmic reticulum kinase
piRNA	piwi-interacting RNA
Ppargc1	Peroxisome proliferator-activated receptor gamma coactivator 1
RNA-seq	RNA sequencing
RT-qPCR	reverse transcription-quantitative polymerase chain reaction
SAA	serum amyloid A component
SAP	serum amyloid P component
siRNA	short interfering RNA
sncRNA	Small non-coding RNAs
SOCS1/3	Suppressor of cytokine signaling 1/3
STAT1/3	Signal transducer and activator of transcription 1/3
T2D	Type 2 diabetes
TNF-α	Tumor necrosis factor alpha
tRNA	transfer RNA
UPR	unfolded protein response
WHO	World health organization

XBP-1	X-box binding protein 1

1 INTRODUCTION

1.1 ENVIRONMENTAL FACTORS AS REGULATORS OF METABOLIC DISORDERS

The technological advancement of the last century has caused a radical shift in the way people are living their lives. Work is generally less physically demanding, caloric intake has increased, and spontaneous sporting activity has decreased. This disequilibrium of calories in vs. calories out has, in turn caused an increase in unhealthy weight gain accompanied by several comorbidities such as cardiovascular and metabolic diseases.

Previously, metabolic diseases, such as obesity and type 2 diabetes mellitus, have been considered a problem mainly for the Western civilization. However, this is no longer the case. More people are suffering from overweight and obesity than undernutrition and starvation (WHO, 2016). During the last decades, the prevalence of obesity has escalated at an alarming rate. The most common way of classifying overweight and obesity is body mass index (BMI) (Table 1). Although BMI has been criticized for being imprecise, especially on the individual level, it is a good and easy way to identify individuals at risk of developing metabolic disease. Unfortunately, the Western lifestyle has been exported to developing countries, such as India and China, causing more harm to those populations.

Table 1 – BMI classification

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal weight	18.5 - 24.9
Overweight	25 - 29.9
Obese Class I	30 - 34.9
Obese Class II	35 - 39.9
Obese Class III	≥ 40

Type 2 diabetes, previously known as adult-onset diabetes, is characterized by insulin resistance and has been increasing in conjunction with obesity. To diagnose an individual, a glucose tolerance test is performed, were a solution containing 75 grams of glucose is consumed in the fasting state. The blood glucose is monitored during the following two hours, and if it is > 11.1 mmol/L at the final measuring time-point the individual is diagnosed with Type 2 diabetes (Table 2). In just 30 years, 1985 – 2015, there has been more than a 10-fold increase of diagnosed individuals and it is estimated to keep on increasing (Smyth and Heron, 2006; GBD, 2016). The disease is caused both by inherited and lifestyle factors. However, since no major change of the human DNA code has occurred in that timeframe, lifestyle factors are commonly considered to be the major causes of the increase.

Table 2 – Type 2 diabetes and impaired glucose tolerance diagnosis criteria's.

Classification	Fasting plasma glucose	2-h plasma glucose
Normal	≤ 6 mmol/L	≤ 7.8 mmol/L
Impaired fasting glucose	6.1 - 6.9 mmol/L	≤ 7.8 mmol/L
Impaired glucose tolerance	7.0 mmol/L	7.8 - 11.1 mmol/L
T2D	7.0 mmol/L	≥ 11.1 mmol/L

Exercise has proven effective both in the prevention and treatment of metabolic disease. Exercise increases insulin sensitivity, insulin-independent glucose uptake, as well as lipid and glucose oxidation (Gabriel and Zierath, 2017). Exercise capacity and general fitness seem to be even more vital to overall health than adiposity, as exemplified by the "obesity paradox" (Lavie *et al.*, 2015). It is worth noting, that there is ongoing debate regarding whether or not there really is such a thing as "healthy obesity" (Fan *et al.*, 2013; Kramer *et al.*, 2013). However, it is clear that exercise is beneficial, if not crucial, for a healthy life, and thus understanding exercise–mediated mechanisms of health improvement is important.

Although lifestyle factors are part of the explanation, there is an increasing amount of evidence indicating inherited biological factors contribute to the escalating rates of obesity and its comorbidities (Lake *et al.*, 1997; Pembrey *et al.*, 2006; Carone *et al.*, 2010). Even more interesting is that there is a missing link between the heritability of metabolic disorder and associated genes. More than 150 genetic loci have been linked to the development of obesity or diabetes though only around 10% of the heritability of type 2 diabetes can be explained, using the significant genome-wide association studies identified in humans with European ancestry (Bonnefond *et al.*, 2010; McCarthy, 2010). The realization that disease etiology may be partially independent of genetic alterations has sparked an increased interest in the field of epigenetics. Epigenetics is defined as inheritable changes of a phenotype that is not caused by a change in the DNA. Furthermore, epigenetic traits have been suggested to be one of the missing pieces that may explain the heritability of the obesity and type 2 diabetes in the current epidemic. The work of this thesis focuses on exploring the genetic alterations in skeletal muscle due to exercise and the influence of transgenerational epigenetic inheritance on metabolic disease.

1.2 INFLAMMATION AND METABOLIC DISEASE

There is a strong connection between development of metabolic disease and a dysregulated inflammatory response. Chronic low-grade inflammation is a trait of both obesity and type 2 diabetes (Donath and Shoelson, 2011; Ouchi *et al.*, 2011). In lean healthy individuals, nutrients are stored and metabolized in metabolically active tissues such as the liver, adipose, and skeletal muscle tissue. However, in obese individuals, adipocytes have been forced to expand in size and numbers to cope with the over consumption of calories. At one point, the adipocytes are no longer able to store the excess lipids and thereby causing a "spill over" of lipids to other compartments in the body, which are less well equipped to story fat. This, in turn, will trigger activation of several stress- and inflammatory signaling pathways in the affected tissues and cells, sometimes referred to as lipotoxicity (Unger, 1995). Other physiological states, potentially caused by over nutrition, which can initiate insulin resistance through inflammation, are glucotoxicity, endoplasmic reticulum stress, and oxidative stress (Rossetti *et al.*, 1987; Evans *et al.*, 2002). Although, there is still an ongoing debate as to whether metabolic diseases cause insulin resistance or if insulin resistance causes metabolic diseases, the link between the two is undisputed.

1.2.1 Inflammation in different metabolically active tissues

Adipose tissue is, besides an energy-storage site, an endocrine organ that can affect the whole body. Furthermore, in addition to containing adipocytes, it is also the residence of immune cells. Macrophages in particular have received attention by the scientific community. Obesity is associated with increased immune cell infiltration and the number of macrophages in adipose tissue correlates with adipocyte size (Weisberg *et al.*, 2003; Xu *et al.*, 2003). Additionally, macrophages in adipose tissue undergo a phenotypic switch in the obese environment, from having anti-inflammatory M2 characteristics to becoming pro-inflammatory M1 macrophages (Lumeng *et al.*, 2007). The enlarged adipocytes also contribute pro-inflammatory cytokines and chemokines (Skurk *et al.*, 2007). Together, all of this adds to systemic inflammation and the development of metabolic disorders (Esser *et al.*, 2013).

Unhealthy weight gain leads to lipid accumulation and inflammation in the liver. Nowadays, non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of liver disease and two thirds of the patients are obese (Bellentani *et al.*, 2004; Vernon *et al.*, 2011). If left untreated, the "simple" hepatic steatosis can develop into non-alcoholic steatohepatitis (NASH). NASH is characterized by, apart from the lipid accumulation, inflammation and fibrosis. It is elicited by several factors such as mitochondrial dysfunction, oxidative stress, and pro-inflammatory cytokines (Day and James, 1998). Unlike the adipose tissue, obesity does not increase the amount of immune cells in the liver but causes primarily the Kupffer cells to be activated (Cai *et al.*, 2005). Weight loss due to lifestyle intervention or bariatric surgery will reduce steatosis and the pro-inflammatory state in the liver (Weiner, 2010; Musso *et al.*, 2012), further demonstrating the close link between inflammation and metabolic disease.

One of the major organs affected by obesity and type 2 diabetes is skeletal muscle. The associated insulin resistance will greatly reduce insulin-stimulated glucose uptake by the muscle cells (DeFronzo, 1988). Despite this, the link between dysregulated metabolism and inflammation in skeletal muscle is less investigated than other tissues such as liver and adipose. Just as in adipose tissue, there is evidence that obesity can increase immune cell infiltration into skeletal muscle and that they appear to exhibit a pro-inflammatory phenotype (Fink *et al.*, 2014; Khan *et al.*, 2015). This is also observed in type 2 diabetes patients (Fink *et al.*, 2013). Local inflammation in skeletal muscle can impair insulin signaling and thereby decrease insulin sensitivity through autocrine and paracrine effects (Austin *et al.*, 2008; Patsouris *et al.*, 2014). The interaction between inflammation and metabolism in skeletal muscle is a relatively unexplored field that is both interesting and has the potential to be fruitful in the search for therapeutic targets against metabolic disease.

1.2.2 Molecular interactions between inflammatory and metabolic pathways

On a molecular level, the NF-κB and JAK/STAT pathways are of interest when exploring the interplay between dysregulated metabolism and inflammation (Figure 1). One of the main activators of NF-κB is TNF-α, though it can also be activated by several growth factors (Lowenthal *et al.*, 1989; Biswas *et al.*, 2000; Bhat-Nakshatri *et al.*, 2002). After binding to its

receptor, TNF-α activates a cascade that will lead to activation of IκB kinase (IKK), which will ultimately free NF-κB from IκB, thereby leaving NF-κB free to migrate into the nucleus and act as a transcription factor. Furthermore, activation of the NF-κB pathway can lead to insulin resistance through IKK or c-Jun N-terminal kinase (JNK) activation (Aguirre *et al.*, 2002; Gao *et al.*, 2002; Lee *et al.*, 2003). JNK prevents activation of the insulin receptor (IR) by phosphorylating inhibitory sites of the insulin receptor substrate (IRS) (Aguirre *et al.*, 2000; Aguirre *et al.*, 2002). The JAK/STAT pathway is primarily activated by interferons or cytokines such as IL-6. Although it is still unclear exactly through which mechanism the JAK/STAT pathway can also induce insulin resistance, it is suggested that this occurs through an increased abundance of SOCS1/3. Activation of JAKs results in increasing the phosphorylation of STAT1 and STAT3, which will lead to increased transcription and translation of SOCS1/3 that can interrupt the interaction of IR and IRS (Grzelkowska-Kowalczyk and Wieteska-Skrzeczynska, 2009; Gorina *et al.*, 2011; Tanti *et al.*, 2012; Mashili *et al.*, 2013)

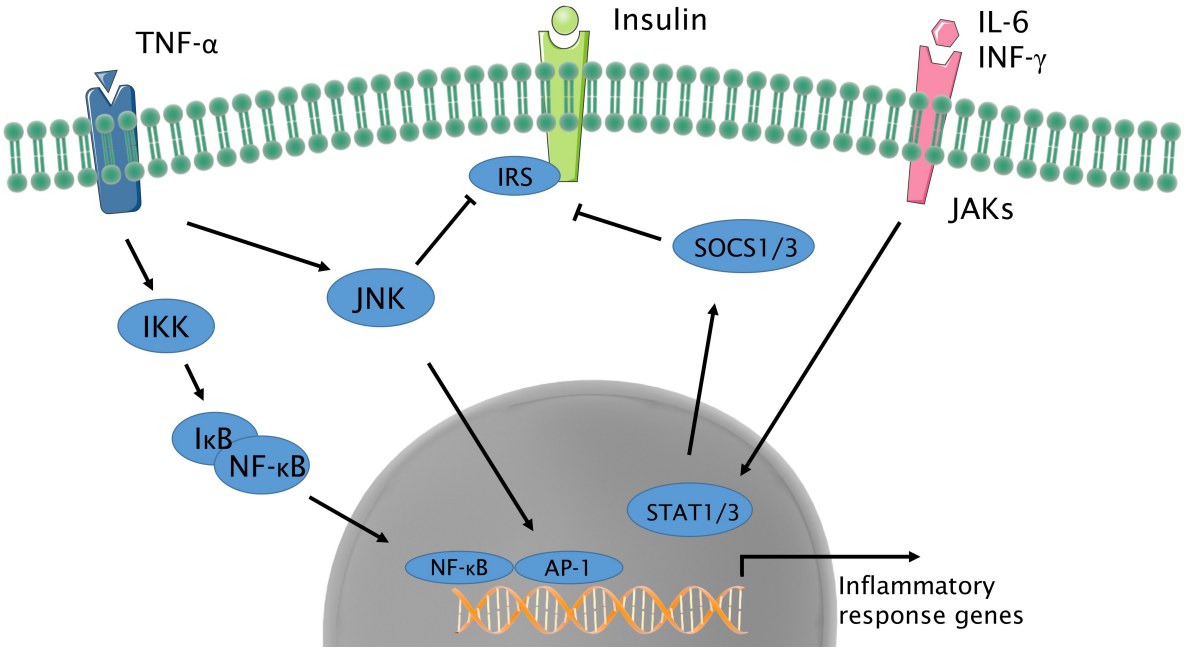

Figure 1 - Schematic illustration of inflammatory pathway connected to metabolism. Abbreviations used in the Figure: AP-1 (Activator protein), IFN-γ (Interferon gamma), IKK (IκB kinase), IL-6 (Interleukin 6) IRS (Insulin receptor substrate), JAK (Janus kinase), JNK (c-Jun N-terminal kinase), NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), SOCS1/3 (Suppressor of cytokine signaling 1/3), STAT1/3 (Signal transducer and activator of transcription 1/3) and TNF-α (Tumor necrosis factor alpha)

Another interaction site between metabolism and inflammation is the endoplasmic reticulum (ER) and the unfolded protein response (UPR) pathway (Figure 2). The ER is the key location for protein folding. If stressed by the accumulation of unfolded proteins the ER will initiate the UPR. Several symptoms of dysregulated metabolism such as lipotoxicity, hyperglycemia, and reactive oxygen species can cause ER stress (Shimoke *et al.*, 2003; Cnop *et al.*, 2010). The UPR consists of three branches: IRE-1, ATF6 and PERK. IRE-1 acts by initiating splicing of

XBP-1, turning it into an important transcription factor and thereby upregulating UPR genes needed to deal with the ER stress. ATF6 is located in the ER membrane and when it senses increased ER stress it is transported to the Golgi apparatus where it is cleaved and further migrates to the nucleus where it promotes transcription of ER chaperones (Wang *et al.*, 2000). PERK activation will cause eIF2α to be phosphorylated, thereby reducing protein synthesis and subsequently reduces the ER stress (Wang *et al.*, 2000). PERK can also cause a transcriptional response by activating ATF4 and, further downstream in the cascade, CHOP (Hotamisligil, 2010). Just as with other of inflammatory responses, the UPR is a crucial system when dealing with short-term stressors, but is harmful if constitutively activated.

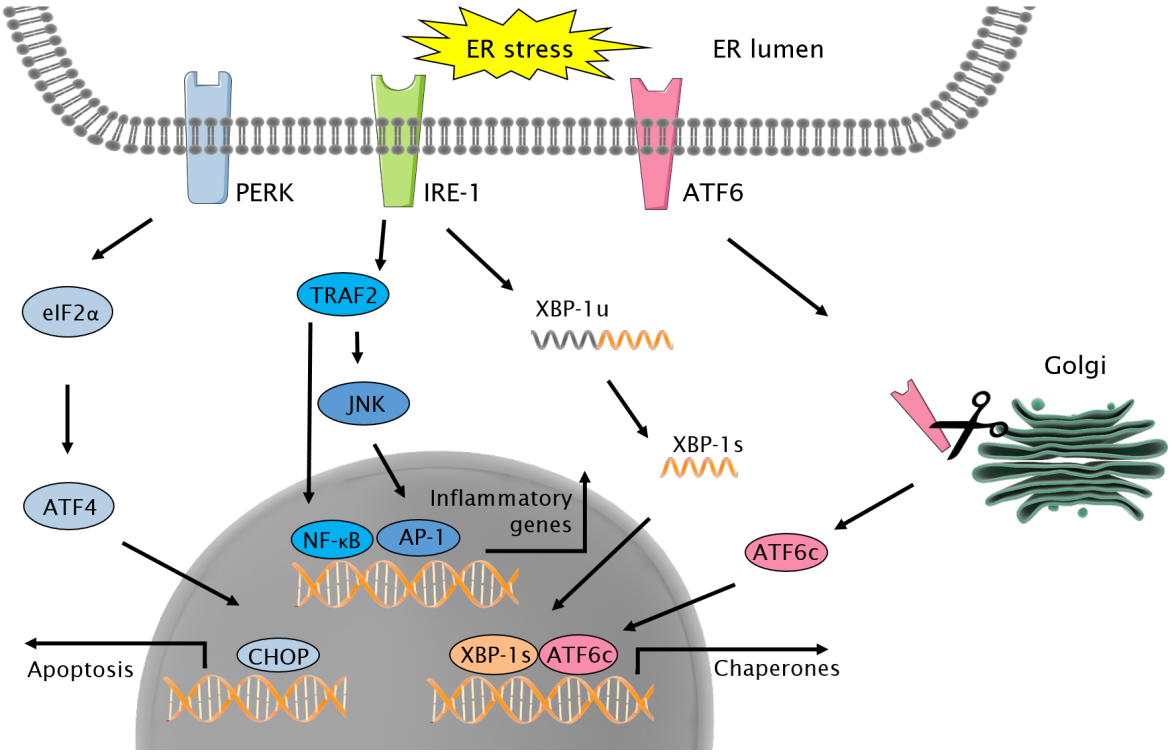

Figure 2 – Schematic illustration of the unfolded protein response signaling pathway. Abbreviations used in the Figure: AP-1 (Activator protein), ATF4 (Activating transcription factor 4), ATF6 (Activating transcription factor 6), cATF6 (cleaved activating transcription factor 6), CHOP (C/EBP homologous protein), eIF2α (Eukaryotic translation initiation factor 2 subunit 1), ER (endoplasmic reticulum), IRE-1 (inositol-requiring enzyme 1 α), JNK (c-Jun N-terminal kinase), NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), PERK (protein kinase R (PKR)-like endoplasmic reticulum kinase), TRAF2 (TNF receptor-associated factor 2), XBP-1s (X-box binding protein 1 spliced) and XBP-1u (X-box binding protein 1 unspliced).

1.2.3 Inflammatory mediators

There are a number of acute phase proteins, cytokines and chemokines, which are mediators of either pro-inflammatory or anti-inflammatory responses. Proteins that can act as inflammatory mediators and are included in the scientific papers of the thesis are summarized in Table 3 with a short description of their function and whether or not their circulatory levels are altered by obesity or diabetes in humans.

Table 3 – Table of inflammatory mediators included work of this thesis. Type 2 diabetes (T2D)

Name	Symbol	Main function	Obesity	T2D	References
Acute phase proteins					
Alpha 2-Macroglobulin	A2M	Protease inhibitor, can bind and carry inflammatory cytokines	na	↑	(James *et al.*, 1980; Rehman *et al.*, 2013)
C-reactive protein	CRP	Role in innate immunity	↑	↑	(Visser *et al.*, 1999; Duncan *et al.*, 2003)
Haptoglobin	Hp	Binding free plasma hemoglobin	↑	↑	(McMillan, 1989; Chiellini *et al.*, 2004)
serum amyloid A component	SAA	Recruitment of immune cells to inflammatory sites	↑	↑	(Pickup *et al.*, 1997; Yang *et al.*, 2006)
serum amyloid P component	SAP	Mediated phagocytosis	na	na	(Wright *et al.*, 1983)
Chemokines					
Chemokine (C-C motif) ligand 2	CCL2	Chemoattracts monocytes & macrophages	↑	↑	(Kim *et al.*, 2006; Liu *et al.*, 2012)
Chemokine (C-C motif) ligand 21	CCL21	Adhesion of naive T cells	na	↑	(Gunn *et al.*, 1998; Van Dyke *et al.*, 2017)
Chemokine (C-X3-C motif) ligand 1	CX3CL1	Chemoattracts T cells and monocytes	↑	↑	(Shah *et al.*, 2011)
Chemokine (C-X-C motif) ligand 1	CXCL1	Chemoattracts neutrophil activity	↑	↑	(Maury *et al.*, 2010; Sajadi *et al.*, 2013)
Chemokine (C-X-C motif) ligand 9	CXCL9	Chemoattracts T cells	na	na	(Muller *et al.*, 2010)
Chemokine (C-X-C motif) ligand 10	CXCL10	Chemoattracts T cells and monocytes	↑	↑	(Dufour *et al.*, 2002; Van Dyke *et al.*, 2017; Hueso *et al.*, 2018)
C-X-C motif chemokine 12	CXCL12	Chemoattracts lymphocytes	na	na	(Bleul *et al.*, 1996)
C-X-C motif chemokine 16	CXCL16	Chemoattracts T cells	↑	=	(Matloubian *et al.*, 2000; Zhao *et al.*, 2014; Lopes *et al.*, 2018)
C-X-C motif ligand 2	CXCL2	Chemoattracts polymorphonuclear leukocytes	↑	na	(Wolpe *et al.*, 1989; Rouault *et al.*, 2013)
Cytokines					
Interleukin 1 alpha	IL-1α	Induces NFκB pathway	↑	=	(Di Renzo *et al.*, 2007; Donath and Shoelson, 2011; Ballak *et al.*, 2015)
Interleukin 1 beta	IL-1β	Induces NFκB pathway	↑	↑	(Spranger *et al.*, 2003; Um *et al.*, 2004; Ballak *et al.*, 2015)
Interleukin 2	IL-2	Induces regulatory T cell proliferation	↓	↓	(Liao *et al.*, 2011; Vargas *et al.*, 2016)
Interleukin 4	IL-4	T-cell activation and promotes a M2 phenotype of macrophages	↑	na	(Egawa *et al.*, 2013; El-Wakkad *et al.*, 2013)

Interleukin 5	IL-5	Induces B cell growth	↑	na	(Takatsu *et al.*, 2009; El-Wakkad *et al.*, 2013)
Interleukin 6	IL-6	Activates a pro- or anti-inflammatory response	↑	↑	(Roytblat *et al.*, 2000; Pradhan *et al.*, 2001)
Interleukin 10	IL-10	Anti-inflammatory action, can block NF-κB activity	↓	na	(Esposito *et al.*, 2003)
Interleukin 12	IL-12	Stimulates TNF-α and INF-γ production	↑	↑	(Tripp *et al.*, 1993; Mishra *et al.*, 2011; Suarez-Alvarez *et al.*, 2013)
Interleukin 13	IL-13	Induces anti-inflammatory pathway, similar to IL4	na	na	(Zurawski and de Vries, 1994)
Interleukin-16	IL-16	Chemoattrats CD4+ immune cells	na	na	(Cruikshank *et al.*, 2000)
Granulocyte-macrophage colony-stimulating factor	GM-CSF	Stimulates monocyte and granulocyte production	na	na	(Burgess and Metcalf, 1980)
Interferon gamma	IFN-γ	Activation of macrophages	na	=	(Dalton *et al.*, 1993; Balducci *et al.*, 2010)
Tumor necrosis factor alpha	TNF-α	Promotion of inflammation (via NFκB pathway)	↑	↑	(Miyazaki *et al.*, 2003; Park *et al.*, 2005)

1.3 EXERCISE

Already the Greeks of the ancient world knew about the beneficial effects of physical activity and exercise. The main differences regarding the definitions of physical activity and exercise is that exercise is a structured, repetitive, planned, intentional movement that is intended to maintain or increase physical fitness. Modern technology has made it possible to replace the physical workforce that was previously needed for the majority of human history. As a result, there has been a decrease in the amount of everyday (and spontaneous) physical activity and an increase in organized exercise. Consider that a "treadmill" once referred to a mill powered by treading men, but now refers to a workout machine. About 24% of the world population is now, according to the WHO definition physically inactive (Sallis *et al.*, 2016). What is even more alarming is that studies based on questionnaires usually underestimate the level of physical inactivity. In an English self-reported study, 39 % of the men and 29 % of the women reported that they were meeting the physical activity guidelines. However, when a subset of the participants were equipped with accelerometers, it turned out that only 6% and 4%, respectively, actually met the requirements (Health and Social Care Information Centre, 2009). A similar accelerometer study from the USA found that 86% of the subjects did not meet the physical activity criteria and are *de facto* inactive (Troiano *et al.*, 2008). In conclusion, it is possible that the prevalence of inactivity is even higher than reported by the WHO.

Physical inactivity is a risk factor for a number of chronic diseases such as coronary heart

disease, accelerated biological aging/premature death, sarcopenia, metabolic syndrome, obesity, insulin resistance, gestational diabetes, type 2 diabetes, peripheral artery disease, hypertension, stroke, arterial dyslipidemia, congestive heart failure, endothelial dysfunction, depression and anxiety, osteoporosis, rheumatoid arthritis, colon cancer, breast cancer, and endometrial cancer (Booth *et al.*, 2012). The onset of type 2 diabetes can be prevented, or at least delayed, by lifestyle interventions that include physical activity/exercise (Tuomilehto *et al.*, 2001). For people with type 2 diabetes, exercise improves overall quality of life, blood glucose levels, blood pressure, body weight, and lipid levels (Gregg *et al.*, 2012; Wing *et al.*, 2013; Espeland *et al.*, 2014).

Besides transforming chemical energy into mechanical energy during exercise, skeletal muscle is also the tissue that takes up a majority of the glucose postprandially. Because of this, it has been the focus for a majority of mechanistic exercise adaptation studies. The primary glucose transporter in skeletal muscle is glucose transporter 4 (GLUT4). Under resting conditions, or in the fasted state, GLUT4 is mainly located in the cytoplasm and is thereby not able to transport glucose through the cell membrane. However, both insulin and exercise can trigger GLUT4 translocation to the cell surface (Figure 3). Increased insulin levels in the plasma will activate the insulin receptor (IR), which in turn activates the IRS. At the end of the signaling pathway TBC1 domain family member 4 (TBC1D4), will trigger the GLUT4 vesicles to translocate to the cell membrane and thereby increasing glucose uptake into the cell. This is known as insulin-dependent glucose uptake. Muscle contraction/exercise is on the other hand known as insulin-independent stimulator of glucose uptake. Exercise triggered translocation of GLUT4 occurs mainly through 1) $Ca^{2+/}$calmodulin-dependent protein kinase II (CaMKII), which is activated by the increased amounts of Ca^{2+}. 2) Through the increased amounts of nitric oxide by nitric oxide synthase (NOS). 3) By an increased AMP:ATP ratio caused by the high energy demand from the exercise, which increases 5' AMP-activated protein kinase (AMPK) activity, that will in turn activate TBC1 domain family member 1 (TBC1D1) and TBC1D4 (Ojuka and Goyaram, 2014).

Since GLUT4 translocation can be activated though these two different mechanisms, exercise is a good alternative to increase glucose uptake in individual that have developed insulin resistance. In addition to the acute effect of exercise on GLUT4 translocation, chronic exercise increases the amount and activity of mitochondria, and leads to metabolic flexibility (Meex *et al.*, 2010; Higashida *et al.*, 2011). Furthermore, exercise training can increase the amount of GLUT4 protein in skeletal muscle of type 2 diabetes subjects (Little *et al.*, 2011). It is still not fully understood through which mechanisms physical activity and exercise make skeletal muscle more insulin sensitive. Learning this can prove crucial in the fight against metabolic disease. In summary, exercise is not only favorable for a healthy life, but failing to meet the recommendations is outright detrimental.

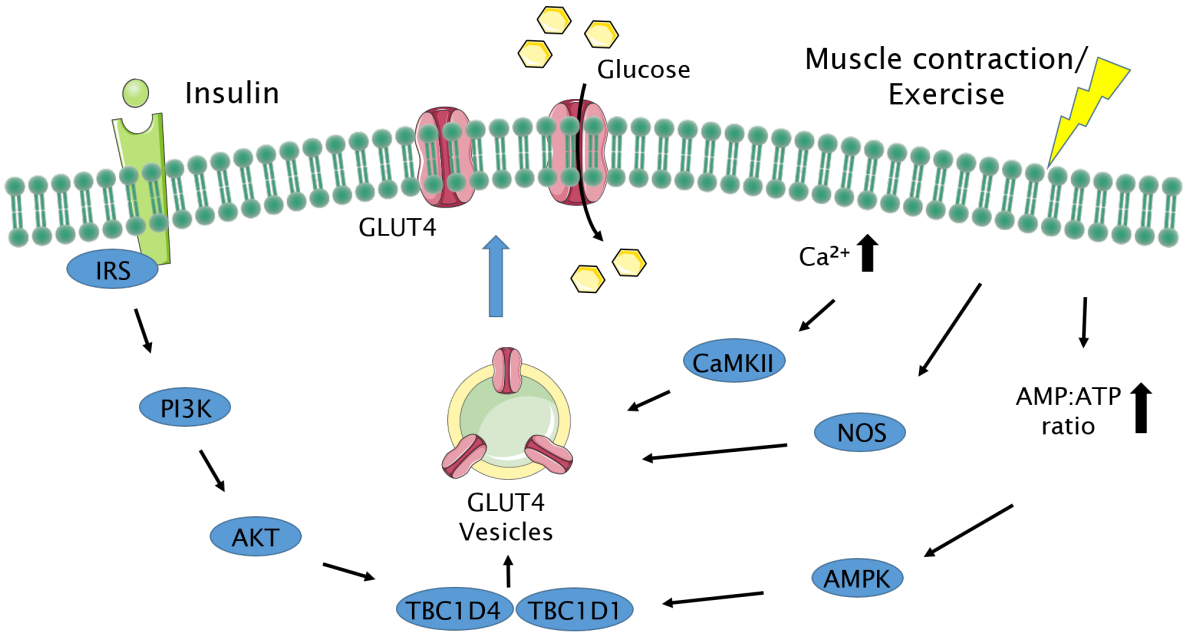

Figure 3 – Schematic illustration of glucose transtporter 4 (GLUT4) translocation in skeletal muscle. AKT (Protein kinase B), AMPK (5' AMP-activated protein kinase), CaMKII (Ca2+/calmodulin-dependent protein kinase II), GLUT4 (glucose transporter 4), IRS (Insulin receptor substrate), NOS (nitric oxide synthase), PI3K (Phosphatidylinositol-4,5-bisphosphate 3-kinase), TBC1D1 (TBC1 domain family member 1) and TBC1D4 (TBC1 domain family member 4).

1.3.1 Exercise and inflammation

Exercise is dependent on muscular contractions and because of this, skeletal muscle has been the tissue in focus for most types of exercise studies, inflammation included. Exercise will increase the amount of reactive oxygen species that in turn will trigger inflammatory signaling pathways such as NF-κB. Previously, this has been viewed as a deleterious process that should be avoided. However, non-steroidal anti-inflammatory drugs will inhibit the protein synthesis after exercise and blunt the exercise induced increase of satellite cells (Trappe *et al.*, 2002; Mackey *et al.*, 2007). This demonstrates how pro-inflammatory processes are crucial for a normal adaptive exercise response. Another part of the inflammatory response after exercise is the accumulation and activation of leukocytes in skeletal muscle (Peake *et al.*, 2005; Neubauer *et al.*, 2008). The inflammatory response can be divided into two different phases, the early and the late phase. In the early phase pro-inflammatory M1 macrophages, are more pronounced and are in charge of "cleaning up" cell debris before switching into more anti-inflammatory M2 macrophages that promote muscle regeneration (Arnold *et al.*, 2007; Varga *et al.*, 2016). Apart from being vital for tissue remodeling, leukocytes are important for maintaining normal glucose uptake in skeletal muscle and are able to enhance muscle performance through GLUT4 translocation (Tsuchiya *et al.*, 2018). Even though inflammation is part of the exercise response, it is still not clear if is dysregulated in people suffering from metabolic diseases.

On a whole-body level, exercise can reduce markers of chronic low-grade inflammation that are associated with type 2 diabetes (King *et al.*, 2003; Wang *et al.*, 2013; Hayashino *et al.*,

2014). This occurs through both direct and indirect actions. One direct effect of exercise is reduce the accumulation of inflammatory-prone visceral fat (Vissers *et al.*, 2013). Furthermore, exercise promotes the secretion of anti-inflammatory cytokines into the blood. Whether IL-6 has a role as a pro- or anti-inflammatory cytokine is still debated. Obese people have increased levels of circulating IL-6, suggesting that it is more of a pro-inflammatory cytokine. However, IL-6 is also increased by exercise and it has been shown to inhibit TNF-α production (Schindler *et al.*, 1990; Northoff and Berg, 1991). Overall, chronic exercise has a proven anti-inflammatory effect. However, an acute pro-inflammatory response is necessary for the beneficial training adaption to occur. Together this highlights the complex interaction between exercise and inflammation.

1.4 EPIGENETIC REGULATION

After the complete human genome sequenced, the expectations were that most, if not all, diseases would be traced to genetic causes. Thereby simplifying disease preventions and drug development. However, this way of thinking completely ignored the plasticity and rapid response of the genome to environmental stimuli that are often observed in biology. Since many diseases cannot be explained by alterations in the genetic code, there has been a paradigm shift that also takes into account epigenetic factors. The interplay between the environment and genome is thereby taken into account (Figure 4). The main epigenetic mechanisms DNA methylation, histone modification, and non-coding RNAs, will be briefly described in this section, (Figure 5**)**.

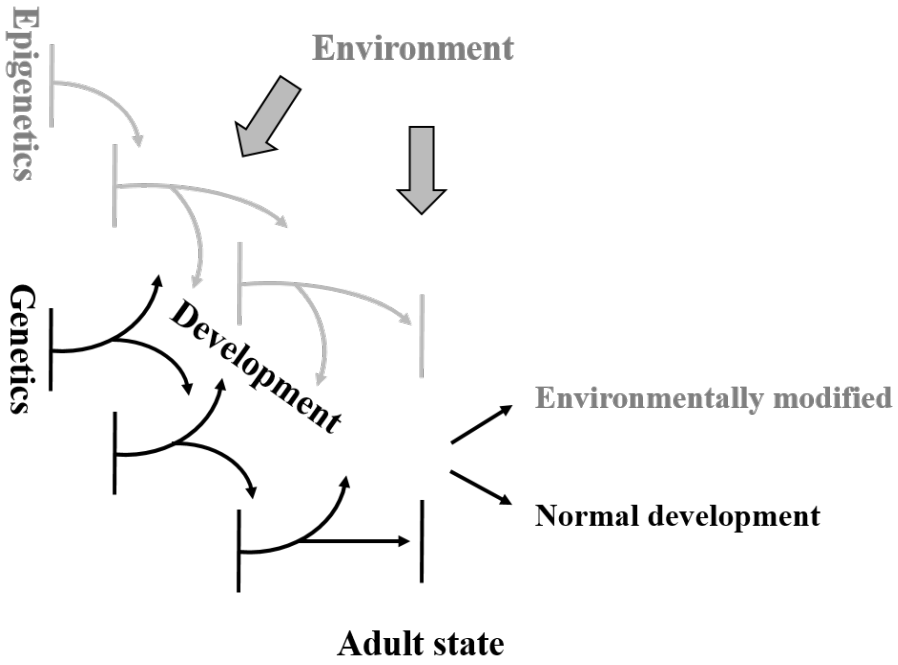

Figure 4 - Epigenetic and genetic cascade of events involved in development of phenotypes.

1.4.1 DNA methylation

DNA methylation was the first discovered and is the currently most investigated epigenetic

factor (Holliday and Pugh, 1975). In mammals, it is the cytosine base that is primarily methylated at the C5 position, but there has also been some observations suggesting that other DNA bases could be methylated (Barres *et al.*, 2009; Ziller *et al.*, 2011; Kulis *et al.*, 2015). In general, DNA methylation leads to repression or silencing of gene transcription, where the inactivation of one of the X-chromosomes in females is a classic example (Riggs, 1975). One of the mechanisms behind the repression/silencing is that methylation can sterically block the binding of transcription factors to the DNA. Although, DNA methylation is stable in somatic cells, there have been several studies demonstrating that the methylation and demethylation process are much more dynamic than previously thought (Shen *et al.*, 2013). Environmental factors can change the methylome of twins (Fraga *et al.*, 2005). Furthermore, exercise and weight loss can alter DNA methylation levels (Barres *et al.*, 2012; Moleres *et al.*, 2013). This data strongly suggests DNA methylation plays a role in mediating environmental changes in the regulation of metabolism.

1.4.2 Histone modification

The DNA is wrapped around protein structures referred to as histones, which can regulate gene expression by making the DNA more or less accessible for transcription. Histones can be modified at their tails in more than 60 ways. Although, it is possible to reduce and simplify this number to 10-15 patterns with specific genetic regulation (Tan *et al.*, 2011; Ko and Susztak, 2013). Histones are modified by adding or removing methylation groups, acetylation, phosphorylation, sumoylation and ubiquitination by several different enzymes (Biel *et al.*, 2005). Unlike DNA methylation, that in general is a more stable/long term repressor of gene transcription, histone modifications are relatively flexible and short-term in their character (Reik, 2007).

1.4.3 Non-coding RNA

Non-coding RNA is transcribed from DNA, but not translated into a protein. Large regions of the genome that do not code for protein have previously been described as “junk-DNA”. However, several of these transcripts have been subsequently revealed to have a regulatory function.

Small non-coding RNAs (sncRNA) is a group of RNAs that are shorter in length than 200 nucleotides, which include a number of different RNA classes such as micoRNAs (miRNAs), short interfering (siRNAs) and piwi-interacting RNAs (piRNAs). miRNAs were first discovered in the early 1990’s and contain around 22 nucleotides (Lee *et al.*, 1993; Wightman *et al.*, 1993). However, it was not until the early 2000’s their functions were better understood (Reinhart *et al.*, 2000; Lee and Ambros, 2001). miRNAs function by silencing mRNA by first base-pairing to the transcript, which will either cleave the mRNA, causing a less efficient translation in the ribosome, or by shortening the poly(A) tail of the mRNA and thereby destabilizing it. Since the role of miRNAs was discovered, numerous studies have been performed linking miRNAs to several diseases such as cancer, type 2 diabetes and obesity (Ortega *et al.*, 2010; Kantharidis *et al.*, 2011; Honardoost *et al.*, 2014). piRNAs are also able

to modulate gene expression by forming RNA-protein complexes and particularly targeting transposons. In mammals, the piRNAs are most abundant in testes and ovaries and have been shown to be crucial for spermatogenesis (Carmell *et al.*, 2007).

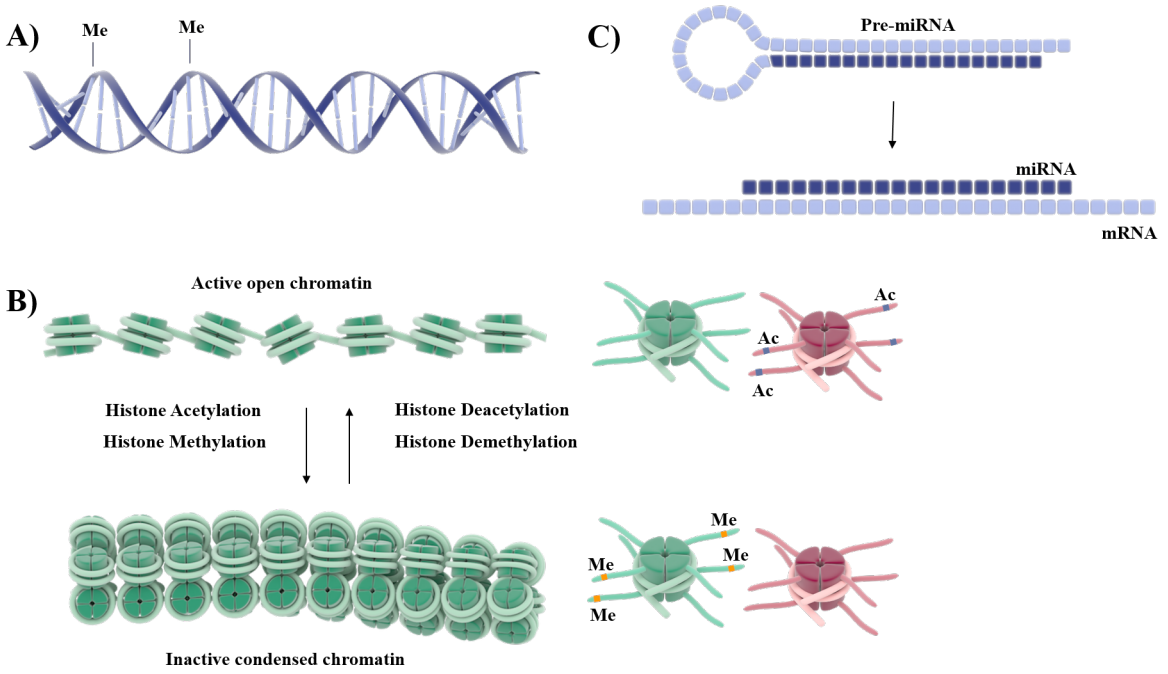

Figure 5 - Schematic of Epigenetic Mechanisms. A) DNA methylation of CpGs. B) Chromatin alteration by histone tail modifications. C) mRNA silencing by miRNA.

1.5 NON-MENDELIAN INHERITANCE

The idea that environmental information can be transferred from one generation to the next is not new; the first suggestions have been attributed to Jean-Baptiste Lamarck. He exemplified his thoughts with an example of the giraffe. According to Lamarck, giraffes developed long necks by reaching towards the treetops to eat and this caused the necks to get progressively longer. This trait would then be inherited by the offspring and so forth. His thoughts were later rejected and forgotten with the discovery of Mendelian inheritance. The discovery of epigenetic mechanisms that might explain how a trait can be inherited without a mutation in the DNA code has led to a rebirth of "Lamarckism". The results from the Överkalix cohort, together with the Dutch Winter Hunger study, implies that this phenomenon of inter- and/or transgenerational epigenetic inheritance also occurs in humans and has received a lot of attention both by the general public and the scientific community (Kaati *et al.*, 2002; Pembrey *et al.*, 2006; Lumey *et al.*, 2007). These studies and their implications for transgenerational epigenetic inheritance will be described in more detail later in section 1.5.2.

The term intergenerational or multigenerational epigenetic inheritance is generally used to

describe a transmission from one generation to the next. On the other hand, transgenerational epigenetic inheritance refers to a transmission of an environmentally triggered phenotype, to a generation that was not exposed to the initiating stimuli. If using a maternal lineage, there has to be a change in the F3 generation for it to be a true transgenerational effect, since also the fetus and its primordial germ cells are exposed together with F0 generation, which will in the end become the F2 generation, with the same stimuli. In contrast, if using a paternal lineage, it is sufficient for the F2 generation to exhibit a phenotype for it to be a true transgenerational effect (Figure 6) (Heard and Martienssen, 2014).

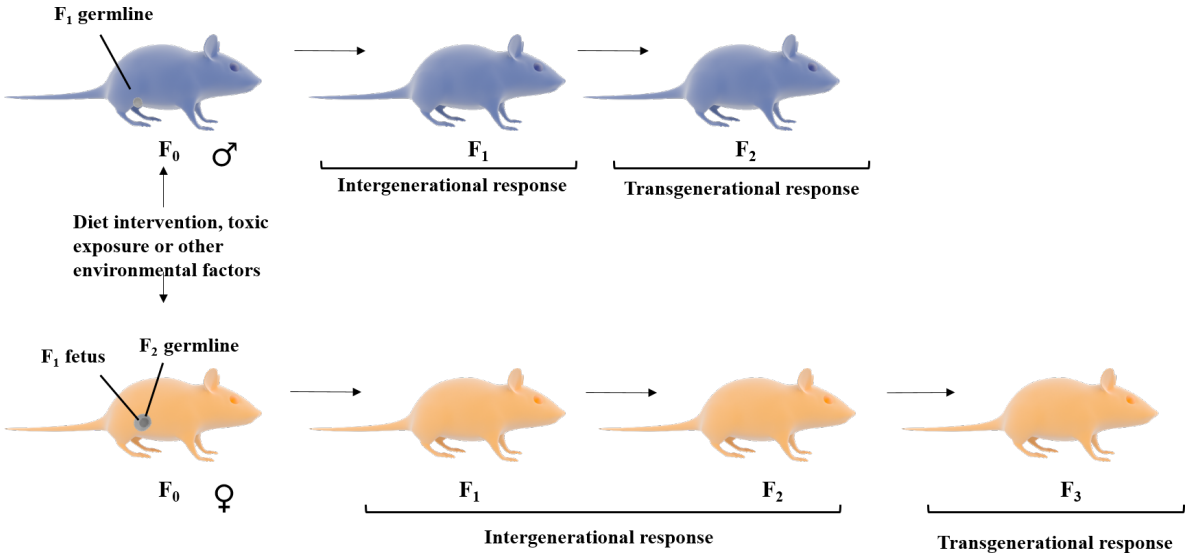

Figure 6 - Environmental effects across generations. Environmental exposure of the F0 generation will also directly expose the F1 generation, and in the case of a maternal linage also the F2 generation. A true transgenerational phenotype needs to be observed in the F2 generation when using a paternal linage or the F3 for a maternal.

Epigenetic inheritance was first described in plants (McClintock, 1961). Others have also shown how traits regulated by epigenetic mechanisms can be inherited by plants and one of the better illustrations of this is how two complex traits, root length and flowering time is due to inheritance of epigenetic quantitative traits loci that can account for 90% of heritability (Cortijo *et al.*, 2014).

One of most well-studied examples of epigenetic inheritance in mammals is the agouti viable yellow (Avy) gene (Morgan *et al.*, 1999; Martin *et al.*, 2008). An insertion of a retrotransposon IAP 100 kb upstream from the agouti gene leads to a yellow fur, obesity and diabetes in these mice. The phenotype can be rescued with supplementation of various methylation donors, i.e. vitamin B12, folic acid and betaine, which results in wild-type coat color and a decrease of morbidities (Cooney *et al.*, 2002; Waterland *et al.*, 2006). Another interesting observation is how this is only inherited through a maternal linage (Daxinger and Whitelaw, 2012).

A more recent example of non-Mendelian triggered obesity is how a Trim28 haploinsufficiency

state can induce bistable obesity and how obesity could be explained by enrichment of paternally expressed imprinted genes in Trim28+/D9 mice. Furthermore, humans with low TRIM28 adipose expression showed increased obesity prevalence together with an imprinted gene dysregulation similar to the Trim28+/D9 mice, suggesting that polyphenism could partly explain the obesity pandemic that the world is experiencing (Dalgaard *et al.*, 2016).

1.5.1 Maternal inter- and transgenerational epigenetic inheritance in mammals

Many studies have investigated the effect of an altered *in uterus* milieu on the offspring (Fowden and Forhead, 2004; King, 2006; Bruce and Hanson, 2010). Depending on the intervention and organism used, there are some differences in the observed phenotype of the offspring. Some methods used to induce intrauterine growth restriction are protein restriction, calorie restriction or ligation of the uterine artery of pregnant females (Simmons *et al.*, 2001; Resnik, 2002; Fernandez-Twinn *et al.*, 2005). When performed in rats, the later method results in a progressive dysfunction in insulin sensitivity and insulin secretion in a similar fashion as observed in people with type 2 diabetics (Stoffers *et al.*, 2003). To cope with the nutritional limitation, the cells of the fetus respond by reprogramming their mitochondrial function, causing the mitochondria to be dysfunctional in adulthood. This has been observed in several different tissues such as skeletal muscle, liver and β-cells, regardless if utilizing a protein restriction model or uterine artery ligation to induce the intrauterine growth restriction (Park *et al.*, 2004; Chang *et al.*, 2013; Sakai *et al.*, 2013). There is evidence of both histone alterations at specific targets, such as the key coactivator *Ppargc1*, as well as global hypomethylation (Fu *et al.*, 2004).

Undernutrition is still a real threat for millions of people around the world (Black *et al.*, 2013). However, the number of people suffering from overweight and obesity have surpassed the amount of people suffering from malnutrition and is predicted to increase (NCD-RisC, 2016). Both animal and human studies have confirmed that exposure of over-nutrition in a prenatal state can lead to maladaptive metabolism in the adult offspring. The obesity affects the oocyte directly and the phenotypes observed in the offspring are not only due to changes in the intrauterine environment (Jungheim *et al.*, 2010; Marquard *et al.*, 2011). This is nicely illustrated by transferring a two-cell embryo from obese mice to normal weight mice and thereby separating the fetus from the obese uterine milieu, which induces changes in the brain reward system and alters gene expression in the placenta (Grissom *et al.*, 2014; Sasson *et al.*, 2015).

Two possible mechanisms that can cause impaired metabolism are oxidative stress and inflammation. Obesity increases whole body inflammation both in a pregnant and non-pregnant state (You *et al.*, 2004; Roberts *et al.*, 2011). Furthermore, placentas of obese women have increased mRNA levels of genes associated with oxidative and inflammatory stress and therefore changes in gene expression can contribute to an inflammatory or state of the uterus (Roberts *et al.*, 2011). Oxidative stress appears to have a direct link with the maladaptive

metabolism developed by the offspring of the obese mother. If antioxidant supplements are added to a pregnant dam's diet, it can avert the negative effects in the offspring's future metabolic health (Sen and Simmons, 2010). Although there is a clear difference in the abundance of a number circulating metabolites and cytokines in normal weight versus obese pregnancies, which may cause negative phenotypes in the offspring, it is still unclear whether these two different signaling pathways are contributing.

An F1 generation can be directly affected by changes in the uterus milieu. However, environmental stress can be inherited to, at least, the F3 generation. Toxins have been used as environmental stressors', causing' different pathologic responses such as obesity, maladaptive social behavior and polycystic ovary syndrome in the offspring (Nilsson *et al.*, 2012; Wolstenholme *et al.*, 2012; Chamorro-Garcia *et al.*, 2013). Furthermore, a uterine exposure of a high-fat diet cause obesity in the F3 female, inherit through the paternal lineage (Dunn and Bale, 2011). Together this demonstrates how different environmental stimuli can cause phenotypic changes that are later inherit by future generations.

1.5.2 Paternal inter- and transgenerational epigenetic inheritance in mammals

Although the maternal environment affects future well-being of the offspring, whether the fathers environment could add to this is less well appreciated. Traditionally, studies on a paternal diet have focused on sperm count and motility since the spermatozoon does not contribute much more to the offspring than the DNA code it is carrying. This view has been challenged and mounting evidence showing how the fathers' lifestyle can be connected to offspring phenotype. Although some of the studies have used toxin exposure as an intervention (Skinner *et al.*, 2010; Lombo *et al.*, 2015; Mao *et al.*, 2015), the following text will focus on role of the dietary interventions and possible mechanisms for the transfer.

The previously mentioned studies from the Överkalix cohort are the most well-known examples of how the fathers' diet could influence his offspring cardiometabolic health in humans (Kaati *et al.*, 2002; Pembrey *et al.*, 2006; Bygren *et al.*, 2014). In Överkalix, the crop harvest records are available for several centuries, and since the village was practically isolated during the winter, researchers were able to estimate the food accessibility around 1900 and then correlate this with morbidities in the grandchildren of the starvation survivors. One of their most striking findings was that the risk of developing metabolic diseases in the grandchildren varied with the grandparental exposure to the food supply during adolescence (Kaati *et al.*, 2002; Pembrey *et al.*, 2006). Few human studies have focused on male-line inheritance of a diet, and the studies from the Överkalix cohort showed how an ample or inadequate paternal diet might influence the offspring phenotype. More importantly, the result from this cohort have triggered an increasing interest of transgenerational epigenetics, which have in turn led to a number of studies in animals confirming this phenomena (Carone *et al.*, 2010; Ng *et al.*, 2010; Fullston *et al.*, 2013; Wei *et al.*, 2014).

The first study to describe how a paternal high fat diet could influence the metabolic health of

the offspring in mammals (in a controlled environment) used F0 male rats fed a high-fat diet before mating with females on a control (chow) diet (Ng *et al.*, 2010). This intervention resulted in β-cell dysfunction in the F1 female offspring, accompanied with changes in the pancreatic transcriptome and methylome. Furthermore, the offspring from high-fat fed males showed increased adiposity, increased body weight, and impaired glucose tolerance (Ng *et al.*, 2010). A follow-up study in the same animal cohort showed five networks, (mitochondrial and cellular response to stress, telomerase signaling, proliferation, cell cycle and cell death and survival) were significantly changed in the retroperitoneal adipose tissue transcriptome (Ng *et al.*, 2014). A similar study published more evidence of an intergenerational inheritance in response to diet (Carone *et al.*, 2010). The founding F0 males were fed a low protein diet that caused an increased expression of hepatic genes involved in lipid and cholesterol biosynthesis. However, the methylome in the spermatozoa was concluded to be for the most part unaffected by the diet (Carone *et al.*, 2010).

The first evidence of a true paternal transgenerational epigenetic inheritance due to a diet intervention in mammals was published in 2013 (Fullston *et al.*, 2013). Earlier mentioned studies did not follow the offspring phenotype further than a F1 and are per definition intergenerational studies. F0 male mice were fed a high-fat diet and then bred with control females. The main phenotype in the mice deriving from the high-fat-fed founder was increased adiposity. Four miRNAs were identified to be altered in the high-fat-fed male founders sperm; miR-133b, miR-340, 196a and miR-205. The investigated predicted targets were down regulated in both the sperm and testis, following the dogma that miRNAs down-regulates the expression of its targets. The alterations in the miRNAs expression were suggested to explain how the environmental information (high-fat diet) was transferred from one generation to the next. (Fullston *et al.*, 2013).

1.5.3 Inter- and transgenerational epigenetic inheritance in other animal models

Animal models such as *Caenorhabditis elegans* (*C. elegans*) and *Drosophila melanogaster* (*D. melanogaster*) have been used to investigate inter-/transgenerational epigenetic inheritance in response to a diet intervention (Rechavi *et al.*, 2014; Öst *et al.*, 2014). These studies are interesting since these models lack DNA methylation, but still show an altered phenotype in the offspring, indicating that methylation does not have to be the main epigenetic marker responsible for transmitting environmental information to future generations.

A paternal high or low sugar diet in *D. melanogaster* can increase the susceptibility of obesogenic diet in the F1 offspring, demonstrating that there is a U-shaped response to the diet intervention (Öst *et al.*, 2014). The two extremes showed altered feeding behavior, with increased food intake and maladaptive alteration of the lipid store mobilization. The phenotype was not transmitted further to the F2 generation making it an intergenerational event. In *C. elegans*, starvation of the F0 founders' increases lifespan and a significant enrichment of genes associated with nutrient reservoir activity in the F3 generation (Rechavi *et al.*, 2014). Thus,

these studies provide evidence that transgenerational epigenetic inheritance is conserved between several species.

1.5.4 Possible mechanisms for paternal non-Mendelian inter- and transgenerational inheritance

The main biological contribution from a male to its offspring is the haploid genome, and this has lead researchers to mainly target the sperm in their search for epigenetic carriers that could explain non-Mendelian inheritance (Rando, 2012). There are also other possible contributing factors such as seminal fluids or unintended transfer of microbiota (Theodorou, 2013; Bromfield *et al.*, 2014). Here the focus will be on the three earlier mentioned epigenetic markers: DNA methylation, histone modifications and sncRNAs.

DNA methylation is the most well-studied of these potential epigenetic carriers. Several researchers investigating paternal non-Mendelian inheritance have measured changes in methylation in both spermatozoa and metabolic active tissues in the offspring (Carone *et al.*, 2010; Ng *et al.*, 2010; Wei *et al.*, 2014). However, in most cases the differentially methylated regions (DMRs) in tissues of the offspring have not been overlapping with DMRs in the father/grandfathers spermatozoa (Carone *et al.*, 2010; Fullston *et al.*, 2013). This appears to undermine the rather simplistic hypothesis that a diet can cause changes in the sperm epigenome that will escape erasure in the early development and is maintained into the somatic tissue, and thereby explaining the offspring phenotype. Furthermore, there are doubts that Lamarckian inheritance is a real phenomenon in mammals (Bromfield *et al.*, 2014; Iqbal *et al.*, 2015; Weiss, 2015). A well-executed study investigating transgenerational inheritance induced by different chemical compounds (vinclozolin, bisphenol A and bis(2-ethylhexyl) phthalate) provided evidence against persistent methylation changes in the non-exposed generation's germ cells (Iqbal *et al.*, 2015). Furthermore, a key caveat against DNA methylation as an epigenetic carrier of paternal environmental information is the almost complete erasure of methylation that takes place right after fertilization (Oswald *et al.*, 2000; Ly *et al.*, 2015). Even though parts of the methylated genome are able to escape erasure, it is unlikely that methylation would be the main epigenetic carrier since the imprinted control regions are neither more protected, nor more responsive to parental undernutrition. However, the imprinted genes that are affected have been proposed to play a role in the fetal response to undernourishment, and potential health issues in the offspring adult life (Radford *et al.*, 2012).

Overall, the evidence that DNA methylation is able to transfer information regarding the paternal milieu is at this point correlative. Functional studies to directly address this issue are currently challenging, although rapid technical advances should make relevant experiments possible in the near future. Until then, the debate will continue as to whether or not DNA methylation is a non-Mendelian mechanism capable of transferring environmental information between generations.

Changes in histone modifications have also been associated with dietary intervention causing an inter-/transgenerational response in the offspring (Carone *et al.*, 2010; Siklenka *et al.*, 2015;

Terashima *et al.*, 2015). Although, a majority of the histone proteins are exchanged into protamine's during spermatogenesis and thereby enabling a tighter packaging of the DNA code, some histones are maintained and might possibly transfer environmental information between generations. A high-fat diet can alter specific histone retention at genes involved in the regulation of development in mice spermatozoa (Siklenka *et al.*, 2015). A paternal high sugar diet is able to modulate the chromatin state in *D. melanogaster* spermatozoa and in their offspring (Öst *et al.*, 2014). Together, this suggests that histone modifications is a possible epigenetic carrier.

Another argument for the possible importance of histone modifications in inter-/transgenerational inheritance context, is that the common models *C. elegans* and *D. melanogaster* do have histones, but lack DNA methylation (Öst *et al.*, 2014). Histone methylation can be inherited in a transgenerational manner. One example of this is how changes of histone methylation in F0 sperm can impair survival and development of the offspring. These effects can be inherited to at least the F3 generation without changes of the genome or DNA methylation (Rechavi *et al.*, 2014). However, the same study also observed changes in sperm RNA, suggesting that an interacting complex mechanism between histone modification and RNA is responsible for transgenerational epigenetic inheritance.

sncRNAs are altered in many in inter-/transgenerational epigenetic inheritance studies (Ng *et al.*, 2010; Fullston *et al.*, 2013; Rechavi *et al.*, 2014). A majority of studies have been focused on miRNAs, but other classes such as piRNAs, siRNAs and tRNA fragments has also been observed to be changed. One logistical advantage is that it is relatively easy to perform functional studies with sncRNA in comparison to DNA methylation and histone modifications, by extracting sncRNAs from F0 male and then injecting them into fertilized oocytes. Mice on a "Western diet", i.e. a diet with both high fat and sugar content, have upregulated levels of several miRNAs, and control one-cell embryos injected with mmu-miR-19 present an impaired metabolic phenotype (Grandjean *et al.*, 2015). Furthermore, RNA-pools extracted from sperm exposed to different treatments have been injected into embryos resulting in similar phenotypes as the offspring conceived in a natural manner (Rodgers *et al.*, 2015), demonstrating that miRNAs can play a role in transferring information from the paternal environment in an inter-/transgenerational manner.

Recently, tRNA fragments have been suggested as mediators of epigenetic inheritance. When the sperm travels through the epididymis the abundance of different sncRNAs changes significantly and the increase of fragmented tRNAs is the most distinctive. Two individual groups have provided evidence of how a paternal diet intervention can alter amounts of the fragmented tRNAs and also how microinjections of these can alter gene expression of metabolic pathways in early embryos and adult offspring (Chen *et al.*, 2016; Sharma *et al.*, 2016). A counterargument against sncRNAs as a carrier of paternal epigenetic information is that sperm carries a small amount of sncRNA's in comparison to an oocyte, thus questioning if sperm could carry enough sncRNA to actually make a difference. However, an increasing number of studies utilizing different animal models and approaches have suggested that even

small amounts of sncRNA can have an impact.

2 AIMS

Metabolism is regulated by a complex interplay between environmental and genetic factors. The human genome has not undergone any radical changes during the last decades, but our way of life has drastically changed. These environmental changes have caused a surge of metabolic diseases such as obesity and type 2 diabetes. The overall aim of this thesis is to study the effect of environmental interventions on metabolism. The specific sub-aims were to:

- Determine if there is a different skeletal muscle trasncriptomic response to an acute exercise bout between healthy individuals and individuals with type 2 diabetes.

- Determine the effect of paternal and grandpaternal high fat diet on tissue-specific and whole body metabolism in subseqent generations.

3 EXPERIMENTAL PROCEDURES AND METHODOLOGICAL CONSIDERATIONS

Selected methods used in the studies of this thesis are presented here. The aim of this section is not to provide detailed information regarding the methods used (for this I refer to the method sections of the specific manuscript), but rather to discuss advantages or drawbacks of the different methods. All studies were approved by the local ethical committee and conducted accordingly.

3.1 HUMAN COHORT

In **study I**, a human cohort consisting of 18 normal glucose tolerant men and 18 men diagnosed with type 2 diabetes was recruited to perform a 30-minute bout of cycling. Blood and skeletal muscle samples were taken before, just after and 3-hours after the bout of exercise. Participants undertook an oral glucose tolerance test to evaluate the status of their glucose metabolism (Table 4).

Table 4 – Anthropometric data of the participants in **study I** that are included in this thesis.

	NGT	T2D
Age	60.3 ± 1.4	61.0 ± 1
BMI (kg/m^2)	27.5 ± 0.7	28.6 ±0.6
Body Fat (%)	28.7 ± 1.3	30.1 ± 1.0
Blood Pressure Diastolic (mmHg)	78.7 ± 1.7	78.8 ± 2.1
Blood Pressure Systolic (mmHg)	130.1 ± 3.0	135.6 ± 3.2
Fat Mass (kg)	25.4 ± 1.5	27.0 ± 1.7
HbA1c(mmol/L)	35.6 ± 0.9	47.8 ± 2.1
Hip (cm)	109.9 ± 1.5	102.3 ± 2.3
HOMA-IR*	1.5 ± 0.2	3.6 ± 0.5
Lean mass (kg)	60.0 ± 1.3	60.1 ± 2.0
Length (m)	1.8 ± 0.0	1.8 ± 0.0
Max heartrate (beats/min)	164.5 ± 2.2	164.1 ± 3.8
Max workload (Watt)	246.6 ± 10.9	201.1 ± 11.1
ALAT (µkat/L)*	0.4 ± 0.0	0.6 ± 0.1
ASAT (µkat/L)	0.4 ± 0.0	0.5 ± 0.0
Cholesterol (mmol/L)*	5.3 ± 0.2	3.9 ± 0.2
Creatinine (µmol/L)	83.2 ± 3.2	80.1 ± 2.7
Glucose OGTT 0 min (mmol/L)*	5.2 ± 0.1	7.4 ± 0.4
Glucose OGTT 120 min (mmol/L)*	5.6 ± 0.4	14.4 ± 0.7
HDL (mmol/L)*	1.4 ± 0.1	1.2 ± 0.1
LDL (mmol/L)*	3.4 ± 0.2	2.1 ± 0.2
Triglycerides (mmol/L)	1.0 ± 0.1	1.2 ± 0.2
Resting Pulse (beats/min)*	58.8 ± 0.1	68.7 ± 2.2
C-peptide (nmol/L)	0.6 ± 0.1	1.0 ± 0.1
Waist/Hip ratio*	1.0 ± 0.0	1.0 ± 0.0
Waist (cm)	98.8 ± 2.1	104.1 ± 1.9
Weight (kg)	88.3 ± 2.3	89.5 ± 3.3
Relative VO_2 max (ml/kg/min)	36.4 ± 2.0	31.4 ± 2.0
Absolute VO_2 max (ml/min)	3162.8 ± 141.1	2753.4 ± 163.9

* Significant difference using unpaired Students *t*-test. Data are represented as mean ± SEM. N = 18

3.1.1 Exercise protocol

The participants performed a maximal VO_2 uptake test as part of the screening process. Maximal heartrate and maximal workload were also recorded. The measured maximal heartrate was later used to individualize the resistance during the exercise bout, with a target workload corresponding to 85% of maximum heartrate. A 5 min warm-up with increasing resistance preluded the bout.

3.1.2 Skeletal muscle biopsies

There are different methods of obtaining a skeletal muscle biopsy; one can use the "Bergström needle", "open muscle biopsy" or the "Weil-Blakeley conchotome", to mention some. All possible methods have different advantages or disadvantages. In **study I**, we used the Weil-

Blakeley conchotome. The subjects' skin is washed and local anesthesia is applied before a small, 2-3 cm, incision is made above the *vastus lateralis*. The surgeon then goes through the subcutaneous fat depot to open the muscle *fascia*. Muscle biopsies weighing ~ 20-70 mg are acquired by pinching the head of the conchotome to the muscle and firmly pulling it out. The procedure is relatively easy to perform, several biopsies can be obtained from the same incision and it is not overly invasive, which reduces both the risk of infection and recovery period. However, the biopsy size is smaller compared to other methods, such-as "open muscle biopsy", and the biopsy is also less intact. Thus, when gathering muscle biopsies to perform RNA or DNA experiments, the Weil-Blakeley conchotome method is an excellent option.

3.2 ANIMAL EXPERIMENTS

In biological research, it is often necessary to utilize different models due to logistical, financial and/or ethical reasons. Furthermore, it makes it possible to exclude confounding factors to a greater degree than it is possible to do when studying humans. Doing so, we can expand and deepen our knowledge regarding physiological processes considerably faster. In **study II, III** and **IV**, we used Sprague Dawley rats as a model to study transgenerational inheritance. Rodents are excellent models when studying physiology. They are mammals, their genomes are sequenced, and the fact that they have been used as models for several decades means that we know a lot about how to translate rodent physiology to human physiology. We chose rats as a model rather than mice. Although, it is more expensive to house rats – due to their size they require more cages and more food – there were other advantages. Since our study design was based on an intergenerational study by (Ng *et al.*, 2010), it meant that we could directly compare our results with theirs. Furthermore, since rats are larger, we obtained more material (tissue) to work with and could thereby reduce the number of animals needed.

3.2.1 Breeding design

In order to investigate the transgenerational effect of a high fat diet we used a paternal line of Sprague Dawley. The founder generation was either fed a high-fat diet (mix of two different high-fat diet pellets consisting of either 42 E % or 45 E % from fat) or a chow diet. As previously mentioned, our study design was based on a study from Margret Morris' group (Ng *et al.*, 2010), hence the choice of the high-fat diet diet and span of treatment. After 12 weeks, male rats bred with chow-fed female rats to generate the F1 generation. To generate the F2 generation, chow-fed F1 male rats (from a high-fat-fed or chow-fed father) mated with independent female rats. Both the F1 and F2 generations were divided into different subgroups and fed either a high-fat diet or a chow diet. Resulting in four different study groups per generation (Figure 7).

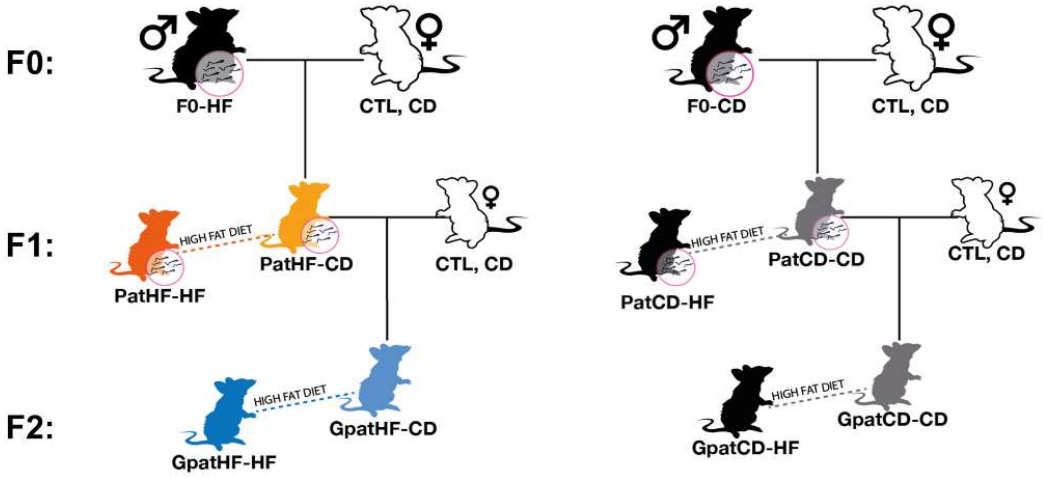

Figure 7 – Schematic illustration of the study design: To engender the F1 offspring, F0 male Sprague-Dawley rats fed a high-fat diet (F0-HF) or a control chow diet (F0-CD) during 12 weeks, before they were mated with control fed females (CTL,CD). F1 male rat, fed a control-diet, mated with females from an independent line to generate the F2 generation. Both generations were studied at 10 weeks of age, a sub-group of rats were subjected to chow or high-fat diet for 12 weeks. Adapted from (de Castro Barbosa *et al.*, 2016) (study II).

3.2.2 Animal phenotyping

Our major tools to assess the offspring's metabolic phenotype were glucose and insulin tolerance tests. Food was removed prior to the tests to ensure that the animals were fasted before injection with a glucose or insulin solution into the peritoneum, and blood from the tail vein was extracted to measure the blood glucose level. Furthermore, we monitored the weight gain of individual animals on a weekly basis, as well as food intake. Another interesting measurement would have been energy expenditure. Unfortunately, we did not have access to metabolic cages that could house rats at this time. At endpoint, we collected and measured the weight of different fat pads, muscles and other relevant tissues, making it possible to estimate body composition. The ideal way of measuring this would had been magnetic resonance imaging (MRI). However, due to logistical reasons, this was not possible.

3.3 METHODS TO MEASURE GENE EXPRESSION

All cells in a single organism share the same genetic code. However, the gene expression of different cell populations (i.e. transcribed RNA molecules) are vastly diverse. The univariate method of reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is one of the most commonly used methods to measure gene expression. It has the advantage of being relatively inexpensive, easy to perform, specific, and highly sensitive. Furthermore, it is relatively easy to perform traditional statistical methods, such as Student's *t*-test and different versions of ANOVAs, with the results from a RT-qPCR. However, it does suffer from the same problem as all univariate methods: the target needs to be previously known, and the method is

more suited for hypothesis testing studies, as opposed to hypothesis generating studies.

It is getting more and more common to perform so-called 'omics studies were the whole genome expression is measured in a sample at once. One way of doing this is by gene array. A gene array contains thousands of DNA probes, which align with specific parts of the genome. When a sample DNA strand hybridizes with the probe, it gives a fluorescent signal that is then measured and quantified. Gene arrays have the advantage of being (for an 'omic approach) inexpensive and nowadays analysis of the data is well established. However, just as with other 'omics methods, the massive amount of data is a double-edged sword. Traditional statistical tests, such as Student's *t*-test and ANOVA, are not designed for this type of data. There have been different approaches to deal with the analysis of this type of data and one of the most common approaches is to apply a multiple comparison test. In addition to this, results from probes that are giving a low signal need to be validated, most commonly by qPCR, to ensure that the signal is not due to 'noise'.

Another method of measuring whole genome expression is by RNA sequencing (RNA-seq). There are different ways of preparing a RNA-seq library, depending on the focus of the study in question. In short, one way is to first perform an rRNA depletion and then fragment the remaining RNA before converting into cDNA. The cDNA is then amplified using PCR and, finally, sequenced. Although the cost of RNA-seq is still relatively high, it is diminishing. Compared to gene arrays, it has higher sensitivity, is more precise, and is much more suited when investigating non-coding RNA. In addition to this, RNA-seq does not require *a priori* knowledge of the samples genome; something that is required for a gene array. In this thesis, all three mentioned methods of measuring gene expression were used. RT-qPCR and gene arrays were used in **studies II, III and IV**; and RNA-seq in **studies I and II**.

3.4 BIOINFORMATICS

Bioinformatics is an interdisciplinary field using mathematical and programing approaches to investigate the massive amounts of data acquired from various 'omics methods into meaningful biologic insights. If a data matrix consists of 20 000 genes and 100 samples, it means that there will be 2 000 000 data cells. It is not possible to analyze this amount of data manually and there is a big risk of "not seeing the forest for the trees" if not using the correct set of tools. One example of how to make sense of this data is to apply different clustering algorithms. Nowadays, there are many well-established pipelines and methods to analyze individual 'omics, but how to integrate the different 'omics types is still challenging. With the increased usage of 'omics-based methods, the need to understand the rapidly expanding field of bioinformatics has increased in equal measure.

Study I is primarily utilizing the statistical program "R" and freeware packages that are available from BioConductor, for the bioinformatics analysis. The RNA-seq alignments were performed by National Genomics Infrastructure, at Science for Life Laboratory, Stockholm. We used a negative binomial distribution method, DESeq2, to determine the differential gene expression of acute exercise in the two groups (Love *et al.*, 2014). After this, we used different

methods of clustering: principal component analysis (PCA), gene ontology over-representation, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (Tarca *et al.*, 2009; Yu *et al.*, 2012). The gene arrays of **studies II-IV** were performed and normalized using the console plier method, at Bioinformatics and Expression Analysis (BEA) core facility, Karolinska Institutet, Huddinge. In **studies I-IV**, we used gene set enrichment analysis (GSEA), from Broad Institute, to assess transcriptomic alterations (Mootha *et al.*, 2003; Subramanian *et al.*, 2005).

3.5 FIGURES INCLUDED IN THE THESIS

The figures included in the introduction were made in PowerPoint using freely available medical images from two online sources: https://smart.servier.com/ and http://www.somersault1824.com/. Figures in the method and result/discussion sections are adaptations from **studies I-IV**.

4 RESULTS AND DISCUSSION

4.1 RESPONSE TO ACUTE EXERCISE IN TYPE 2 DIABETES

The benefits of exercise are well known (Gabriel and Zierath, 2017). However, it is not fully understood through which pathways these beneficial adaptations occur or if they are differently regulated in people diagnosed with type 2 diabetes. Previous studies investigating the effects of exercise in patients with type 2 diabetes have mainly focused on the effects of training, rather than acute effects of exercise (Boule *et al.*, 2001)**.** It is clear that long-term effects, such as decreased HbA1c and increased aerobic capacity, are positive for both healthy and diabetic individuals. However, less is known about the acute effects of exercise in individuals with a type 2 diabetes diagnosis.

To determine the exercise-induced pathways in skeletal muscle we used an unbiased approach by sequencing RNA from skeletal muscle biopsies of healthy or type 2 diabetesmiddle-aged men in **study I**. Data were then analyzed in the statistical program "R", (Figure 8). The initial transcriptomic response of both the healthy control group and the type 2 diabetes group were similar. Some of the top gene ontologies were expected gene pathways related to blood vessel development and regulation of MAPK cascade (Gomez-Cabrera *et al.*, 2005; Hoier *et al.*, 2012) (Figure 9A-B). Several of the genes upregulated just after the exercise were associated with gene transcription, such as early growth response (EGR), protein 1, EGR2 and MYC. Increased expression of transcription inducing genes immediately after exercise is expected (Yu *et al.*, 2001; Safdar *et al.*, 2011)**.** Our results suggest that in this early post-exercise phase, the skeletal muscle of both groups is primed for further transcription and remodeling. Supporting this notion, another study with a similar design found that most genes upregulated directly after exercise are either transcription factors or genes related to RNA transport (Hansen *et al.*, 2015). This study combined a metabolic and transcriptomic analysis. This integrative 'omics approach has shown the insulin signaling pathway to be highly affected by acute exercise. In contrast, we did not observe any transcriptomic changes in the insulin singling pathway. However, it is likely that these alterations would have been uncovered by an integrated approach with phosphoproteomics or metabolomics.

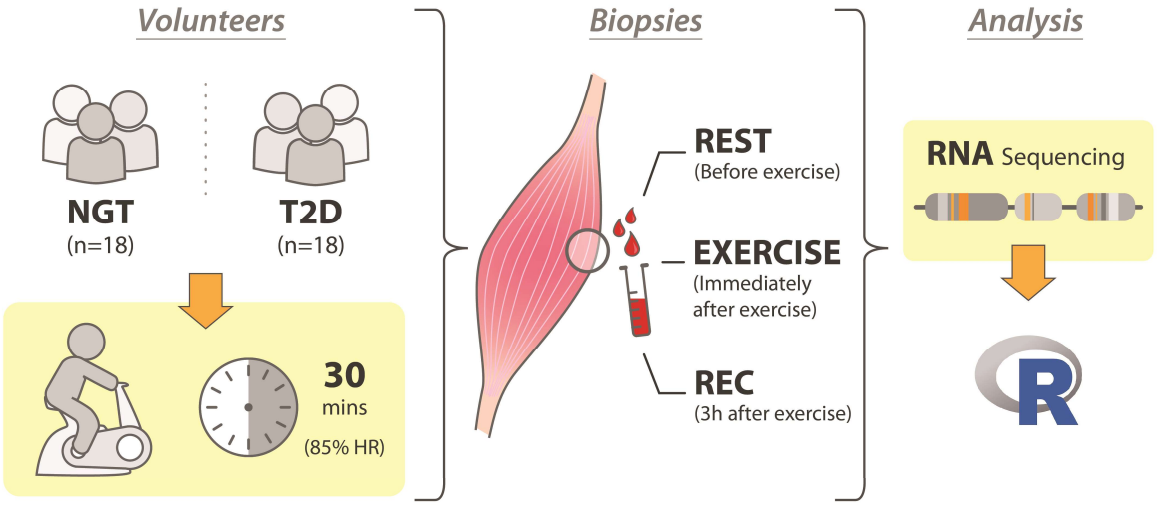

Figure 8 – Schematic illustration of the study design. Normal glucose tolerant (NGT) and type 2 diabetic (T2D) subjects were recruited and performed an acute bout of aerobic exercise at 85% of maximal heartrate. RNA sequencing was performed on skeletal muscle biopsies taken at three time-points: Before exercise, "rest"; immediately after exercise, "exercise"; and after three hours of resting after the exercise bout; "recovery". RNA sequencing data were analyzed using different bioinformatics tools, mainly in the statistical program "R".

Strikingly, the type 2 diabetes group had a much higher number of differentially expressed genes compared to the control group at recovery (Figure 10A). The majority of genes altered in the control group are also altered in the type 2 diabetes group, indicating that the exercise response is still somewhat shared between the two diagnosis groups. This pattern of acutely increased transcription after exercise is in line with previous observations in both healthy and diabetic populations (Hansen *et al.*, 2015; Rowlands *et al.*, 2016). Thus, it is not suprising to see an increase in the amount of altered transcripts in the recovery time-point. Indeed, one of the most highly upregulated ontologies in the exercise time-point was related to gene transcription. After the initial high energy demand from the actual work has reduced, the muscle cell can redistribue more energy to both transcription and translation.

When performing a gene ontology over-representation test on the upregulated genes identified at the recovery time-point, we identified the "inflammatory response as the most significant ontology in both groups (Figure 10B). Even if inflammation is often associated with deleterious conditions such as the metabolic syndrome and obesity, it is a key component required for myogenesis and remodeling of skeletal muscle following exercise (Neubauer *et al.*, 2014). The necessity of inflammation for an adaptive exercise response is demonstrated by how the use of non-steroidal anti-inflammatory drugs can blunt positive adaptations to exercise (Trappe *et al.*, 2002). Therefore, an inflammatory exercise response is expected. However, Hansen et al., did not observe a similar inflammatory response (Hansen *et al.*, 2015), which may be due to a lower exercise intensity as compared to our study.

The number of genes we observed to be altered in the type 2 diabetes subjects could be a result of infiltration by non-muscle cells and the gene ontology over-representation test indicated

leukocytes as a possible source of transcripts. Whether the heightened inflammatory response in the diabetic individuals is deleterious remains to be determined. However, as prolonged training is benifical for diabetic patiens, the inflammatory response is either benign or lessens over time as the subjects get acclimated to the exercise during the training period.

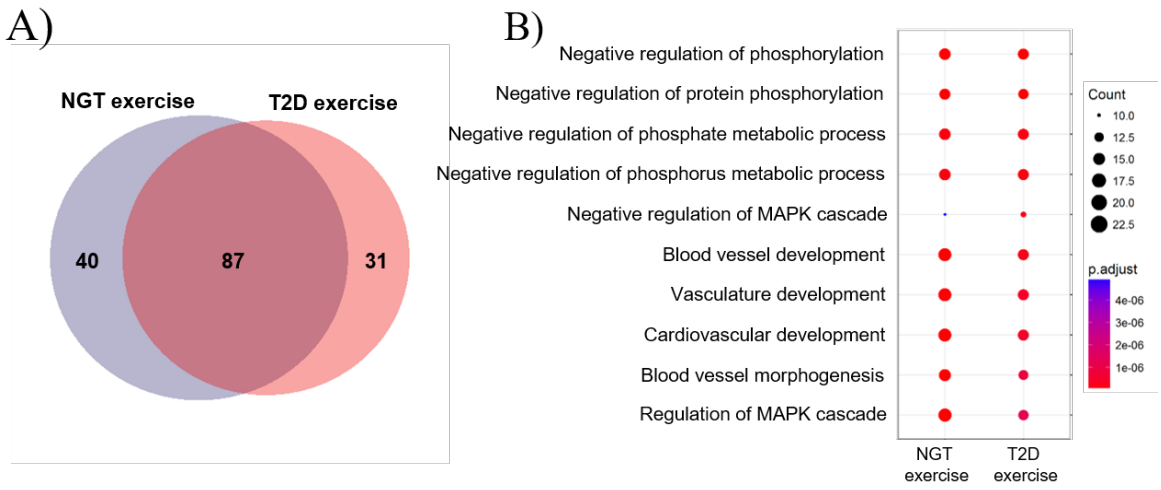

Figure 9 – A) Overlap of differentially expressed genes at the exercise time point between T2D and NGT subjects. Differentially expressed gene inclution criteria, false discory rate ≤ 0.05 and ± 0.5 log2 fold change. B) Gene ontolgy over-representation plot of upregulated genes in the exercise timepoint.

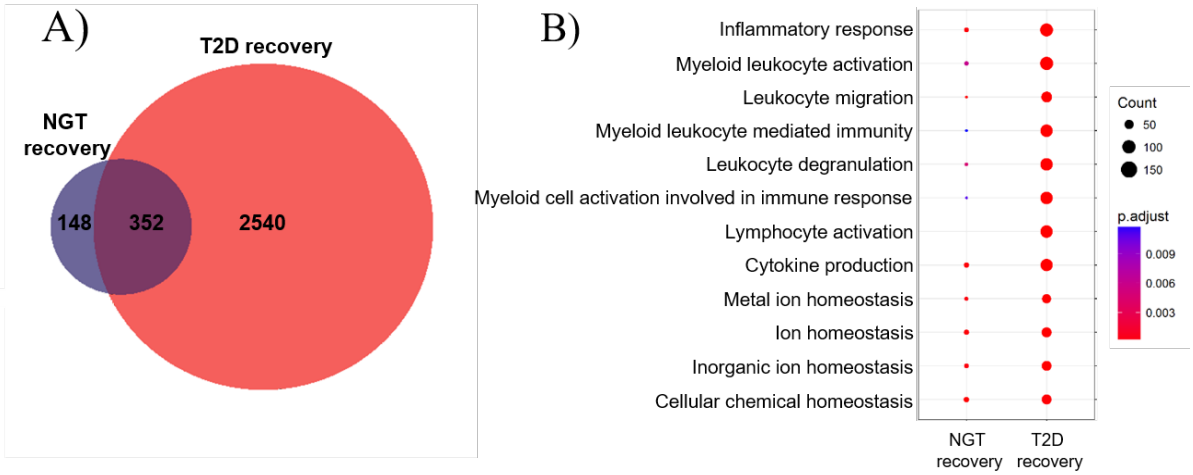

Figure 10 – A) Overlap of differentially expressed genes at the "recovery" time point between T2D and NGT subjects. Differentially expressed gene inclution criteria, false discory rate ≤ 0.05 and ± 0.5 log2 fold change. B) Gene ontolgy over-representation plot of upregulated genes in the "recovery" timepoint.

Cytokines and chemokines are crucial mediators of inflammation and leukocyte migration. Although the transcriptomic results indicated a strong inflammatory effect, it is important to validate these type of results on a protein level. It is, in the end, the proteins that will act as attractants and an increase in transcription does not always lead to a similar increase of translation. Thus, we sought to evaluate whether exercise or disease state altered the abundance of CCL2, CCL21, CX3CL, CXCL1, CXCL9, CXCL10, CXCL12, CXCL16 and IL-16 protein levels (Figure 11). Most of the cytokine and chemokine levels were altered by exercise. Even more interesting was the finding that there was an overall difference between the control and type 2 diabetes groups in CCL2, CCL21, CXCL1, CXCL10 and CXCL16 proteins. The *post hoc* tests revealed that the abundance of CCL2, CCL21, CXCL10 and CXCL16 was different between the diagnosis groups in the recovery time-point. Several of these chemokines have previously been shown to be upregulated at both protein and mRNA levels by exercise (Della Gatta *et al.*, 2014; Gjevestad *et al.*, 2017). However, to our knowledge this is the first study to show a difference between normal glucose tolerant controls and type 2 diabetic subjects. In summary, both the protein abundance and RNA sequencing results show comparable effects, favoring enhanced leukocyte migration into the skeletal muscle of the diabetic subjects.

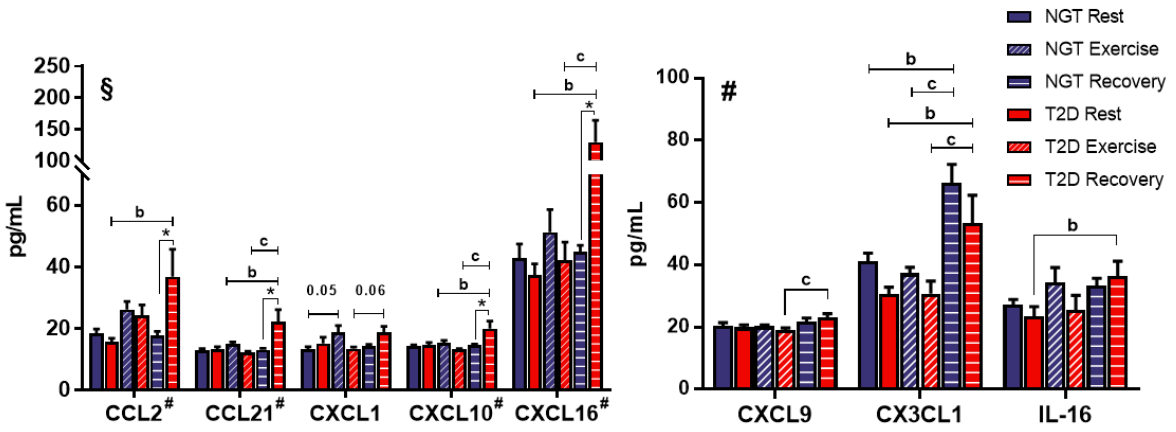

Figure 11 - Chemokine measured in skeletal muscle after an acute bout of exercise. Data are represented as mean ± SEM. § = interaction effect ‡ = overall effect of group, # = overall effect of exercise, Sidak's multiple comparison test * = NGT vs T2D; a = Rest vs Exercise; b = Rest vs Recovery; c = Rest vs Recovery.

In conclusion, we found that men with type 2 diabetes experienced an increased inflammatory response to an acute bout of exercise compared to age- and BMI-match normal glucose tolerant men. It is possible the inflammatory response will be reduced after a training period, as is the case for adipose tissue (Fabre *et al.*, 2018). Additionally, it is possible that ingestion of either a low dose of inflammatory nonsteroidal anti-inflammatory drugs or supplementation of protein-leucine supplements after the exercise bout could normalize the inflammatory response in the diabetic subjects. Consumption of high protein-leucine supplements after exercise can increase anti-inflammatory networks in the recovery phase in healthy trained men (Rowlands *et al.*, 2016). Even though high doses of nonsteroidal anti-inflammatory drugs lead to decreased

protein synthesis post-exercise, moderate doses do not inhibit muscle hypertrophy or increased strength (Trappe *et al.*, 2002; Krentz *et al.*, 2008). However, since nonsteroidal anti-inflammatory drugs can inhibit myoblast differentiation into myotubes, long-term use is probably not recommended (Mendias *et al.*, 2004). Finally, our finding highlight the complexity of the pathology of type 2 diabetes and raises questions regarding the role of inflammation as detrimental or a necessity in exercise and diabetic skeletal muscle.

4.2 THE IMPACT OF A PATERNAL/GRANDPATERNAL HIGH-FAT DIET ON OFFSPRING'S WHOLE BODY METABOLIC PHENOTYPE

As described in the method section, we fed male Sprague Dawley rats a high-fat diet for 10 weeks before mating them with chow-fed female rats to generate the F1 generation. To mimic the current world scenario, where many children adopt their parents' dietary habits (Isma *et al.*, 2012), we further challenged a subgroup of the offspring to a high-fat diet in addition to the ancestral dietary intervention. Doing so we found, surprisingly, that female offspring from high-fat-fed fathers (Paternal high-fat diet on a high-fat diet (PatHF-HF)) did not increase body weight as much as the females from a father on a chow diet (Paternal chow-diet on a high-fat diet (PatCD-HF)) (Figure 12A). The PatHF-HF weight resembled the weight of the chow-fed rats, even though they were eating the same amount of kcal as the PatCD-HF. Additionally, glucose tolerance were worse in PatHF-HF female rats as compared with PatCD-HF rats, despite their lower body weight (Figure 12B). Male F1 rats on a chow diet were used to generate the F2 generation, which was also subdivided and challenged with a high-fat diet. The whole body metabolic phenotype of the F2 animals were comparable with the F1 generation (Figure 12C-D), supporting the assertion that a high-fat diet can cause transgenerational alteration of metabolism.

The phenotype we observed was surprising in several ways. A lower body weight is usually associated with a healthier phenotype, decreased mortality risk and higher insulin sensitivity (Chan *et al.*, 1994; Adams *et al.*, 2006). In contrast, the PatHF-HF and GpatHF-HF had decreased adiposity coupled with increased insulin resistance compared to their PatCD and GpatCD littermates. Together, this suggests glucose handling to be dysregulation in the adipose tissue, or a redistribution of fat to less metabolically favorable sites. Although, we cannot be certain that the decreased whole-body glucose tolerance can be solely attributed to any of the metabolically active tissues (adipose tissue, skeletal muscle or liver) investigated in this thesis. To elucidate this, we would have had to perform a hyperinsulinemic-euglycemic clamp followed by immediate harvesting of the tissues of interest (Hughey *et al.*, 2011) which would have enabled us to evaluate the insulin sensitivity and glucose uptake tissue-specifically. Unfortunately, due to logistic reasons this was not possible in this study. Another useful experiment would have been to place the animals in a metabolic cage to monitor their physical activity and energy expenditure (Whittaker *et al.*, 2016). We know that the high-fat fed female offspring ate the same amount of calories without gaining as much weight. In light of equivalent energy intake, the differences would likely be due to changes in energy expenditure. Utilization of the metabolic cages could have shed light on whether this is due to increased activity or

increased metabolic turnover. At the time, we did not have access to metabolic cages large enough to house rats. Thus, in order to further understand the resultant phenotype, studies including these methods are warranted.

Interestingly, the phenotype was only noted in the female offspring and not the male. This sex-specific phenotype corresponds with other studies of both inter- and transgenerational inheritance (Pembrey *et al.*, 2006; Dunn and Bale, 2011; Dunn *et al.*, 2011; Fullston *et al.*, 2013). Although many studies report sexual dimorphisms, there are several differences. An intergenerational study in rats also reported that the daughters, and not sons, of high-fat fed fathers had impaired glucose tolerance (Ng *et al.*, 2010). However, they observed this in chow fed female offspring, whereas we only observed this difference in the high-fat fed and not chow fed offspring. Another transgenerational study in mice, using a high-fat diet as the intervention reported that female offspring, but not male, had increased adiposity (Fullston *et al.*, 2013), in contrast to our findings of decreased adiposity in the female offspring. Studies using maternal linages to investigate epigenetic inheritance have also found sex differences in the transmission of the phenotypes (Jimenez-Chillaron *et al.*, 2009; Dunn and Bale, 2011). The Överkalix studies show that epigenetic inheritance in humans may be sex-specific (Pembrey *et al.*, 2006). Together, this is a strong argument that epigenetic inheritance is sex-specific in its character. Since the inherited phenotypes do not coincide with each other, it is likely that either the different animal models or the different interventions are the cause of these differences. If the differences are due to the models, it means that it is not fully conserved between species and this raises the question: how come? On the other hand, the notion that different interventions (for example high-fat diet vs. a low protein diet) would cause different phenotypes is perhaps not that strange. In the end, it is likely that a combination of differences in both species and intervention type trigger different responses.

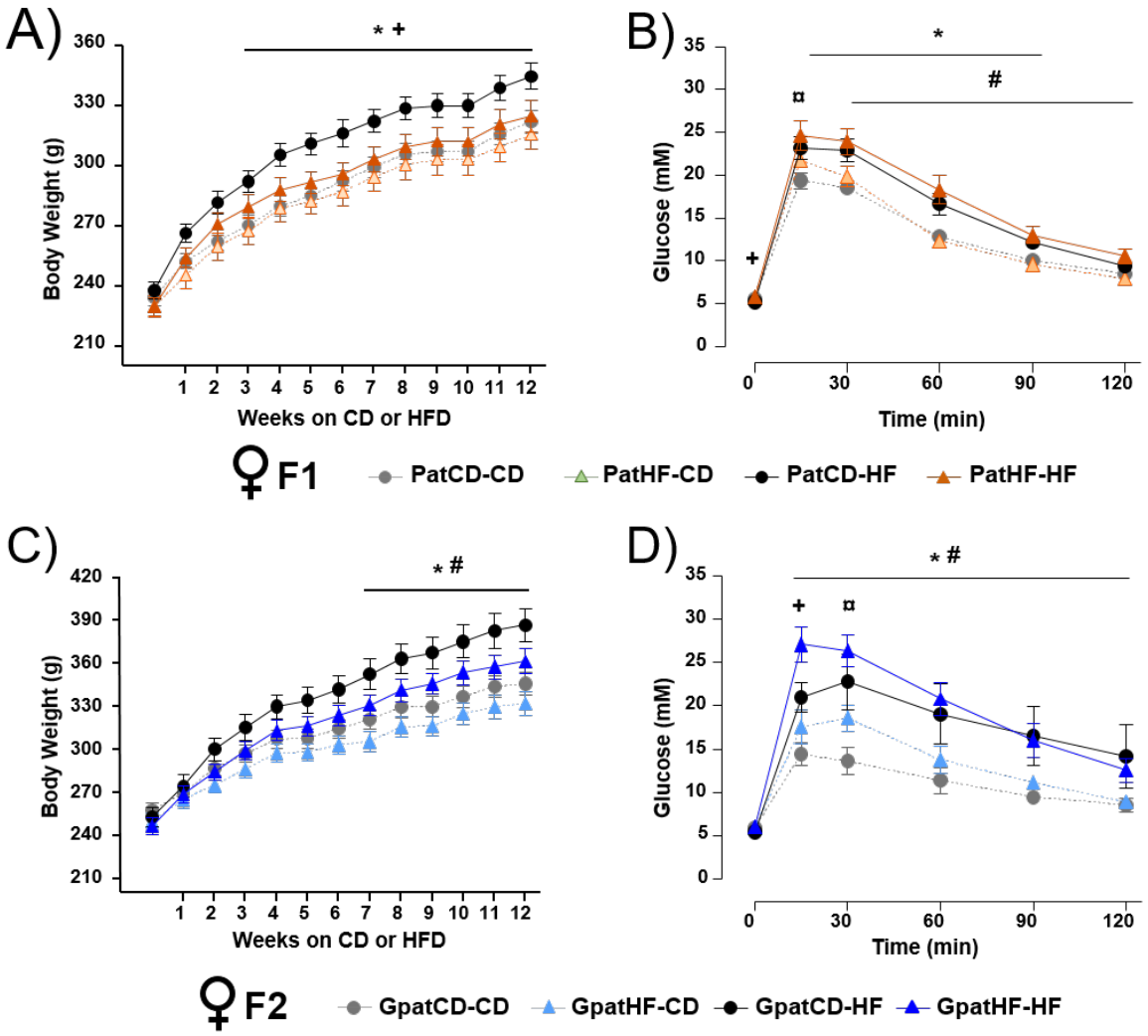

Figure 12 - A) Glucose levels during an intraperitoneal glucose tolerance test (ipGTT) (n=9-11 animals; 1 sibling per litters) and B) Body weight curve (n=14-22 animals from 14-17 litters; used more than 1 sibling per litter) of F1 females. D) Metabolic profiling of F2 female offspring after 12 weeks of high fat feeding. D) Blood glucose levels during an ipGTT (n=5-6 litters; 1 sibling per litter) and (D) Body weight curve (n=10-13 animals from 5-6 litters; used more than one sibling per litter). Results are represented as mean ± SEM. Two-way ANOVA followed by Bonferroni post-hoc test: *p≤0.05: PatCD-HF vs PatCD-CD or GpatCD-HF vs GpatCD-CD; ¤p≤0.05: PatHF-CD vs PatCD-CD or GpatHF-CD vs GpatCD-CD; #p≤0.05: PatHF-HF vs PatHF-CD or GpatHF-HF vs GpatHF-CD; +p≤0.05: PatHF-HF vs PatCD-HF or GpatHF-HF vs GpatCD-HF. PatCD-CD: Paternal-Chow on Chow; PatHF-CD: Paternal-HFD on Chow; PatCD-HF: Paternal-Chow on HFD; PatHF-HF: Paternal-HFD on HFD; GpatCD-CD: Grandpaternal-Chow on Chow; GpatHF-CD: Grandpaternal-HFD on Chow; GpatCD-HF: Grandpaternal-Chow on HFD; GpatHF-HF: Grandpaternal-HFD on HFD. Adapted from (de Castro Barbosa *et al.*, 2016).

4.3 POSSIBLE EPIGENETIC CARRIERS OF A PATERNAL/GRANDPATERNAL HIGH FAT DIET

As previously stated, minor environmental stimuli such, as a high caloric intake, does not alter the genetic code. Thereby, there must be another inheritable factor in place that can explain inter and –transgenerational inheritance metabolic features based on food consumption. Epigenetic changes have been suggested as putative mechanisms. Paternal models of transgenerational inheritance have the advantage of limiting possible epigenetic sites to the spermatozoa. Therefore, we extracted DNA and sncRNA from the spermatozoa of both F0 and F1 breeders. The methylome was investigated by sequencing of MBD-captured DNA fragments. Using DiffBind we identified 18 differential methylated regions that were altered due to the paternal high-fat diet, i.e. PatHF-CD vs. PatHF-CD, and overlapped the high-fat diet in the F0 breeder (Figure 13). However, when investigating a number of different somatic tissues of the F2 offspring, only a few of the differential methylated regions were found changed in a similar manner. Furthermore, none of the regions corresponded with altered levels of mRNA. Our data suggests that methylation plays a minor role in mediating the transgenerational effect of paternal high-fat diet. In contrast, the spermatozoa methylome is reported to be different between obese and lean men (Donkin *et al.*, 2016). Pre-diabetic male mice have also been reported to have an altered sperm methylome (Wei *et al.*, 2014). Furthermore, the altered sperm methylome in mice was connected to a dysregulated metabolism in their offspring. However, other studies have concluded that methylation is an unlikely carrier of epigenetic inheritance (Carone *et al.*, 2010; Iqbal *et al.*, 2015). The facts that the zygote undergoes several steps of demethylation and remethylation, makes methylation a less likely candidate for transferring environmental information between generations.

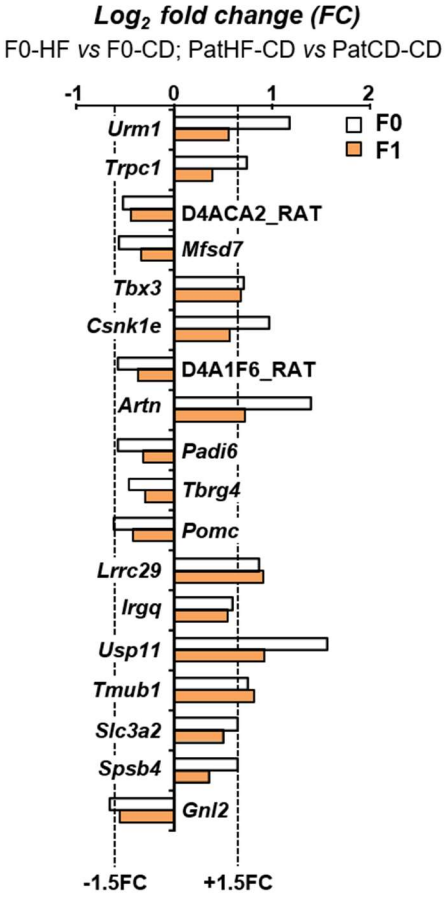

Figure 13 - Differentially methylated regions common in sperm of F0 and F1 indicates a direct gametic transgenerational reprogramming. Data are represented as log2 of the fold change in F0 (HF vs CD) or F1 (PatHF-CD vs PatCD-CD) founders. Adapted from (de Castro Barbosa *et al.*, 2016).

Another epigenetic mechanism that has been suggested as an epigenetic carrier is the non-coding RNAs, especially sncRNAs such as miRNA. The software packed edgeR was used to make a differential expression analysis of the sncRNA extracted from F0 and F1 spermatozoa. This revealed that piRNAs were the biotype with the highest amount of differential expressed genes, ranging between 307 - 1091 affected genes in the different comparisons (Figure 14).

Although, pi-RNAs have been implicated in transgenerational epigenetic studies (Ashe *et al.*, 2012; de Vanssay *et al.*, 2012), we chose to further investigate the miRNAs, since their functions and actions in metabolism are better understood. In addition, the piRNAs exhibit lower counts than the miRNAs which indicates that they are less likely to influence the development of the zygote.

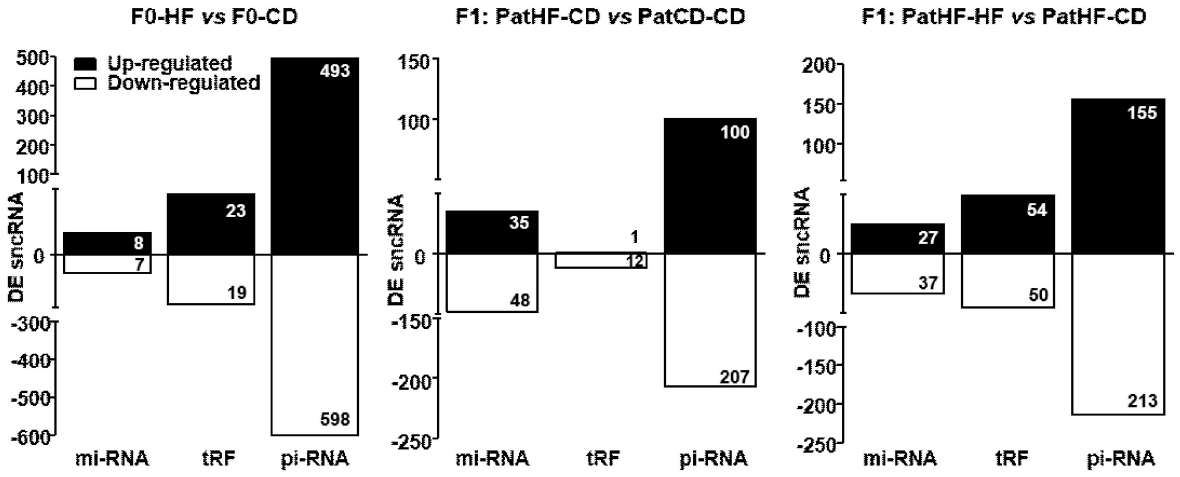

Figure 14 - Amount of differentially expressed sncRNAs i.e. miRNAs, tRFs and piRNAs. In sperm of F0-HF vs F0-CD (n=8-10), F1 offspring of F0-HF (PatHF-CD) vs F1 of F0-CD (PatCD-CD) fed a chow diet or a high fat diet (PatHF-HF vs PatCD-HF) (n=7 per group; 1 sibling per litter). Adapted from (de Castro Barbosa *et al.*, 2016).

We found three miRNAs to be changed in the same direction in the F0-HF vs F0-CD and the PatHF-CD vs PatCD-CD rats. Two of these miRNA's, miR-293-5p and miR-880-3p, were downregulated in both comparisons. Whereas let-7c-5p was found to be upregulated (Figure 15). The let-7 family is associated with predisposition to type 2 diabetes and has a role in the regulation of insulin sensitivity and glucose homeostasis (Frost and Olson, 2011; Zhu *et al.*, 2011). Therefore, we were interested in evaluating *Let-7c*s functional role in somatic tissue from the female offspring, presented in section 4.4.1.

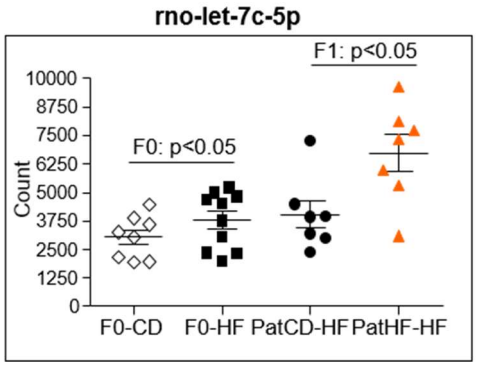

Figure 15 - Let-7c expression in spermatozoa. Values are represented as mean ± SEM. F0-CD: F0 on chow diet; F0-HF: F0 on HFD; PatCD-CD: Paternal-Chow on Chow; PatHF-CD: Paternal-HFD on Chow; PatCD-HF: Paternal-Chow on HFD; PatHF-HF: Paternal-HFD on HFD Adapted from (de Castro Barbosa *et al.*, 2016).

There are other potential epigenetic carriers such as microbiota (Elgart and Soen, 2018). However, in our study the offspring sharing the same diet with a different paternal background (i.e. GpatCD-HF and GpatHF-HF) were housed in the same cage. Rodents are known coprophages and all offspring would thereby ingest the same ancestral microbiome. Therefore, we can reasonably rule out the

microbiome as an epigenetic carrier in our study. More recent studies have also highlighted the potential role of tRNA fragments as an epigenetic carrier (Chen *et al.*, 2016; Sharma *et al.*, 2016). Sharma et al., also found several members of the *Let7* family to be altered by their dietary intervention. In line with previous findings, our study strongly suggests that the main epigenetic carrier in a paternal linage could be the sncRNAs. However, at this point it is not possible to single out only one molecule, or even a specific class of the sncRNAs. It is likely that several different tRNA fragments and miRNAs are all contributing to the offspring phenotype.

4.4 TISSUE-SPECIFIC ALTERATIONS AS A RESULT OF PATERNAL/GRANDPATERNAL HIGH-FAT DIET

At the end of **study II**, we collected liver, adipose and skeletal muscle tissue to evaluate the tissue-specific alterations introduced by the paternal/grandpaternal high-fat diet. Our main approach for exploring these tissues were to perform gene arrays and thereby utilizing an unbiased approach when investigating the transcriptomic alterations.

4.4.1 Adipose tissue

As previously stated, *Let-7c* had been observed to be upregulated in the spermatozoa in both the high-fat fed F0 male founders and F1 male that had derived from a high-fat fed father. We investigated the expression levels of *Let-7c* in several tissue, including the gonadal white adipose tissue, of the F1 and F2 female rats. We found *Let-7c* to be upregulated in the offspring derived from a father/grandfather fed high-fat diet, independent of their own diet (Figure 16). The let-7 family of miRNA are involved in glucose metabolism and the insulin signaling pathway (Zhu *et al.*, 2011). *In vivo* overexpression of let-7 causes decreased adiposity and impairs glucose tolerance (Frost and Olson, 2011). This is in line with our findings in white adipose tissue and the whole body phenotype. The whole body phenotype is only occurring in the PatHF/GpatHF animals when they are fed a high-fat diet. However, *Let-7c* is upregulated due to the paternal/grandpaternal high-fat diet in the white adipose tissue regardless of the offspring's diet. Suggesting that upregulation of *Let-7c* is not enough to cause the impaired glucose tolerance. This makes sense from an evolutionary perspective. If the impact of ancestral environment on the offspring would surpass the impact of the current environment, the offspring would not be able to adapt. Transgenerational epigenetic inheritance should be looked upon as a fine-tuning system and it is therefore only natural that it does not come to light until the offspring were further metabolically stressed by their own high-fat diet.

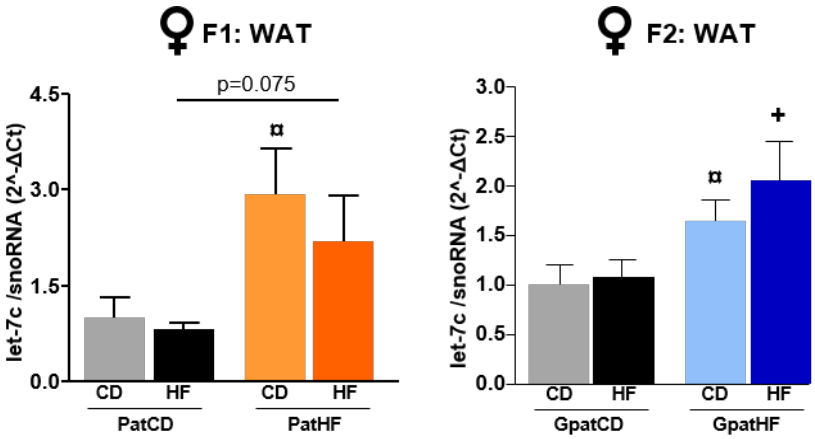

Figure 16 - *Let-7c* expression in gonadal white adipose tissue (WAT) in F1 and F2-female offspring. Two-way ANOVA followed by Bonferroni post-hoc test: *p≤0.05: PatCD-HF vs PatCD-CD or GpatCD-HF vs GpatCD-CD; ¤ p≤0.05: PatHF-CD vs PatCD-CD or GpatHF-CD vs GpatCD-CD; #p≤0.05: PatHF-HF vs PatHF-CD or GpatHF-HF vs GpatHF-CD; +p≤0.05: PatHF-HF vs PatCD-HF or GpatHF-HF vs GpatCD-HF. Results are represented as mean ± SEM. PatCD-CD: Paternal-Chow on Chow; PatHF-CD: Paternal-HFD on Chow; PatCD-HF: Paternal-Chow on HFD; PatHF-HF: Paternal-HFD on HFD; GpatCD-CD: Grandpaternal-Chow on Chow; GpatHF-CD: Grandpaternal-HFD on Chow; GpatCD-HF: Grandpaternal-Chow on HFD; GpatHF-HF: Grandpaternal-HFD on HFD. Adapted from (de Castro Barbosa *et al.*, 2016).

To investigate whether the increase of *Let-7c* had an effect on it target genes we performed a gene array on the adipose samples and correlated the predicted targets gene expression versus the *Let-7c* expression. The analysis revealed how the predicted targets of *Let-7c* were downregulated, following the dogma that miRNAs function in a gene-suppressing manner (Valinezhad Orang *et al.*, 2014). Our data is consistent with increased *Let-7c* expression acting as a suppressor. The transcriptomic data, together with the whole body phenotype, suggests that the adipose tissue is the metabolically active tissue most affected by an ancestral high-fat diet. Of note, an ancestral high-fat diet mainly affects the adipose tissue in mice (Fullston *et al.*, 2013).

4.4.2 Skeletal muscle

In **study III**, we used *Extensor Digitorum Longus* (EDL) muscle from the female F1 and F2 offspring. The initial GSEA on the transcriptome revealed that there was no effect of the grandpaternal diet between the F2 females' offspring, when fed a chow diet. However, when comparing the GpatHF-HF vs. GpatCD-HF females, we saw that the unfolded protein response was enriched in skeletal muscle of GpatHF-HF females (Figure 17). The result from the GSEA was also confirmed using RT-qPCR. The unfolded protein response consists of three different branches (Walter and Ron, 2011). We examined which of these signaling pathways could have caused the transcriptomic enrichment with Western blots. This analysis revealed an increased abundance of the chaperone GRP94 and cleaved ATF6-α in the GpatHF-HF animals. This suggests that the ATF6-α branch is the driving arm activation of the unfolded protein response. However, when we evaluated the unfolded protein response genes that were confirmed to be affected by the grandpaternal diet by RT-qPCR, in the EDL from the F1 females, there were no difference in expression of these genes. It is possible that the targeted approached missed the potential differences and that a full transcriptomic approach would find detect this. Another possibility is that the response to the ancestral diet is different depending on whether it is an intergenerational or a transgenerational response.

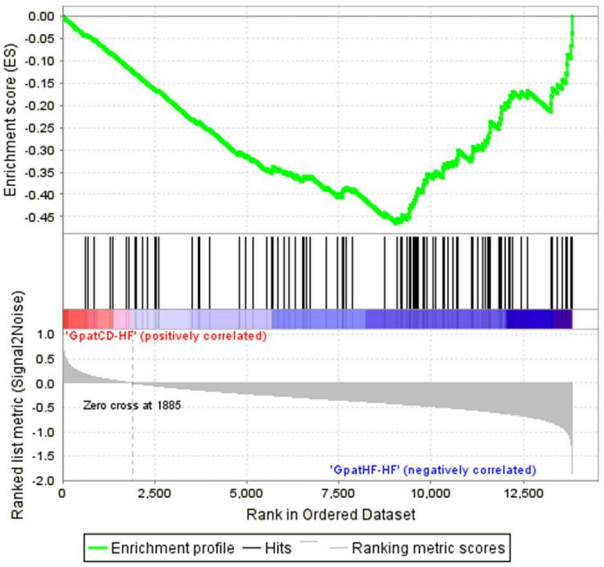

Figure 17 - Gene set enrichment score plot of unfolded protein response comparing GpatCD-HF and GpatHF-HF females. GpatCD-HF: HFD-fed offspring from grandfathers fed a chow diet; GpatHF-HF: HFD-fed offspring from grandfathers fed a HFD. Adapted from (Alm *et al.*, 2017).

In contrast to the adipose tissue (**study II**) and liver (**study IV**), the grandpaternal high-fat diet did not induce any differences on the skeletal muscle transcriptome while the grandoffspring were fed a chow diet. Thus, skeletal muscle is less sensitive to changes by diet induced transgenerational alterations. Nonetheless, the grandpaternal high-fat diet did affect the grandoffspring's response to their high-fat diet.

4.4.3 Liver

In **study IV**, we explored the effects of a grandpaternal high-fat diet in the liver. Using the same approach as in **study III**, we found several upregulated gene sets in the GSEA. However, in the liver we also found gene sets being differently enriched in the offspring on a chow diet. Among these, "interferon alpha response", "interferon gamma response" and "TNF-α signaling via NFκB" were enriched in liver of the offspring from chow-fed grandfathers, i.e. downregulated in the females from high-fat-fed grandfather, independent on their own diet (Figure 18). On a transcriptomic level, the grandpaternal high-fat diet impact was stronger in

the liver than the skeletal muscle. In line with this, the liver exhibits gene sets affected solely by the grandpaternal high-fat diet. This is likely reflecting the plastic nature of the liver. A paternal low protein diet can affect the fatty acid metabolism in offspring livers (Carone *et al.*, 2010). Interestingly, we found that a grandpaternal high-fat diet altered genes associated to fatty acid metabolism. Since the fatty acid metabolism pathway is affected in two different animal models, by two different dietary interventions suggests that it is the site of epigenetic reprograming.

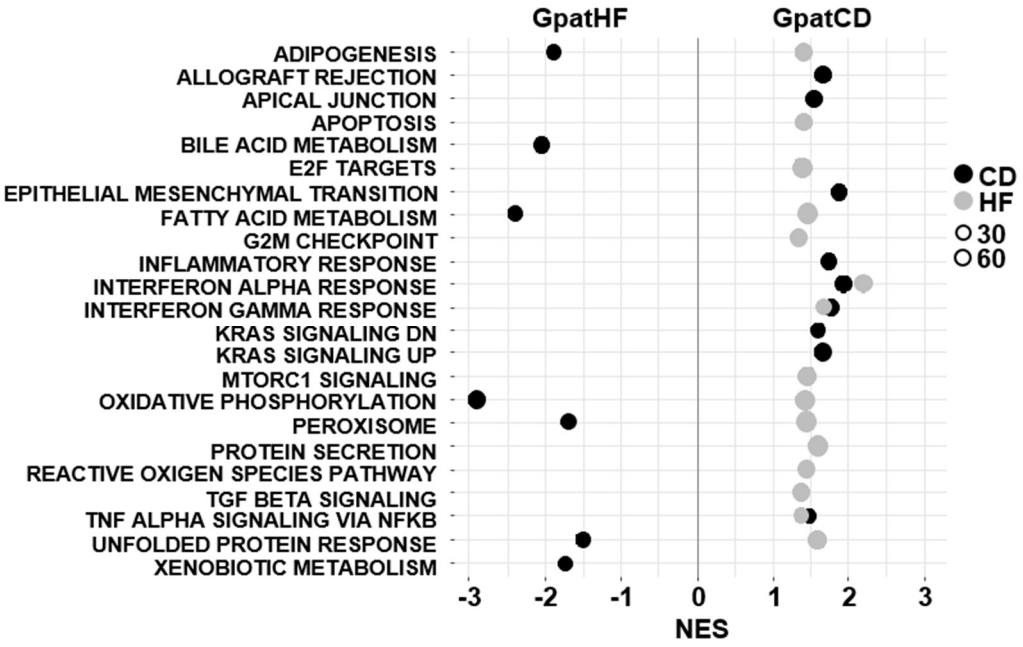

Figure 18 - Significantly altered gene sets obtained by GSEA in the liver of F2 female rats from either chow- (GpatCD) or high-fat diet-fed (GpatHF) grandfathers. F2 rats were also subjected to either a chow (-CD) or high-fat diet (-HF). Gene sets with a negative normalized enrichment score (NES) are enriched in F2 females from GpatHF, while gene sets with a positive NES are enriched in F2 females from GpatCD. Dot sizes are scaled according to the percentage of core enriched genes in the respective gene set, and colored according to the F2 diet (black dot: chow diet and gray dot: high-fat diet).

We chose to target the TNF-α signaling since it is closely related to metabolism and showed a grandpaternal-effect independent of the offspring's own diet. RT-qPCR confirmed the gene array results. However, only one of the genes selected for the validation, *G0s2* (G0/G1 Switch 2), was found to be altered in a similar manner in the F1 liver. Next, we evaluated cytokine levels of the F2 livers. The grandpaternal high-fat diet reduced the level of TNF-α IL-5, IL-10, IL-13 and the granulocyte-macrophage colony-stimulating factor (GM-CSF) (Figure 19). TNF-α is a crucial adapter of chronic inflammation and is linked to both insulin resistance and obesity (Hotamisligil *et al.*, 1993; Dandona *et al.*, 1998). Animals with a GpatCD background exhibit a normal and expected increase of TNF-α by the high-fat diet. However, in animals with a GpatHF background the TNF-α response was blunted, suggesting that the GpatHF animals are not able to activate an appropriate inflammatory response. Furthermore, the expected increase of IL-5, IL-6, IL-10, IL-13 and GM-CSF weres also diminished in the

offspring with a grandfather on a high-fat diet. Together these results suggest that the TNF-α pathway is markedly affected by the grandpaternal diet.

In both the liver and the skeletal muscle, we found several genes were altered by the grandpaternal high-fat diet in the F2 females, but not in the F1 generation. This indicates an alternative mechanism, in addition to the paternal gametic reprogramming that is regulating the transcriptomic response in these two tissues. One possible explanation is so called serial programming (Hur *et al.*, 2017). In short, an intervention in the F0 causes one phenotype in the F1 that subsequently alters the epigenetic carrier and thereby causes a new phenotype in the F2. We utilized a paternal model of epigenetic inheritance and we did not observe a whole body phenotype in the male offspring. Thus, it is uncertain whether serial programing is causing a "skip" in the transcriptomic response. However, even though we did not observe a body weight or glucose tolerance phenotype in the male offspring, it is still possible that the paternal high-fat diet caused a phenotype that we did not measure, and in turn could have caused this serial programming. In conclusion, our studies indicate that the gametic epigenome is amenable to dietary interventions and that it is able to adjust gene expression between generations. Additionally, variations in the ancestral diet seem to affect the transcriptome in the decedents in a tissue- specific manner and further influence the offspring response to its own diet. An interesting next step would be to combine the availed transcriptomic information and construct an atlas of genes that are responsive to ancestral diets. Although our ancestral dietary heritage can affect our metabolic health, it is important to recognize that our own lifestyle choices still triumph over our ancestors'.

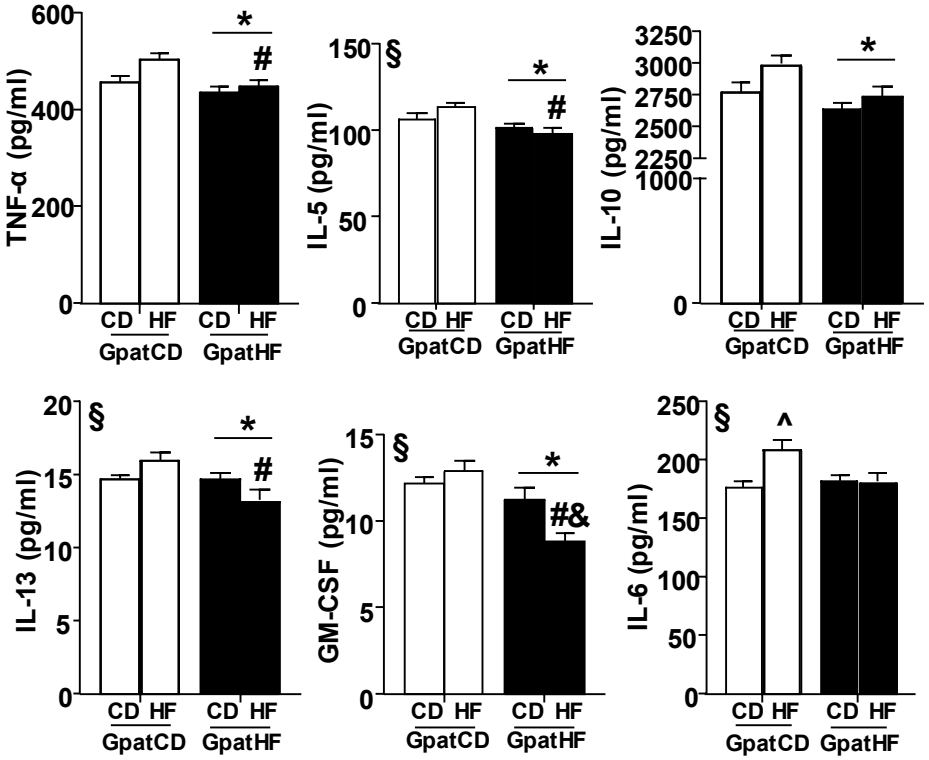

Figure 19 - Abundance TNF-α, IL-5, IL-10, IL-13, GM-CSF and IL-6 in liver of GpatCD: grandpaternal chow; GpatHF: grandpaternal HFD; -CD: F2 chow; -HF: F2 HFD. *p≤ 0.05: GpatHF vs GpatCD; #p≤ 0.05: GpatHF-HF vs GpatCD-HF; ^p≤ 0.05: GpatCD-HF vs GpatCD-CD; &p≤ 0.05: GpatHF-HF vs GpatHF-CD; §p≤0.05: interaction effect. GpatCD: grandpaternal chow; GpatHF: grandpaternal HFD; -CD: F2 chow; -HF: F2 HFD.

5 SUMMARY AND CONCLUSIONS

This thesis focuses on the role of environmental factors, exercise and grandpaternal diet, as factors that influence and regulate metabolism (Figure 20). It is well know that exercise is beneficial and is recommended for treatment and prevention of type 2 diabetes. Our results in **study I** clearly show that acute exercise causes an increased inflammatory response in the skeletal muscle of individuals with type 2 diabetes. However, it is unclear whether this is an adaptive or detrimental response. Thus, this area of research has ample room to increase its understanding of these complex processes. Further knowledge might prove to be key in developing treatments or a possible cure for metabolic disease.

Additionally, our transgenerational studies (**II-IV**) provide further evidence on how ancestral lifestyle can lead to detrimental effects of environmental stressors in the future generations. We found that both the adipose tissue and liver transcriptome were affected by the grandpaternal high-fat diet, independent of the decedents own diet. Although, worth noting, is that we did not observe a strong inter- or transgenerational dietary effect on whole body metabolism or skeletal muscle without introducing a further stressor, namely, a high-fat diet. Thus, our results highlight that the individual's own lifestyle is still of great importance to their metabolic health.

The specific conclusions of this thesis are:

- Acute aerobic exercise induces an heightened inflammatory response in skeletal muscle of men diagnosed with type 2 diabetes.

- *Let-7c* is a possible epigenetic molecular carrier underlying gametic inheritance of metabolic dysfunction.

- A paternal/grandpaternal high-fat diet affects whole body metabolism of future generations.

- Adipose tissue, skeletal muscle and liver are all susceptible genomic modifications induced transgenerationally by dietary factors.

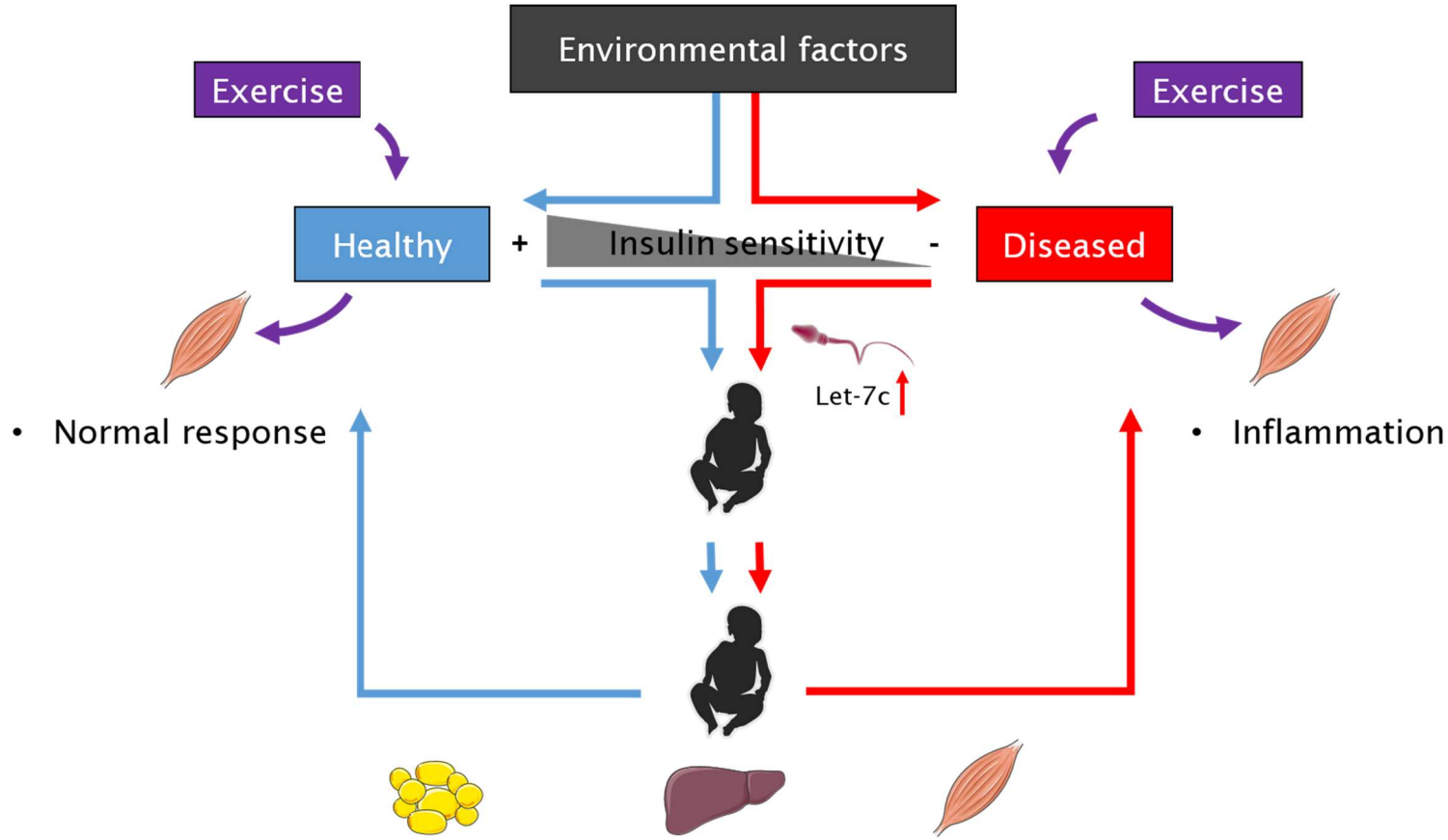

Figure 20 – Summary of the main finding of this thesis. Environmental factors directly influence the metabolic health of the exposed generation. These changes can be passed down through a paternal linage via the spermatozoa, possible through the miRNA *Let-7c*, and affect metabolically active tissues and whole-body metabolism. The insulin resistant skeletal muscle will trigger a heightened inflammatory response after exercise.

6 STUDY LIMITATION

There are several limitations in the studies of this thesis, some of more practical nature and some more of an intrinsic. In **study I** our subjects had a smaller breakfast in the morning of the exercise bout (exercise and recovery biopsies), whereas they did not have this during the first biopsy (rest). Furthermore, there were 7-10 days in between the rest biopsy and the exercise and recovery biopsies. Both the smaller breakfast and the fact that all of the biopsies were not taken the same day could influence the results. This was done for two main reasons. 1) We did not consider it ethical to take a muscle biopsy just before our upper middle-age unhealthy patients would perform a relatively tough bout of exercise or have them fasted for a total of 12-14 hour. 2) Facilitate the recruitment of subjects. We wanted to avoid "intimidating" potential subjects away by using a too severe intervention. Although the minor breakfast could possible impact the result, it is not that likely. Our readouts were on a protein and RNA level, which are both not as sensitive as when measuring metabolites. Furthermore, both the control group and the type 2 diabetes group were subjected to the same protocol. Thereby, any co-founding effect that was added would be added to both groups and can therefore, to the most part, be ruled out.

The biggest limitation of **study II – IV** is that it is performed in a rodent model. Not because that it is a rodent model, but because it is a model. If one is mainly working with models, may it be an animal or a cell model, it is easy to forget that it is merely a tool to understand human physiology. The big question is; how much of these results can be extrapolated to humans? Since rats and humans share around 80% of their protein coding DNA, it is fair to assume that the comparable (Fong *et al.*, 2013). Thus, animal models is still a good, even crucial, resource for the scientific community to utilize. In additions, it makes it possible to investigate scientific questions that we could not otherwise do, due to ethical, logistical and financial reasons.

Another limitation with **study II – IV** is the relatively low n number for the investigated adipose tissue, skeletal muscle and liver. In these three studies, we have utilized gene arrays to investigate the transcriptomic response to a grandpaternal high-fat diet. Omic' methods are useful and powerful tools that are helping to increase gathered knowledge of the world. In our studies, we used them mainly as screening tools that was later validated using other univariable tools. Considering that the actually effect of the grandpaternal high-fat diet was mild, we would have greatly benefited by having a higher amount of (tissue) samples. This would have reduced the multiple comparisons problem and allowed us to more powerful bioinformatic tools, which could have given us more useful results.

7 CLINICAL IMPLICATIONS

Transferring basic research into clinic practice is a time consuming and usually not an easy process. As the cause with **study I**, we found that type 2 diabetic skeletal muscle responded with an increased inflammatory response. At a first glance, this imply that patients diagnosed with type 2 diabetes perhaps should use non-steroidal anti-inflammatory drugs to normalize their inflammatory response after exercising. However, since training has a proven beneficial effect in this population, it is likely that the training itself will normalize the inflammatory response. Furthermore, chronic drug use is almost always associated with negative side effects and should therefore, if possible, be avoided. Thus, our findings highlights the need for more studies before any recommendation can be given.

We cannot influence the diet of our ancestors but we can affect our own. In **study II – IV**, we found that a grandpaternal high-fat diet has a negative impact on whole body metabolism and in specific tissues of future generations. However, on the bright side, the results are suggesting that the detrimental effect only occurs if the offspring is also subjected to an unfavorable diet. Thus, even if our parents or grandparents have been eating an unhealthy diet, this will not necessary have a negative impact on our metabolic health. Therefore, individuals and society should keep on promoting a balanced and healthy diet. We are not only eating for ourselves, but for our future generations' metabolic health.

8 SVENSK POPULÄRVETENSKAPLIG SAMMANFATTNING

Under de senaste decennierna har andelen överviktiga och feta ökat i en alarmerande hastighet och ökningen fortgår ännu. Detta leder till stora kostnader för samhället och ansenligt lidande för de drabbade individerna. Det mänskliga genomet har inte ändrats avsevärt under denna period. Det är snarare vår miljö och vårt sätt att leva som har ändrats. Våra arbeten är mycket mindre fysiskt krävande, vardaglig motion minskar och kaloririk mat har aldrig varit så lätt att få tag på som idag.

Vikten utav fysiskaktivitet för ett hälsosamt leverne har varit känt sedan de gamla grekernas tid. Men även om detta har varit känt sedan länge, så är det oklart genom vilka mekanismer som fysisk aktivitet leder till positiva adaptioner. Dessutom vet vi inte hur, eller ens om, dessa mekanismer är förändrade vid metabola sjukdomar såsom typ 2 diabetes. Att ytterligare kartlägga den molekylära responsen av träning kan ge ny kunskap som kan bli avgörande i kampen för att behandla och förebygga metabol ohälsa.

Utöver detta, så har det under senare år framkommit att förfäders livsstil kan påverka framtida generationer metabola hälsa. Dessa fynd visar att Lamarcks idéer om så kallad mjuk ärftlighethet är, åtminstone delvis, sanna. Däremot är det fortfarande inte klarlagt till vilken grad eller genom vilka mekanismer våra förfäders livsstil kan påverka oss idag. Att undersöka detta har varit ett av huvudsyfterna med denna avhandling.

I **studie I**, har vi undersökt skelettmuskulaturens genuttryck efter en träningssession hos friska medelåldersmän samt män diagnostiserade med typ 2 diabetes. Vi fann att förändringar som kunde uppmätas efter 30 minuters cykel arbete var liknande mellan de två grupperna. Däremot skiljde sig diabetikernas respons tre timmar efter träningen avsevärt från kontrollgruppens. Hos typ 2 diabetiker var inflammationsresponsen uppreglerad till en mycket högre grad. Vi kunde konfimera dessa fynd på såväl mRNA som på proteinnivå, och kunde visa genom mikroskopi att tre timmar efter träning fanns det en ökad mängd av vita blodceller i muskeln hos diabetiker

I **studie II**, fick hanråttor äta en högfettsdiet under 10 veckor innan de parades med honor som hade ätit vanligt foder. Avkomman till dessa råttor (F1 generationen) fick antingen äta högfettsdiet eller vanligt foder. De hanar i F1-generationen som hade ätit en vanlig foder-diet användes för att generera F2-generationen. Delar av F1 och F2-avkomman tilldelades även en högfettsdiet. Vi fann att honor från en högfettsdiet-fader/farfar inte ökade i vikt till samma grad som honor med en far/farfar på en vanlig foder-diet, när de själva åt en högfettsdiet. De var med andra ord skyddade från den förväntade viktuppgången av högfettsdieten. Trots detta var deras glukostolerans till och med lite försämrad. Således hade deras farfars kost påverkat hur de svarade på olika sorters kost. För att hitta en möjlig mekanism som kan förklara hur farfaders kost kan ändra det metabola svaret två generationer senare så sekvenserade vi RNA från spermierna från den första generationen. Vi fann förändringar i antal av små, och kodande RNA (micorRNA). Speciellt fann vi evidens för att microRNA:t *Let-7c* kan vara en möjlig epigenetisk bärare av det kostbaserade fädernearvet.

I **studie III och IV**, utvärderade vi vävnadspecifika skillnader i fram för all skelettmuskelatur och lever från djuren i **studie II**. I båda studierna undersökte vi initialt det globala genutrycket från vävnaderna med så kallade gene arrays. I **studie III** fann vi att att ”unfolded protein response” var uppreglerad i skelettmuskelaturen från F2 honor med en farfar som hade ätit en högfettsdiet, när det själva åt en högfettsdiet. I **studie IV**, fann vi att farfädernas högfettdiet förändrade TNF-α signaleringen i lever, oberoende av avkommans egna diet.

Tillsammans visar dessa studier hur, och genom vilka möjliga mekanismer, miljön (både den vi ärvt och den vi utsätts för direkt) kan påverka vår metabolism och metabola hälsa.

9 POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ PITMÅLE

Sisstå:ra jena håva döm som väga för mötche å döm som våra fääit euöke falit fort, å hä fortsätt åt euök änn. Hä djär:r åt hä kosst möche för samhälle å döm som våre drabbe fåra iill. Jenå:me öte fåLke hav et endrese jusst na: under den här tijdn. Hä jär mässte miljön:n å sätte vä leva opa som jär endre. Jobba våre våra minnder taång, man rö:r sä minnder, å fäijtma:tn hav åller vöre så jett åt få ta:g i som nöförtijdn.

Man har lèeng, ja redan i Grekland veta omm, vöre viktigt hä jär åt rö:r opa: sä om man vell leva hällsosamt. Men fastann hä hav vöre tjennt, hav man et fåta vo hä jär för mekanissmom som djär:r åt ge:nen förendres del na bätter. Vä veta et hälar vöre, älär ensch hur de dära mekanisma våra förendre bårte ämnesommsättningsschu:kdaåma, som dell exämpäL typ-to: sokkerschu:ka. Åt man ännu mäijr undersch:ök molekylä:r-reakschåon som vàaL bårt hä man trään, kan vàaL mötche vikitit då hä djäll åt arrbaijt måt ämnesomsättningsschu:kdaåoma.

Mäijr än dòoj, have opa sisså:ra vorte tjennt åt vöre gambLen förr i ti:jdn lèevd kan innvärk opa vöre jeneraschaåna lenger framm å ämnesommsättninga dömersch. Hä hav bevijse åt hä n´Lamarck ment om ”mju:k ärftlighäijt”, åtminnschtone däijlvijs jär sannt. Däremaåot jär e et änn riktit kLart vöre mötche älär djuning vo mekanissmom gameLfåLkes sätt åt leva kan på:värk åss ida:. Åt skoda hä: hav vöre na: båRte de viktigaste vä avhandlingen jena.

Öte **förschtstu:dien** håva vä underschökt jenå:me borte skelättmusskulateurn öte frijska/kroija håLvgamLa karom, å nest karan som håva ’ty:p to:sokkerschu:ka, äätt ha döm håva tre:ne, vä mäRt åt förscht reakschaån var gaåde li:jk mila bå: gruppa, men åt sokkerschu:kgruppen äätt to: ti:jmsthilld sä mötche frå kontrollgruppen. Dömärsch infLammaschaånsschwa:r var oppreglä:re mötche mäijr. De resultata konfirmä:reses seda opa protein:nnivå me to: andre metoder.

Öte **andra stu:dien** gåv vä matn vä mötch fäijta åt ha:nrotten i ti: vecko, öte döm pa:re sä vä ho:nen, som hadd ite vannlit faåor. Ha:na båRte F1-generaschaån, som hadd ite vannlit faår, anvendese del åt jenrär F2-generaschaåon. Nager båRte F1- och F2- aåonga feng åkksa en hö:gfäijtsdiet. Vä sååg åt ho:nen ät ”högfäijtspapp-älär-farfar” inte euöke leka mötche i vekt, som hon:nen vä ’n papp/farfar vä vannlit faåor ,då döm schöölv åt hö:fäijtsdietn. Döm vaår alltså som ”vaksinä:re” frå åt vàaL så fäijt som döm sku håva böt bLi:j. Ennda var gLukå:stoleranschn dömersch del å mä:ngen nallta försemmre. Jams anne, fonn vä åt microRNA:t *Let-7c* som en måjli epijene:tisk bärar å ma:tarve opa fa:rsch-sijda.

Öte **tredje- å fjö:Lstu:dien**, jämfo:L vä stjilnèen öte särschilda vävnadom, bäinrangeL-muskulateurn å levra, bårte dju:ra öte **àannstu:dien**. B`åå: gangen underschökt vä förscht ”de gLoba:la jen-auttrökke” frå vä:vnada vä hä som kå:les ”gene arrays”. Vä hitte öte **tredje stu:dien** åt ”unfolded protein response” var oppreglä:re ine bäinrangeL-muskulateurn bårte F2-ho:nen vä ’n farfar som hadd ite en hö:gfäijtsdie:t, jämföRt vä ho:nen vä ’n farfar:r vä vannli foderdie:t, då döm schölLv åå:t ’n högfäijtsdie:t. Öte **fjö:Lstu:dien**, så:g vä, åt farfa:ras

hö:gfäijtsdie:t förendre TNF-α signaleringen, vöre änn aåonga schö:lv `ååt.

Ållt häijn, ihaåoptåje, vi:js stu:dien jena vöre å djuning vo måjliga mekanismom, miljö:n kan på:värk ämnesommsättningen våre å hä som hav vä töker schukdaåomom åt djära.

Texttolkning:
Understrykning är en markering för kortstavighet. Kolon (:) efter en bokstav visar att ljudet är långt. Versalt L, är ett tecken som visar att det uttalas med "tungslag"som i svenskans "pärla".

10 ACKNOWLEDGEMENTS

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