Energy metabolism as a target for new treatment strategies in Huntington's disease

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Energy metabolism as a target for new treatment strategies in Huntington's disease

by

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LUND
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DOCTORAL DISSERTATION

By due permission of the Faculty of Medicine, Lund University,
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Energy metabolism as a target for new treatment strategies in Huntington's disease

Abstract

Huntington´s disease (HD) is a fatal, genetic neurodegenerative disorder caused by an expanded CAG triplet repeat in the gene encoding the huntingtin protein. HD pathology is considered to arise from degeneration of basal ganglia and cortex, giving rise to cognitive, motor and psychiatric problems. However, accumulating evidence suggests an altered energy metabolism as a key feature in HD, as a result of mHTT expression throughout the body. Hyper-catabolism, including weight loss, muscle atrophy, fat mass and endocrine alterations are seen in HD patients and HD mouse models. One of the most prominent features of HD is the progressive weight loss, that occurs despite adequate caloric intake, contributing to general sickness and decreasing quality of life. Higher BMI has been demonstrated to be associated with slower disease progression, including functional, cognitive and motor decline independent of mHTT CAG repeat length and disease stage. In this thesis, we utilized the R6/2 mouse model in order to investigate the importance of metabolic disturbances in HD using genetic and pharmacological approaches. The R6/2 mouse model mimics many of the features seen in HD patients, e.g. progressive central pathology, weight loss, skeletal muscle atrophy and altered body composition. Ghrelin, an orexigenic gut-peptide hormone, is known to stimulate appetite and affect whole-body energy metabolism. Ghrelin and ghrelin receptor agonists have been shown to have beneficial effects on muscle wasting, cancer cachexia, cognitive decline, diabetes and metabolic disorders. Liraglutide is an efficient anti-type 2 diabetes incretin drug, and neuroprotective effects have been demonstrated in AD and PD.

In paper I, we show that by using a genetic approach, placing transgenic R6/2 mice on a leptin-deficient background, we were able to decrease the metabolic rate, and increase body weight and fat mass compared to and R6/2 littermates. This approach did not affect neuropathology.

In paper II, we targeted peripheral energy metabolism with ghrelin administration in R6/2 mice. Beneficial effects on both skeletal muscle wasting and cognitive deficits was found. Ghrelin administration (subcutaneous daily injections of 150 µg/kg for 2, 4 and 6 weeks) normalized skeletal muscle gene expression and morphological alterations involved in atrophy and muscle injury. Importantly, ghrelin administration normalized behavioral deficits in R6/2 mice.

In paper III, we evaluated the effects of liraglutide and ghrelin administration on both central and peripheral energy metabolism in R6/2 mice. We found that liraglutide, alone or together with ghrelin (subcutaneous daily injections of 150 µg/kg (ghrelin) and 0.2 mg/kg (liraglutide), for 2 weeks), normalized circulating glucose levels, decreased insulin resistance and improved pancreatic β-cell function. In addition, administration of liraglutide and ghrelin normalized cortical insulin and cholesterol levels, and activation of caspase-12 was observed.

Lastly, in paper IV, possible muscle progenitor (satellite cells) dysfunctions was evaluated *in vivo* and *in vitro*. In addition, the effect of ghrelin administration was studied on cultured satellite cells derived from R6/2 mice and WT littermates. In this study, we investigated the effect of mutant HTT on proliferation and differentiation capacity of satellite cells. We found significant reduction in myofiber diameter in differentiated satellite cells from skeletal muscle (gastrocnemius) cultured from R6/2$^{(CAG\ 242\text{-}257)}$ mice compared to WT mice, which was attenuated with ghrelin administration.

Taken together, the results included in this thesis suggests that targeting whole-body energy metabolism may exert beneficial effects on HD disease progression.

Key words

Huntington´s disease, energy metabolic alteration, weight loss, skeletal muscle, adipose tissue, R6/2, ghrelin and liraglutide

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To my family

"Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less"
— Marie Curie

"Science is a way of thinking
much more than it is a body of knowledge"
— Carl Sagan

Table of Contents

Original papers and manuscripts included in the thesis

I. **Marie Sjögren**, Rana Soylu-Kucharz, Unali Dandunna, Tiberiu Loredan Stan, Michele Cavalera, Åsa Sandelius, Henrik Zetterberg, Maria Björkqvist. Leptin deficiency reverses high metabolic state and weight loss without affecting central pathology in the R6/2 mouse model of Huntington´s disease. *Neurobiology of Disease 132C, 104560, doi:10.1016/j.nbd.2019.104560 (2019)*

II. **Marie Sjögren**, Ana I. Duarte, Andrew C. McCourt, Liliya Shcherbina, Nils Wierup & Maria Björkqvist. Ghrelin rescues skeletal muscle catabolic profile in the R6/2 mouse model of Huntington's disease. *Scientific reports 7, 13896, doi:10.1038/s41598-017-13713-5 (2017)*

III. Ana I. Duarte, **Marie Sjögren**, Maria S. Santos1,4, Catarina R. Oliveira, Paula I. Moreira & Maria Björkqvist. Dual Therapy with Liraglutide and Ghrelin Promotes Brain and Peripheral Energy Metabolism in the R6/2 Mouse Model of Huntington's Disease. *Scientific reports 8, 8961, doi:10.1038/s41598-018-27121-w (2018)*

IV. **Marie Sjögren**, Naomi Franke, Åsa Petersén, Maria Björkqvist, Rana Soylu. Satellite progenitor cell dysfunction in the R6/2 mouse model of Huntington disease. (*Manuscript*)

Published papers outside the thesis

I. Rana Soylu-Kucharz, Åsa Sandelius, **Marie Sjögren**, Kaj Blennow, Edward J. Wild, Henrik Zetterberg & Maria Björkqvist. Neurofilament light protein in CSF and blood is associated with neurodegeneration and disease severity in Huntington's disease R6/2 mice. *Scientific reports 7, 14114, doi:10.1038/s41598-017-14179-1 (2017)*

Summary

Huntington´s disease (HD) is a fatal inherited neurodegenerative disorder caused by a CAG triplet repeat expansion in the huntingtin (HTT) gene and characterized by cognitive and motoric dysfunctions as well as psychiatric problems. Accumulating evidence, however, suggests altered energy metabolism and a hyper-catabolic state as key features of HD pathology. In clinical HD, body weight has been shown to be a predictor of HD progression and higher BMI is demonstrated to be associated with a slower rate of functional, cognitive and motor decline. Body weight loss, muscle atrophy, body composition and endocrine alterations have been reported in HD patients and animal models of HD prior to weight loss. Different HD mouse models have been used to study disease progression with the R6/2 mouse as the most commonly used mimicking many features of HD clinical symptoms, such as weight loss and muscle atrophy, as well as cognitive and motoric dysfunctions.

In paper I, we studied the effect of a higher BMI on HD disease progression in the R6/2 mouse model. By crossing the R6/2 mouse model with the obese leptin-deficient mouse model (Ob/Ob) we generated a novel obese R6/2 mouse model with leptin-deficiency. We showed that it was possible to dramatically increase body weight in R6/2 mice by decreasing energy metabolism. Although, a sign of improvement of repetitive and anxiety-like behavior was seen, this was not associated with improved neuropathological measures.

In paper II, we evaluated the effect of ghrelin administration on metabolic disturbances seen in HD. We found that ghrelin reversed the catabolic gene expression profile with increased expression of *Caspase 8, Traf-5 and Creb1* seen in R6/2 skeletal muscle, and improved skeletal muscle morphology. Interestingly, behavior deficits were also rescued.

In paper III, the effect of liraglutide administration alone or together with ghrelin on brain and peripheral metabolic disturbances was evaluated. We found that liraglutide alone or together with ghrelin normalized glucose homeostatic features in the R6/2 mice. Liraglutide alone decreased brain cortical active GLP-1 and IGF-1 levels, alongside higher ADP levels, while co-administration of liraglutide and ghrelin decreased brain insulin, lactate, AMP and cholesterol levels in R6/2 mice.

In study IV, we studied the mechanism underlying a possible satellite cell dysfunction of HD mouse models, using in vivo and in vitro studies. We utilized the R6/2 mouse model to study proliferation and differentiation of satellite cells. Here we found that R6/2 mice exhibit reduced myofiber diameter both in vivo and in vitro, and altered gene expression (*Irs2, Myh2, MyoG, Sirt2*) and protein levels (MyoD1) in vitro. We found that ghrelin administration increased myofiber diameter and normalized MyoD1 protein levels in differentiated satellite cells derived from R6/2 mice.

Taken together, findings provided in this thesis encourage further studies targeting metabolism, giving rise to potential therapeutically interventions in HD.

Populärvetenskaplig sammanfattning

Huntingtons sjukdom (HS) är en ärftlig fortskridande neurodegenerativ sjukdom som orsakas av en förlängd CAG sekvens i *huntingtin* (*HTT*) genen, vilket leder till motoriska och kognitiva symptom samt personlighetsförändringar. Eftersom den muterade genen och proteinet den kodar för finns uttryckt i kroppens alla celler resulterar det i att även organ utanför hjärnan påverkas. En förhöjd ämnesomsättning är ett vanligt fenomen vid HS vilket kan leda till viktnedgång, förlust av muskelmassa och även förändringar i fettvävnaden. Forskningsresultat har påvisat ett senarelagt sjukdomsförlopp hos HS patienter med ett högre BMI, och är förknippat med en långsammare grad av funktionell, kognitiv och motorisk nedsättning. Förlust av muskelmassa, förändringar i kroppssammansättningen och hormonella förändringar har rapporterats hos HS-patienter och djurmodeller av HS före viktminskning. Olika HS musmodeller har använts för att studera sjukdomsförloppet. Den transgena R6/2 musmodellen är den mest använda, eftersom sjukdomsförloppet efterliknar förloppet hos HS patienter, med viktnedgång, förlust av muskelmassa såväl som förlust av kognitiv och motorisk förmåga.

Ghrelin är ett hormon som utsöndras från magsäcken och som stimulerar aptiten, men som även har visats öka fettmassan hos möss. Ghrelin och andra ghrelin-receptor agonister har påvisat gynnsamma effekter vid många kliniska problem, såsom förlust av muskelmassa, försämrat allmäntillstånd vid cancer, försämrad kognitiv förmåga och diabetes.

Liraglutid är ett läkemedel som har visat gynnsamma effekter vid diabetes, genom att sänka blodsockerhalten, men även ha skyddande effekt vid neurodegenerativa sjukdomar som Alzheimers, Parkinsons och Huntingtons sjukdom.

I artikel I studerade vi effekten av ett högre BMI på sjukdomsförloppet i R6/2-musmodellen. I denna studie använde vi oss av R6/2 möss och Ob/Ob möss, som har en hög respektive låg ämnesomsättning, för att försöka skapa en R6/2 musmodell med en normal ämnesomsättning. Ob/Ob möss har förutom en låg ämnesomsättning, även brist på leptin, vilket leder till fetma. Vi kunde här påvisa att det var möjligt att dramatiskt öka kroppsvikten och fettmassan i R6/2 möss, men ett högre BMI associerat med ökad fettmassa var inte tillräckligt för att ge en skyddande effekt på de patologiska förändringarna som finns i hjärnan vid HS.

I artikel II utvärderade vi effekten av ghrelin-behandling på de fenomen som är associerat med en ökad ämnesomsättning, som tex viktnedgång och förlust av muskelmassa, hos R6/2 mössen. Vi fann att ghrelin-behandling gav ett normalt utseende på skelettmuskulaturens form och uppbyggnad, men även de genuttryck som är associerat med skada och förlust av muskelmassa var normaliserade hos R6/2

mössen. Minnesförmågan förbättrades också hos R6/2 mössen efter ghrelin-behandling.

I artikel III utvärderades effekten av liraglutid- och ghrelin-behandling på de förändringar som finns både i hjärnan och i kroppen, involverade i den förhöjda ämnesomsättningen. En försämrad förmåga att hantera glukos är vanligt hos både HS patienter och hos R6/2 möss, vilket i HS möss leder till höga blodsockernivåer och insulinresistens. Vi fann att behandling med liraglutid ensam eller tillsammans med ghrelin normaliserade blodsockernivån, förbättrade funktionen hos de celler som producerar insulin och normaliserade insulinresistensen hos R6/2-mössen. Liraglutid- och ghrelin-behandling gav också en skyddande effekt i hjärnan genom att sänka de ökade insulin- och kolesterolnivåerna hos R6/2 mössen.

I studie IV odlades satellitceller, föregångare till muskelfibrer (myofibrer), från HS musmodellen R6/2 för att studera mekanismen bakom eventuell nedsatt satellitcellfunktion. Här fann vi att differentierade satellitceller (utvecklade till myofibrer) från R6/2-möss uppvisade en minskad myofiber diameter, förändringar i gener och proteiner som är involverade i differentiering, vilket tyder på en försämrad funktionell kapacitet hos satellitcellerna. Ghrelin-behandling ökade myofiber diametern och normaliserade MyoD1 proteinnivåerna *in vitro*. Ghrelin-behandling ledde till ökad storlek på myofibrerna och ledde dessutom till att proteiner som är involverade i differentieringen uttrycktes i normala nivåer.

Resultaten som visas i denna avhandling uppmuntrar framtida studier som fokuserar på ämnesomsättningen vid HS, vilket kan leda till potentiella behandlingsstrategier för sjukdomen.

Abbreviations

18S	18S ribosomal RNA
ADP	Adenosine diphosphate
AgRP	Agouti-related protein
AMP	Adenosine monophosphate
ANS	Autonomic nervous system
ARC	Arcuate nucleus in the hypothalamus
AMPAR	α-amino-3-hydroxi-5-metyl-4-isoxazol-propansyra receptor
ATP	Adenosine triphosphate
Atp5b	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide
BAC	Bacterial artificial chromosome
BAT	Brown adipose tissue
BDNF	Brain-derived neurotrophic factor
BMI	Body Mass Index
CAA	Cytosine adenosine adenosine
CAG	Cytosine adenosine guanine; glutamine
CAG140	Knock-in mouse models 140 CAG repeats introduced into exon 1 of the mouse Htt gene
Canx	Calnexin
Casp3	Caspase 3
Casp8	Caspase 8
cDNA	complementary DNA
C/EBP	CCAAT-enhancer-binding proteins
CNS	Central nervous system
CREB1	Cyclic AMP response element binding protein 1
DAPI	4',6-diamidino-2-phenylindole
Darpp32	Dopamine- and cAMP-regulated neuronal phosphoprotein 32
Dat	Dopamine transporter
DMH	Dorsomedial part of the hypothalamus
DNA	Deoxyribonucleic acid
Drd1a	Dopamine receptor D_{1a}
Drd2	Dopamine receptor D_2
DRPLA	Dentatorubral-pallidoluysian atrophy
GABA	γ-aminobutyric acid
GH	Growth Hormone
GHRH	Growth hormone–releasing hormone
GLP-1	Glucagon-like peptide-1
Gusb	Glucuronidase beta
HD	Huntington's disease

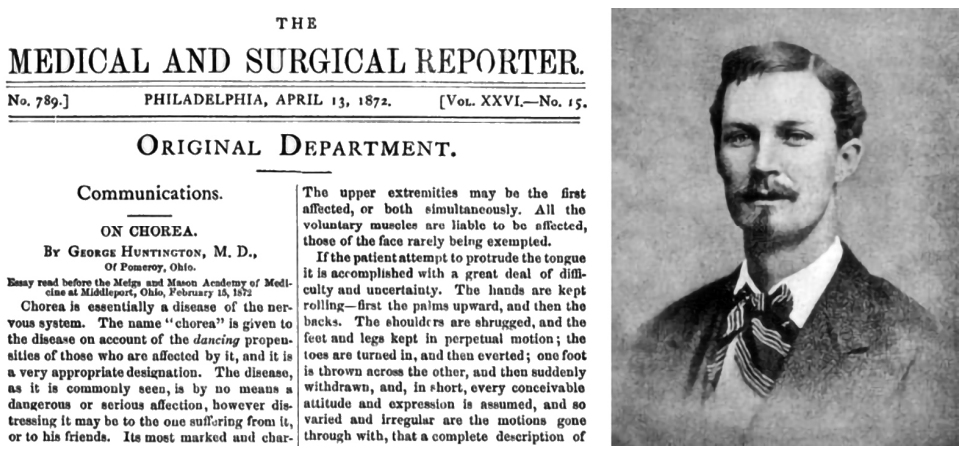
HdhQ150	Knock-in mouse models 150 CAG repeats introduced into exon 1 of the mouse Htt gene
HEAT	Huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A), and the yeast kinase TOR1
HPA	hypothalamic–pituitary–adrenal axis
HPG	hypothalamic–pituitary–gonadal axis
Hsp90ab1	Heat shock protein 90 alpha (cytosolic), class B member 1
HTT	Huntingtin
IGF-1	Insulin-like growth factor 1
IHC	Immunohistochemistry
IL	Interleukin
IRS2	Insulin receptor substrate 2
IT15	Interesting transcript 15
kDA	Kilo dalton
Lep	Leptin
LH	Lateral part of the hypothalamus
mHTT	Mutant huntingtin
MSN	Medium spiny neurons
Myf5	Myogenic factor 5
MYH2	Myosin Heavy Chain 2
MyoD	Myoblast determination protein 1
MyoG	Myogenin (myogenic factor 4)
NADH	Nicotinamide adenine dinucleotide (reduced form)
N171-82Q	Transgenic mouse expressing exon 1 of the human HD gene
NMDAR	N-metyl-D-aspartat receptor
NPY	Neuropeptide Y
Ob/Ob	Leptin-deficient obese mouse model
OXPHOS	oxidative phosphorylation
PCR	Polymerase chain reaction
Pde10a	cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A
PGC-1α	Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
polyQ	Polyglutamine
PPARγ	Peroxisome proliferator activated receptor gamma
PPIA	Peptidylprolyl isomerase A (cyclophilin A)
PVDF	Polyvinylidene difluoride
PVH	Paraventricular nuclei of the hypothalamus
PYY	Peptide YY
R6/1	Transgenic mouse expressing exon 1 of the human HD gene
R6/2	Transgenic mouse expressing exon 1 of the human HD gene
REST/NRSF	RE1-Silencing Transcription factor/Neuron-Restrictive Silencer Factor

RNA	Ribonucleic acid
Rpl13a	60S ribosomal protein L13a
RT-qPCR	Real time quantitative polymerase chain reaction
SBMA	Spinal and bulbar muscular atrophy
SCA	Spinocerebellar ataxia
Sirt2	Sirtuin 2
TBP	TATA Box Binding Protein
TCA	Tricarboxylic acid cycle or Citric acid cycle
TNF-α	Tumor necrosis factor α
Tradd	Tumor necrosis factor receptor type 1-associated DEATH domain
Traf-5	TNF receptor-associated factor-5
UCP1	Uncoupling protein 1
VMH	Ventromedial part of the hypothalamus
WAT	White adipose tissue
WB	Western Blotting
WT	Wildtype
YAC	Yeast artificial chromosome
Zfp516	Zinc finger protein 516
zQ175	Knock-in mouse with exon 1 of the human HD gene introduced into the native mouse gene

Introduction

Huntington's disease

The definition “epidemic dancing mania” was mentioned already in 1374, which was later on referred to as “chorea” (from the Ancient Greek word “χορεία” for dance) (Vale and Cardoso, 2015, Walker, 2007). However, it wasn´t until 1872, that the classical symptoms of Huntington´s disease (HD) was first described by the American physician George Huntington (Huntington, 1872). By studying the medical history of several generations of a family exhibiting similar symptoms, he suggested it to be a hereditary disease of the nervous system causing involuntary twitching muscle movements (Huntington, 1872, Huntington, 2003). The families he studied were ancestors of Jeffrey Francis, an immigrant from England in 1634, carrying the gene with him (Bhattacharyya, 2016).



THE

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ORIGINAL DEPARTMENT.

Communications.

ON CHOREA.

By George Huntington, M. D.,
Of Pomeroy, Ohio.

Essay read before the Meigs and Mason Academy of Medicine at Middleport, Ohio, February 15, 1872

Chorea is essentially a disease of the nervous system. The name "chorea" is given to the disease on account of the *dancing* propensities of those who are affected by it, and it is a very appropriate designation. The disease, as it is commonly seen, is by no means a dangerous or serious affection, however distressing it may be to the one suffering from it, or to his friends. Its most marked and characteristic features are developed in the muscular system. The upper extremities may be the first affected, or both simultaneously. All the voluntary muscles are liable to be affected, those of the face rarely being exempted.

If the patient attempt to protrude the tongue it is accomplished with a great deal of difficulty and uncertainty. The hands are kept rolling—first the palms upward, and then the backs. The shoulders are shrugged, and the feet and legs kept in perpetual motion; the toes are turned in, and then everted; one foot is thrown across the other, and then suddenly withdrawn, and, in short, every conceivable attitude and expression is assumed, and so varied and irregular are the motions gone through with, that a complete description of

Figure 1.
George Huntington, M.D., picture and the publication “On Chorea” published in 1872 describing the clinical symptoms of the hereditary disorder now known as Huntington´s disease.

In 1979, members of the Venezuela Collaborative Research Project, started to annually travel to Maracaibo in Venezuela, to document the extent of HD and progression of the disease (Wexler, 2004). It was found that the population had a very high prevalence of HD, and was therefore suitable for finding the causative

gene (Wexler, 2004). This long-term study made it possible to identify the locus of the gene on chromosome 4 in 1983 using DNA linkage analysis (Gusella et al., 1983). However, it wasn´t until 1993, ten years after the discovery and more than a century after George Huntington´s observations, that the gene mutation causing HD disease onset was discovered (Group, 1993). The gene was identified as the "interesting transcript 15" (*IT15*) gene, now known as the huntingtin (*HTT*) gene (Group, 1993).

Huntington´s disease, named after George Huntington, is a fatal progressive autosomal dominant neurodegenerative disorder (Huntington, 1872), associated with progressive motor dysfunction, cognitive decline, personality changes and metabolic disturbances (Novak and Tabrizi, 2010, Ross and Tabrizi, 2011, Walker, 2007, van der Burg et al., 2009, van der Burg et al., 2011). HD is the most common genetic neurodegenerative disorder with a prevalence of 5–10 cases per 100 000 worldwide (Landles and Bates, 2004), with a higher prevalence in North America, Europe, and Australia (5.70 per 100 000) compared to Asia (0.40 per 100 000) (Phillips et al., 2008, Pringsheim et al., 2012). There is also evidence that prevalence has increased over the past 50 years in Australia, North America and in Western Europe (Rawlins et al., 2016).

The pathology results from an expanded CAG (cytosine-adenine-guanine) trinucleotide repeat in the *HTT gene* on chromosome 4p16.3, leading to a mutated gene and a misfolded protein with a long stretch of polyglutamine (polyQ) (Group, 1993, Ross and Tabrizi, 2011, Sathasivam et al., 1999, Tippett et al., 2017). The prolonged polyQ stretch is not a unique feature of HD. Instead, HD belongs to a CAG–polyQ disease family containing different neurodegenerative diseases caused by the same mutation in their specific target gene, including dentatorubral-pallidoluysian atrophy (DRPLA), spinal and bulbar muscular atrophy (SBMA) and the spinocerebellar ataxias (SCAs) 1–3, 6, 7 and 17 (Landles and Bates, 2004). Typical onset of HD is between the ages of 35 and 45 years and progresses over a period of 15 to 20 years (Walker, 2007). The number of CAG repeats in the *HTT* gene plays a key role in the age of onset of the disease, where the CAG repeat is negatively correlated with disease onset (Duyao et al., 1993). CAG repeat length below 27 is seen in healthy individuals, whereas CAG repeat expansions of 40 and above cause an adult onset of the disease with full penetrance (Duyao et al., 1993). Individuals carrying 36-39 CAG repeats exhibit reduced penetration of the disease with a slower disease progression (Ehrlich, 2012, Duyao et al., 1993). Repeat expansions above 60–70 CAGs lead to a juvenile form of HD, with a much more aggressively progressive form of the disease (Bates et al., 2015). A range between 27 and 35 is considered to be in the intermediate range with subtle disease manifestations (Ha et al., 2012), which might be expanded from generation to generation due to CAG repeat instability (Brocklebank et al., 2009, Telenius et al., 1994, Trottier et al., 1994). Although a higher CAG repeat length is correlated with

an earlier onset, environmental factors are also thought to influence the timing of onset (Wexler, 2004).

The mutation causes the protein to gain toxic functions and aggregate and/or the loss of wildtype function of HTT, leading to neuronal dysfunction and death (Bates et al., 2015, Ross and Tabrizi, 2011). Medium spiny neurons (MSNs) in the striatum are most vulnerable and severely affected accompanied by degeneration of the pyramidal cells in the cortex (Ehrlich, 2012, Rosas et al., 2008, Vonsattel et al., 1985), but when the disease progresses a widespread central pathology is evident (Rub et al., 2015). This neurodegeneration leads to the characteristic pathology with motoric and cognitive impairment, and psychiatric and behavioral problems (Walker, 2007). Since the causative mutant huntingtin protein is ubiquitously expressed (Sathasivam et al., 1999), this results in peripheral pathology evident from early stage of the disease (McCourt et al., 2016, Strand et al., 2005, van der Burg et al., 2009, van der Burg et al., 2011). Increased energy metabolism with a hyper-catabolic state, is a key feature in HD, leading to symptoms such as weight loss, muscle atrophy, fat mass alterations and endocrine alterations (Carroll et al., 2015, Ribchester et al., 2004, Strand et al., 2005, Trejo et al., 2004, van der Burg et al., 2008, van der Burg et al., 2009, van der Burg et al., 2011). Body weight has recently been shown in a large observational study to be a predictor of clinical HD progression, where higher BMI is demonstrated to be associated with a slower rate of functional, cognitive and motor decline (van der Burg et al., 2017).

Although a lot is known about HD, there is no existing cure treating the disease. Up to date, only treatments to help manage the symptoms are available. Future strategies targeting peripheral pathology and energy metabolism could possibly provide better understanding and may exert a beneficial effect on HD disease progression and increase the quality of life for the patients.

Huntingtin protein

Normal function of HTT

The *HTT* gene is located on chromosome 4p16.3 encoding the huntingtin protein (Group, 1993). Normal HTT is a large protein with 3144 amino acids and a molecular weight of approximately 348 kDa (Group, 1993). HTT is ubiquitously expressed, with highest levels in the brain and gonads (DiFiglia et al., 1995, Landwehrmeyer et al., 1995, Li et al., 1993a, Sathasivam et al., 1999, Strong et al., 1993a). HTT contains a series of up to 36 HEAT domains, which mediates interaction with numerous proteins involved in gene expression, endocytosis, vesicle trafficking, intracellular signaling, and metabolism (Caviston and Holzbaur,

2009, Harjes and Wanker, 2003, Li and Li, 2004). HTT is localized mostly inside the cytoplasm, but it can also be shuttled between the cytoplasm and the nucleus (Landles and Bates, 2004, Bates et al., 2015) in both neuronal and non-neuronal cells (Hoogeveen et al., 1993, Trottier et al., 1995). Exact function of normal HTT protein is not yet elucidated, but it is suggested to be involved in many vital processes in many tissues; such as axonal, vesicle and mitochondrial transport (Cattaneo et al., 2005, Velier et al., 1998), synaptic activity (Smith et al., 2005), controlling cortical Brain-derived neurotrophic factor (BDNF) production (Zuccato and Cattaneo, 2007) and having an anti-apoptotic role (Rigamonti et al., 2000, Rigamonti et al., 2001). Homozygous HTT knockout mice (Hdh−/−) are embryonic lethal (Duyao et al., 1995, Nasir et al., 1995, Zeitlin et al., 1995), suggesting that wildtype HTT is necessary for cell survival and that the loss of function can be involved in neurodegeneration (Bates et al., 2002). Brain defects as well as behavioral changes have also been detected in animals heterozygous for HTT knockout (Hdh+/-) (Nasir et al., 1995, O'Kusky et al., 1999, White et al., 1997). An overexpression of normal HTT has been shown to be neuroprotective (Leavitt et al., 2006, Rigamonti et al., 2000), as well as reduce toxicity in peripheral cells overexpressing mutant HTT (Ho et al., 2001, Leavitt et al., 2001). Most interestingly, overexpressing normal HTT in mouse models leads to an increased body weight (Pouladi et al., 2010).

Mutant HTT in disease

While wildtype HTT have neuroprotective properties (Leavitt et al., 2006, Rigamonti et al., 2000), the mutated form leads to widespread neurodegeneration (Rub et al., 2015). Reduced levels of HTT have been shown to sensitize neurons to the toxic effect of mHTT (Auerbach et al., 2001). Some researchers suggest that HTT aggregates are toxic and that the impaired folding and degradation of misfolded proteins contribute to HD pathology (Sakahira et al., 2002), while others suggest that large mHTT inclusions are not correlated with cytotoxicity (Kim et al., 1999), and might even be neuroprotective (Arrasate et al., 2004). Although both toxic gain-of-function of mHTT and loss-of-function of the normal HTT has been suggested to contribute to HD pathogenesis, most studies trying to determine the mechanisms underlying neurodegeneration has been driven by the toxic gain-of-function hypothesis (Bates et al., 2015, Ross and Tabrizi, 2011).

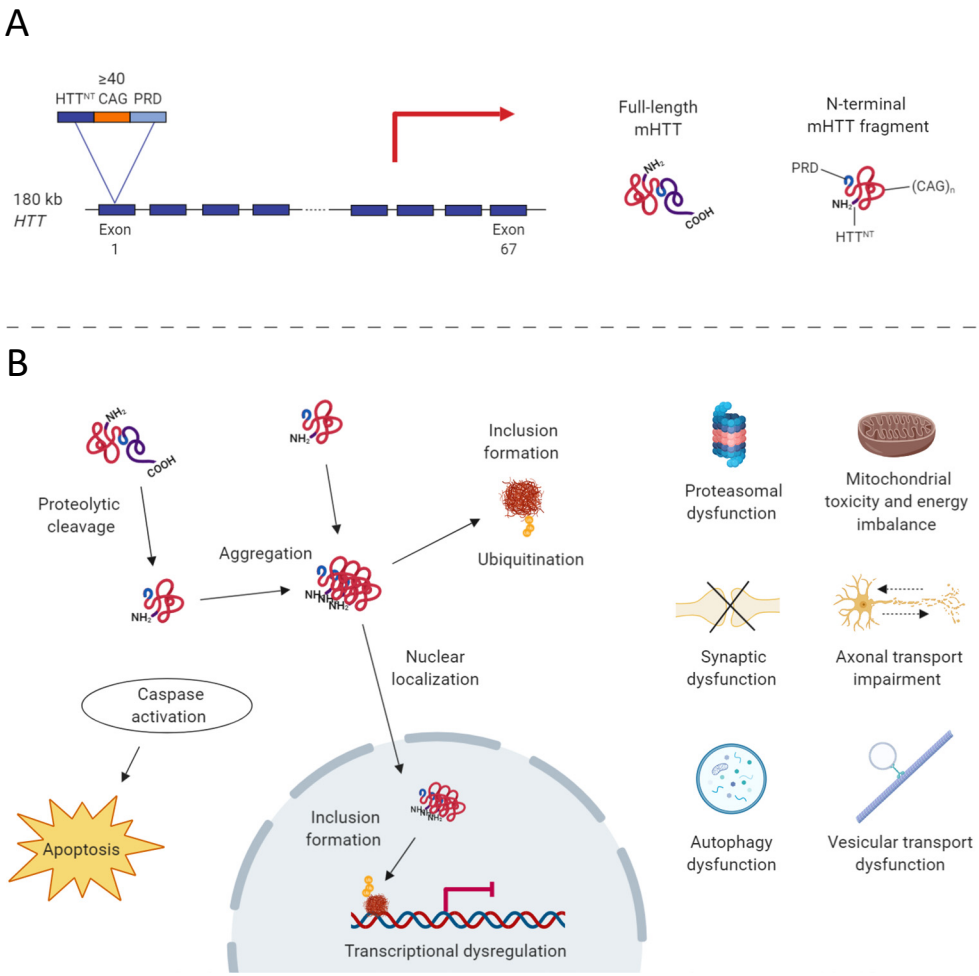

Figure 2. Huntingtin gene and pathogenic cellular mechanism in HD.
The mutated huntingtin gene containing 40 CAG repeats or more gives rise to a misfolded protein. *HTT* is translated into a full-length huntingtin protein as well as an amino-terminal mHTT exon1 fragment **(A)**. Full-length HTT protein is further processed into an amino-terminal mHTT exon1 fragment through proteolytic cleavage, which are more prone to aggregate and form inclusions in both cytoplasm and nucleus. Dysfunctions in folding and degradation by chaperones and proteasome leads to an overload of misfolded proteins and inclusions inside the cell, which can further affect vital functions suh as synaptic transmission, axonal and vesicular transport, mitochondrial function, endocytosis and autophagy. Intranuclear inclusions can affect the function of important transcription factor and thereby impair gene transcription **(B).** (Bates et al., 2015, Cha, 2000, Cortes and La Spada, 2014, Landles and Bates, 2004, Ross and Tabrizi, 2011, Sugars and Rubinsztein, 2003) (*Created with BioRender.com*).

The *mHTT* gene containing more than 40 CAG repeats in exon 1 is translated into either a truncated form of N-terminal fragments or a full-length misfolded huntingtin protein, which is further cleaved through proteolysis to generate additional protein fragments (Bates et al., 2015). These fragments of mHTT are released into the extracellular matrix and are more prone to aggregate (Bates et al., 2015, Chen et al., 2001). They manifest both intracellular and intranuclear

inclusions (Li et al., 2003, Lunkes et al., 2002) leading to a cytotoxic form of mHTT (Bates et al., 2015, Chen et al., 2001). In HD brain, intranuclear aggregates and inclusions of mHTT have been shown to positively correlate with the number of CAG repeats (DiFiglia et al., 1997). However, the distribution of aggregates in different brain areas was shown not to correlate with neuropathology in HD (Gutekunst et al., 1999). The cytotoxic form of HTT interfere both directly and indirectly through different cellular pathways, affecting vital functions such as synaptic transmission, axonal and vesicular transport, mitochondrial function, endocytosis and autophagy, as well as gene transcription (Bates et al., 2015, Cha, 2000, Cortes and La Spada, 2014, Jones and Hughes, 2011, Landles and Bates, 2004, Ross and Tabrizi, 2011, Sugars and Rubinsztein, 2003).

Mutant HTT has been shown to have dysregulatory effect on mRNA levels of different transcription factors and regulatory genes (Seredenina and Luthi-Carter, 2012). It was also shown that mHTT inclusions sequester transcription factors such as cAMP response element binding protein (CREB) and thereby inhibiting downstream factor disturbing cellular signaling (Landles and Bates, 2004, Li and Li, 2004, Marx, 2005, Rubinsztein et al., 2006).

As previously mentioned, loss of the normal HTT might also play a role in HD pathogenesis (Bates et al., 2002), where the absence of BDNF plays an important role in neurotoxicity and survival (Pardo et al., 2006). Production of BDNF is inhibited by transcription factors such as RE1-Silencing Transcription factor/Neuron-Restrictive Silencer Factor (REST/NRSF) with the absence of normal HTT (Zuccato et al., 2003). The reduction of BDNF due to the loss of normal HTT is suggested to play a part in the loss of MSNs in the striatum, since they are particular dependent on this neurotrophic factor (Cattaneo et al., 2005, Landles and Bates, 2004, Li and Li, 2004, Marx, 2005).

Inclusions formed by the mutant HTT is not a unique feature of the brain. Instead, since it is ubiquitously expressed throughout the body, peripheral pathology is also evident as a consequence of mHTT inclusions located in peripheral tissue (Ciammola et al., 2006, Orth et al., 2003, Moffitt et al., 2009, Sathasivam et al., 1999, Strong et al., 1993a). Studies have shown that peripheral pathology might even contribute to degeneration in the brain (Chiang et al., 2007, Martin et al., 2009). Studies in HD mouse models demonstrate that mHTT inclusions contribute to transcriptional dysregulation in adipose tissue (McCourt et al., 2016, Phan et al., 2009) and skeletal muscle (Magnusson-Lind et al., 2014, She et al., 2011, Sathasivam et al., 1999) leading to adipose tissue alterations and muscle pathology. In addition to brain, muscle and adipose tissue; mHTT inclusions are also present in other tissues and organs such as heart, liver, kidney, gastro-intestinal tract and pancreas (Bjorkqvist et al., 2005, Sathasivam et al., 1999), exerting whole-body effects.

Mitochondrial dysfunction due to interference from mHTT inclusions is a common feature in the HD brain (Browne et al., 1997, Tabrizi et al., 1999), as well as non-neuronal tissue such as skeletal muscle in both clinical and mouse models of HD (Panov et al., 2002, Parker et al., 1990, Saft et al., 2005). Energy deficiency due to mitochondrial dysfunctions may, in part, be responsible for the observed metabolic alterations with progressive weight loss in HD patients (Trejo et al., 2004) and mouse models of HD (Stack et al., 2005).

Central pathology

A neuropathological hallmark of HD is the selective degeneration of the striatum, especially γ-aminobutyric acid (GABA)-ergic MSNs (Graveland et al., 1985, Vonsattel et al., 1985). At end stage, approximately 95% of MSNs are lost, but the direct cause and consequence are not yet fully understood (Vonsattel et al., 1985, Yager et al., 2015). Studies suggest that excitotoxicity, in response to the over-activation of glutamate receptors (NMDA- and AMPA receptors) on MSNs, result in neuronal damage and are one of the pathological pathways involved in degeneration in striatum (Cicchetti et al., 2011). It has also been suggested that deprivation of trophic factors from the cortex, especially the loss of BDNF, might have a detrimental effect (Strand et al., 2007, Zuccato and Cattaneo, 2007).

MSNs expressing enkephalin and dopaminergic D2 receptor are the most vulnerable neurons in the striatum at early stage of disease (Bateup et al., 2010, Graveland et al., 1985, Menalled et al., 2000, Sapp et al., 1995). Degeneration of these neurons leads to the inability to control voluntary movements and thereby the choreic movements and tremor (Bateup et al., 2010, Graveland et al., 1985, Menalled et al., 2000, Sapp et al., 1995). While at later stages when rigidity, akinesia and bradykinesia appears, the MSNs expressing substance P, dynorphin, and dopaminergic D1 receptor are lost (Albin et al., 1992, Graveland et al., 1985).

Other regions such as cerebral cortex (Rosas et al., 2008, Vonsattel et al., 1985) and hypothalamus (Aziz et al., 2007, Petersen and Gabery, 2012, Politis et al., 2008) are also affected, and as the disease progresses a more widespread central pathology is seen (Rub et al., 2015). Cortical alterations are evident from an early stage in HD. Several studies suggest that degeneration of pyramidal neurons in the cortex proceed in parallel with striatal degeneration (Halliday et al., 1998, Macdonald and Halliday, 2002).

Peripheral pathology

Accumulating evidence supports the concept that pathology is not limited to the brain (Hoogeveen et al., 1993, Trottier et al., 1995). Mutant HTT is ubiquitously expressed, both in brain and in peripheral tissues and organs in both HD patients and animal models of HD (Bjorkqvist et al., 2005, Ciammola et al., 2006, Li et al., 1993b, Moffitt et al., 2009, Orth et al., 2003, Sathasivam et al., 1999, Sharp et al., 1995, Strong et al., 1993a, van der Burg et al., 2009). It has been suggested that peripheral features might even contribute to brain pathology as a result of toxic effects of mHTT expressed in peripheral cells and tissue (Chiang et al., 2007, Martin et al., 2009). Peripheral features sometimes appears early in disease progression, and can lead to morbidity and eventually to mortality (Lanska et al., 1988).

Alongside weight loss; severe muscle wasting and altered adipose tissue distribution, as well as systemic low-grade immune response and endocrine disturbances are found in clinical HD and mouse models of HD (Aziz et al., 2007, Carroll et al., 2015, Phan et al., 2009, Ribchester et al., 2004, Strand et al., 2005, Trejo et al., 2004, van der Burg et al., 2009, van der Burg et al., 2011)(shown in Figure 3).

Skeletal muscle wasting with an altered gene expression (She et al., 2011, Magnusson-Lind et al., 2014), as well as atrophic morphological features, are prominent features progressing with the disease (Ciammola et al., 2006, Moffitt et al., 2009, Orth et al., 2003, Sathasivam et al., 1999). Gene expression alterations observed in the muscle has been shown to reflect those alterations seen in HD brain (Luthi-Carter et al., 2002), and might therefore be a valuable biomarker of the disease (Strand et al., 2005).

Other symptoms such as cardiac failure, testicular atrophy, osteoporosis, gastrointestinal abnormalities and glucose intolerance could also be considered as HD features (Carroll et al., 2015, Sassone et al., 2009, van der Burg et al., 2009, van der Burg et al., 2011).

Cardiac failure is the second leading cause of death in HD patients (Lanska et al., 1988, Sørensen and Fenger, 1992), and a common cause of death in the R6/2 mouse model of HD (Mihm et al., 2007). Mechanism behind cardiac failure in HD patients is still unclear, but studies in both patients and mouse models suggest that it might be a consequence of a disturbed autonomic nervous system (Bar et al., 2008). It has been demonstrated that expression of mHTT under a cardio-myocyte promoter in wildtype (WT) mice could induce aggregate formation leading to cardiac pathology in HD (Pattison and Robbins, 2008, Pattison et al., 2008).

Systemic activation of the immune system in HD, with increased concentration of circulating pro-inflammatory cytokines, e.g IL-6 and IL-8, has been shown in HD

(Bjorkqvist et al., 2008, Dalrymple et al., 2007). Inflammatory alterations in the periphery have been shown to mirror pathological processes occurring in HD brain (Bjorkqvist et al., 2008, Khoshnan et al., 2004). An altered cytokine signaling may even be involved in the increased metabolic rate and weight loss seen in HD, as well as muscle wasting (Fong et al., 1989, Langen et al., 2001, Spiegelman and Hotamisligil, 1993). Pro-inflammatory signaling molecules such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are associated with muscle wasting in HD, through its inhibition of myogenic differentiation and enhanced apoptosis characterized by an altered gene expression (Magnusson-Lind et al., 2014, Spiegelman and Hotamisligil, 1993, Strand et al., 2005).

Increased interest in pathological effects of mHTT in peripheral tissues and organs has evolved over the last two decades (Carroll et al., 2015, Sassone et al., 2009, Sathasivam et al., 1999, van der Burg et al., 2009). Studies indicate that peripheral tissues are affected in HD as a direct result from mHTT, and further research targeting the periphery might contribute to a better understanding of pathological mechanisms involved in the disease, and lead to new biomarkers and beneficial treatments.

Motor and non-motor symptoms

Clinical diagnosis of HD is based on the criteria of motor symptoms with or without psychiatric or cognitive changes, in combination with genetic testing and family history of HD (Roos, 2010).

Motor symptoms are a prominent feature of HD starting with small twitches in the muscles of the face and extremities (Huntington, 1872). As the disease progresses, larger muscles are affected leading to tremor and involuntary dance-like movements, called chorea, causing problems in everyday activities (Huntington, 1872). At later stages of the disease, choreic movements decline and other motor dysfunctions such as rigidity, slow movement (bradykinesia) and difficulty initiating movements (akinesia) become more distinct (Bateup et al., 2010, Ghosh and Tabrizi, 2013, Huntington, 1872, Novak and Tabrizi, 2010).

Symptoms such as cognitive impairment and psychiatric problems appear many years prior to motor dysfunctions (Paulsen et al., 2008, Paulsen, 2011, Witjes-Ane et al., 2003). The cognitive impairment progresses slowly over time and is correlated with both striatal and cortical degeneration (Montoya et al., 2006). Deficits in planning and solve problems due to slowness in initiating thought processes and attentional problems, difficulties in executive functions as well as impaired episodic memory are common features in HD patients, which in later stages develop into dementia (Butters et al., 1978, Wolf et al., 2009, Zakzanis, 1998). Another common

cognitive impairment at early stage of the disease is the lack of insight regarding the patient´s own symptoms, where the patient denies that they have any motor or cognitive problems (Ho et al., 2006, Hoth et al., 2007). Personality changes with depression, anxiety, apathy and suicidal thoughts, as well as an obsessive compulsive behavior are also commonly displayed by HD patients (Wetzel et al., 2011, Duff et al., 2007, van Duijn et al., 2007).

Hypothalamic damage seen in HD patients contribute to symptoms such as sleep disturbances, alterations in circadian rhythm, and disturbed energy metabolism resulting in weight loss (Aziz et al., 2007, Hult et al., 2010, Morton et al., 2005, Petersen and Bjorkqvist, 2006, Petersen et al., 2005).

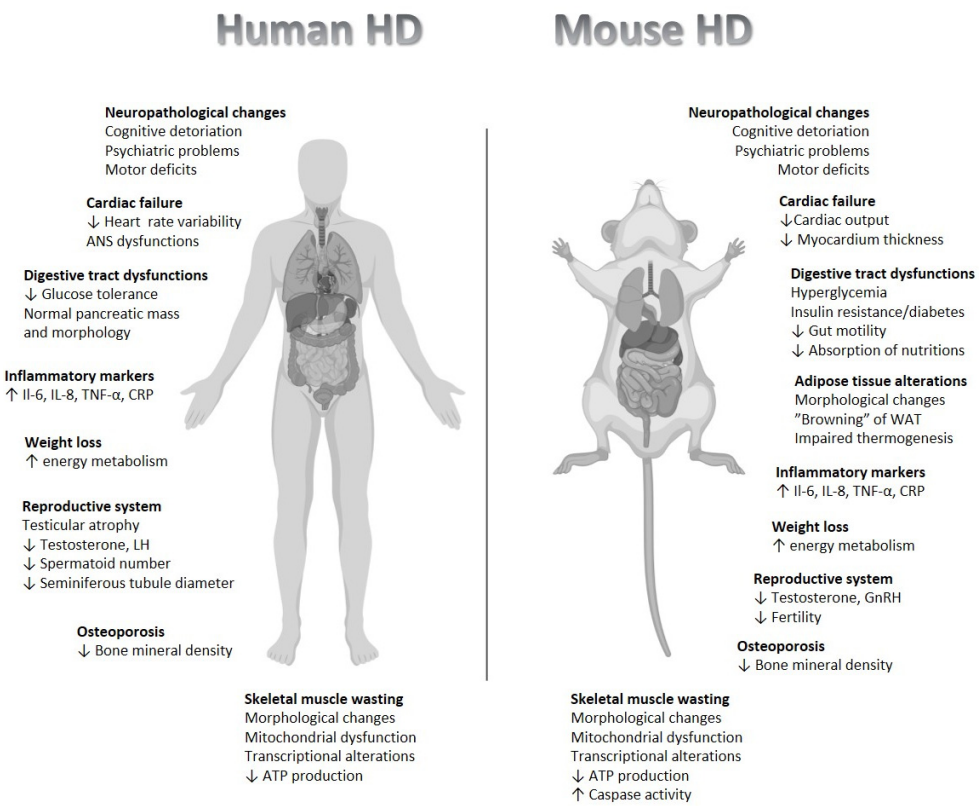

Figure 3. A summary of pathological dysfunctions in Huntington´s disease.
Pathological changes in CNS and periphery in HD patients and mouse models. Progressive weight loss and skeletal muscle atrophy, as a result of increased energy metabolism, is prominent in both human and mouse HD. Other pathological changes seen in mouse HD, such as cardiac tissue alterations, testicular atrophy, bone abnormalities, as well as brain are reminiscent to human HD. (Carroll et al., 2015, van der Burg et al., 2009, van der Burg et al., 2011)

Energy metabolism

Energy metabolism is the process of generating energy in form of adenosine triphosphate (ATP) from nutrients, e.g. carbohydrates, lipids and proteins (Cloutier and Wellstead, 2010, Green and Zande, 1981). ATP is synthesized in the mitochondria either by oxidative phosphorylation (OXPHOS) or via the tricarboxylic acid (TCA) cycle, or by glycolysis in the cytoplasm (Cloutier and Wellstead, 2010).

Complex mechanisms contributing the homeostatic effects on body weight involves interactions between the central nervous system (CNS) and peripheral tissues and organs, such as pancreas, white adipose tissue and the gastrointestinal tract (Yi and Tschop, 2012). The brain is continuously monitoring peripheral energy status, which is extremely important for the brain's survival, since it is depending on a continuous nutrient supply from the periphery (Yi and Tschop, 2012).

Energy homeostasis is primary controlled by different nuclei and areas in the hypothalamus, such as the arcuate nucleus (ARC), the paraventricular nuclei of the hypothalamus (PVH), the dorsomedial (DMH), the ventromedial (VMH) and the lateral hypothalamus (LH) (Wilson and Enriori, 2015). Metabolic responses, involving neural networks located in the hypothalamus, such as ARC, sense whole-body energy status and regulate food intake (Keesey and Powley, 2008). Activation of anabolic pathways increase food intake via mechanisms that involve activation of neuropeptide Y (NPY) and agouti-related protein neurons (NPY/AgRP neurons), by the gastric hormone ghrelin, in both rodents (Tschop et al., 2000) and humans (Wren et al., 2001). The same neurons are inhibited during a catabolic state by the adipocyte hormone leptin, the pancreatic hormone insulin and the intestinal peptide YY (PYY) inhibiting food intake and adiposity (Morton et al., 2006).

Every organ in the body has their own metabolic profile, contributing to lipid, protein and carbohydrates metabolism. The liver is a key metabolic organ regulating the whole-body energy metabolic balance, and connecting various tissues, e.g. brain, skeletal muscle and adipose tissue (Rui, 2014). The connection between brain and liver is especially important since the role of the liver is to remove toxins such as ammonia, and thereby prevent damage of the brain (Felipo, 2013). It also helps to maintain circulating glucose levels during fasting via gluconeogenesis and glycogenolysis, which is essential for the glucose-dependent brain (Han et al., 2016). Another organ essential for regulating energy homeostasis is skeletal muscle, acting as a regulator of energy, glucose and protein metabolism throughout the body. It serves as the key site for uptake and storage of glucose, but also as an amino acid reservoir supporting protein synthesis and energy production when needed (Delezie and Handschin, 2018, Meyer et al., 2002, Wolfe, 2006).

Creating a balance between energy intake, energy expenditure and energy storage to maintain energy homeostasis is essential for all living species (Galgani and Ravussin, 2008, Hill et al., 2012, Hill et al., 2013). Basal metabolic rate is the amount of energy per unit of time that a person needs to keep the body functioning at rest, e.g. processes such as breathing, body temperature control, blood circulation, cell growth, brain function and muscle contractions (Ballesteros et al., 2018).

An imbalance in energy homeostasis leads to disturbances in body weight and body composition (Duan et al., 2014, Fonseca et al., 2018), which can be seen in both obesity (Faria et al., 2012) and cachexia syndrome (Porporato, 2016).

Metabolic disturbances in HD

Alterations in both central and peripheral energy metabolism have been demonstrated in HD patients and mouse models (shown in Figure 4)(Carroll et al., 2015, van der Burg et al., 2009, van der Burg et al., 2011). These alterations contribute to an increased energy expenditure and weight loss, as well as severe muscle wasting, adipose tissue alterations, systemic low-grade immune response and endocrine disturbances (Bjorkqvist et al., 2006, Ribchester et al., 2004, Strand et al., 2005, Trejo et al., 2004, van der Burg et al., 2008).

One of the most prominent feature in HD is the progressive weight loss as a result of the disturbed metabolic rate, despite adequate or even increased caloric intake (Djousse et al., 2002, Farrer and Meaney, 1985, Farrer and Yu, 1985, Mochel et al., 2007, Morales et al., 1989, Robbins et al., 2006, Sanberg et al., 1981, Trejo et al., 2004, van der Burg et al., 2009). Body weight has recently been shown in a large observational study by van der Burg and colleagues to be a predictor of clinical HD progression (van der Burg et al., 2017). Higher BMI was demonstrated to be associated with a slower rate of functional, cognitive and motor decline independent of mHTT CAG repeat size and disease stage (van der Burg et al., 2017).

Mitochondrial dysfunction, resulting in energy deficits in brain and periphery, is a common feature in the pathogenesis of HD in both humans and animal models (Mochel and Haller, 2011). Various mechanism underlying energy deficits due to mitochondrial dysfunction has been suggested; including dysregulation of key factors of mitochondrial biogenesis, such as the transcriptional coactivator PPARγ coactivator-1α (PGC-1α) (Cui et al., 2006, Weydt et al., 2006), impaired OXPHOS (Milakovic and Johnson, 2005), as well as TCA cycle dysfunction (Mochel et al., 2007), ATP deficit (Mochel and Haller, 2011, Seong et al., 2005, Lodi et al., 2000), increased oxidative stress (Tabrizi et al., 1999, Browne et al., 1999) and apoptosis (Mochel and Haller, 2011). Observations of a low ATP/ADP ratio was found in a lymphoblastoid cell lines derived from HD patients (Seong et al., 2005), which

mirrors the findings in striatal cells, and is inversely correlate with the length of the CAG repeat (Seong et al., 2005). Energy deficits induced by mitochondrial dysfunctions in both brain and peripheral tissue could explain the progressive weight loss seen in HD (Mochel and Haller, 2011).

Another possible contribution to altered metabolism and weight loss observed in HD might be a dysfunctional digestive tract (Goodman et al., 2008, van der Burg et al., 2011). The digestive tract and its hormones, e.g. ghrelin amongst others, are important for the communication with hypothalamus and other organs (Coll et al., 2007). In R6/2 mice, gastrointestinal dysfunctions with reduced number of ghrelin producing neurons were found, resulting in weight loss (van der Burg et al., 2011). The gastric hormone, ghrelin, and the adipose hormone, leptin, are two peptide hormones regulating body energy homeostasis by targeting hypothalamic neurons in the arcuate nucleus, ventromedial and lateral hypothalamus (Wynne et al., 2005). Ghrelin stimulate appetite (Inui et al., 2004), increase body weight and adiposity (Bianchi et al., 2016, Tschop et al., 2000), and has been shown to rescue the catabolic profile seen in skeletal muscle in HD (paper II)(Sjögren et al., 2017). Leptin, on the other hand, has been found to induce weight loss by suppressing the appetite and stimulate an increased metabolic rate (Nogueiras et al., 2008, Wynne et al., 2005). High circulating ghrelin and low leptin levels has been observed in HD patients, suggesting a state of negative energy balance (Popovic et al., 2004). The perturbations of ghrelin and leptin levels might be a compensatory effect to preserve body weight and maintain a balanced energy homeostasis (Mochel et al., 2007). Alongside the secretion of leptin and other adipokines, white adipose tissue (WAT) is also responsible for storage and subsequent release of energy, during key metabolic processes e.g lipogenesis, lipolysis and glycolysis (Rutkowski et al., 2015). Adipose tissue alterations have been reported prior to weight loss in animal models of HD (Phan et al., 2009), with altered fat mat mass, lipolytic function, as well as dysregulated gene expression in mature adipocytes (Fain et al., 2001). Hyperplasia of brown/beige cells in WAT of R6/2 mice, resulting in browning of WAT, has been reported to enhance energy expenditure, and contribute to weight loss (McCourt et al., 2016).

In addition to ghrelin and leptin, the pancreatic hormone, insulin, act to reduce hunger and to lower circulating glucose levels after a meal, and is positively correlated with body fat proportions (Bagdade et al., 1967). Insulin resistance coinciding with increased fat mass is found in R6/2 mice at early stage (Bjorkqvist et al., 2006), which is further developed into diabetes at later stages due to decreased β-cell mass and deficient insulin secretion (Bjorkqvist et al., 2005). In HD patients, on the other hand, contradictory results have been shown (Boesgaard et al., 2009, Lalic et al., 2008, Podolsky et al., 1972, Popovic et al., 2004, Wang et al., 2014). In some studies, lower insulin response and sensitivity and an increased incidence of diabetes was found (Lalic et al., 2008, Podolsky et al., 1972), while other studies

showed normal fasting levels of insulin and glucose in premanifest and manifest HD with no implications to develop diabetes (Boesgaard et al., 2009, Popovic et al., 2004, Wang et al., 2014). Increased brain insulin levels has previously been observed, and is suggested to have compensatory effects to overcome systemic insulin resistance in R6/2 mice (White, 2014), and another study show that elevated insulin signaling in the brain could contribute to neurodegeneration in HD due to mitochondrial dysfunction and oxidative stress (Sadagurski et al., 2011).

Glucose is important for the production of energy, and a transient decline in the metabolism of glucose would cause a serious disruption of brain function (Han et al., 2016, Mergenthaler et al., 2013). Deficits in glycolytic pathways have been associated with HD and other neurodegenerative diseases, and are often related to brain pathogenesis (Camandola and Mattson, 2017, Mochel and Haller, 2011). In HD brains, the efficiency of converting glucose to energy in form of ATP is unchanged in a resting state (Powers et al., 2007), whereas during increased brain activity, the induction of energy producing pathways and increased ATP levels, does not occur or is suggested to be limited (Mochel et al., 2012, Lou et al., 2016). In presymptomatic HD, reduced glucose consumption in the basal ganglia has been found (Antonini et al., 1996, Grafton et al., 1992, Kuwert et al., 1993). Further support for an energy deficiency in HD brains are the findings of increased lactate concentrations in the basal ganglia and the occipital cortex (Jenkins et al., 1998, Jenkins et al., 1993), as well as an elevated lactate-to-pyruvate ratio in the CSF (Koroshetz et al., 1997).

In addition to brain energy deficits, skeletal muscle shows energetic disturbances with impaired function of PGC-1α in HD similar to those evident in HD striatal neurons (Gizatullina et al., 2006, Ribchester et al., 2004, Strand et al., 2005). A reduced phosphocreatine/inorganic phosphate ratio and low ATP levels in resting muscle of presymptomatic individuals and symptomatic HD patients has also been found (Lodi et al., 2000, Saft et al., 2005). Low anaerobic threshold and increased skeletal muscle lactate production during exercise has also been found in HD patients (Ciammola et al., 2011).

The liver has an important role in regulating whole-body energy metabolism (Rui, 2014). It removes toxins such as ammonia, thereby preventing damage to the brain, and is responsible for maintaining necessary plasma glucose levels (Rui, 2014). Hepatic mitochondrial dysfunction has also been reported in individuals with both pre-manifest and manifest HD (Stuwe et al., 2013, Hoffmann et al., 2014), and was found to be correlated with striatal atrophy, as well as functional and cognitive deficits (Stuwe et al., 2013). In R6/2 mice, reduced lactate clearance and hepatic glucose production has been found, which suggests that hepatic glucose production might be impaired (Josefsen et al., 2010).

The hypothalamus has been shown to be the major contributor to the metabolic abnormalities that take place in Huntington´s Disease (Hult et al., 2011). In addition to metabolic disturbances induced by peripheral hormones, neuroendocrine alterations in the hypothalamic-pituitary-adrenal (HPA), hypothalamic-pituitary-gonadal (HPG) and somatotropic axis are found in HD patients and HD mouse models (Bjorkqvist et al., 2006, Hult et al., 2010, Petersen and Bjorkqvist, 2006).

The HPA axis has been shown to be activated with increased cortisol levels as a result in both presymptomatic HD and symptomatic HD patients, which might contribute to muscle wasting, mood changes and cognitive deficits (Aziz et al., 2009, Bjorkqvist et al., 2008). An affected HPG axis has been found in HD patients as well as in HD mouse models, with decreased testosterone levels and deficits in spermatogenesis, and testicular abnormalities (Bird et al., 1976, Markianos et al., 2005, Van Raamsdonk et al., 2007). The somatotropic axis (GHRH/GH/IGF-1 axis), in which growth hormone dependent release of IGF-1 from the liver (Schneider et al., 2003) regulates energy metabolism by regulating protein, carbohydrate and lipid metabolism together with enhancing insulin sensitivity (Lewitt et al., 2014), has been found to be altered in HD patients. However, contradictory results of either increased or unchanged levels of growth hormone (GH); either decreased, increased or unchanged levels of IGF-1 have been found, and further studies are therefore needed (Aziz et al., 2010, Chalmers et al., 1978, Mochel et al., 2007, Phillipson and Bird, 1976, Popovic et al., 2004, Saleh et al., 2009).

Above described findings highlight the importance of considering HD as a whole-body disorder, involving metabolic disturbances. Alterations in energy metabolism contribute to the progression of the disease, which is reflected in the progressive weight loss seen in HD patients as well as HD mouse models. Most importantly, these alterations can also be traced in the periphery.

Further understanding of underlying pathogenic mechanisms involving energy deficits would open up for targeting systemic energy metabolism. This could possibly have beneficial effects on the onset and progression of the disease.

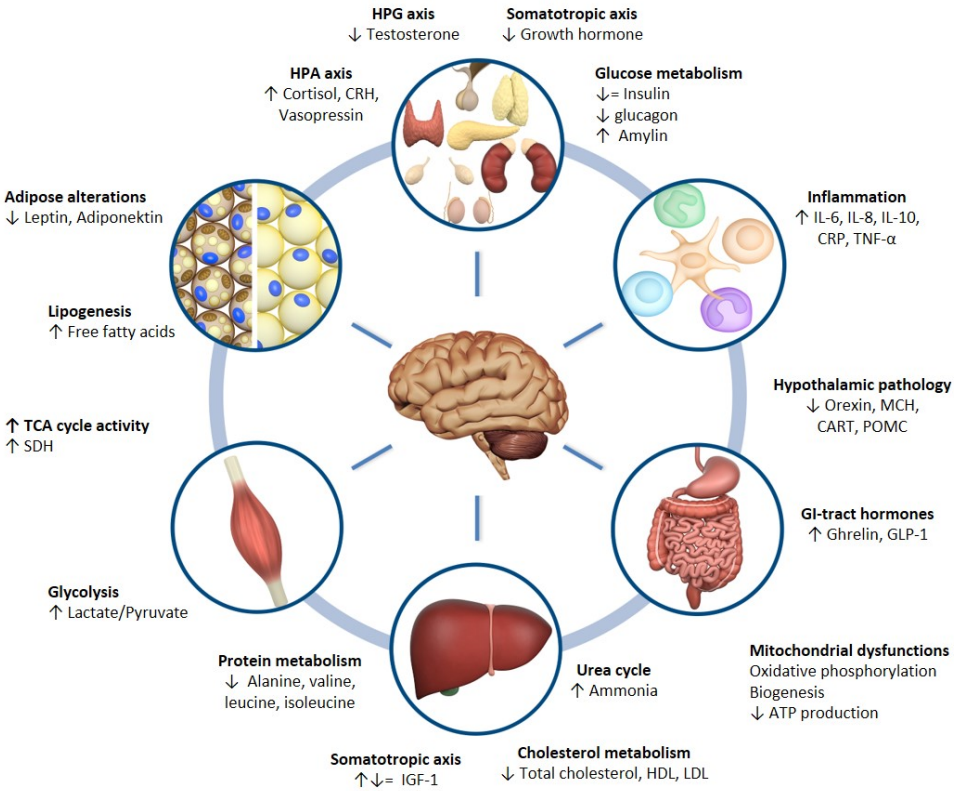

Figure 4. Alterations in central and peripheral energy metabolism in Huntington´s disease.
Energy metabolism homeostasis involves the interaction between CNS and peripheral tissues and organs. Key metabolic processes within each tissue and organ, contributing to lipid, protein and carbohydrate metabolism, as well as energy in the form of ATP, are also involved in keeping a balanced energy metabolism. Metabolic disturbances in both brain and periphery are observed in HD patients and mouse models, contributing to an increased energy expenditure and weight loss, as well as alterations in the brain and periphery. Neuroendocrine alterations is a result of changes in the HPA, HPG and somatotropic axis. Mitochondrial dysfunction, such as impaired oxidative phosphorylation and biogenesis, as well as TCA dysfunction, resulting in energy deficits in the whole body, is also evident in HD. (Carroll et al., 2015, van der Burg et al., 2009, van der Burg et al., 2011)

Tools to affect energy metabolism in HD

Effective cures and disease-modifying treatments are currently not available for HD. Only symptomatic treatments for chorea and dyskinesia have been approved for clinical use by U.S. Food and Drug Administration (FDA). Other promising treatments are at the moment in clinical Phases I–III, and some treatments exert beneficial effects in HD mouse models (Kumar et al., 2015).
HD has traditionally been considered a movement disorder associated with degeneration of striatum and cortex (Graveland et al., 1985, Vonsattel et al., 1985).

However, more attention on the disturbed energy metabolism involving peripheral tissues has been seen over the years. HD has evolved to be considered a metabolic disorder involving energy deficits both in the CNS and in the periphery. Targeting whole-body energy metabolism might therefore have beneficial effects on disease progression, but also increase the quality of life.

The liver is for example an important organ involved in energy homeostasis, by removing toxins and regulates glucose homeostasis (Rui, 2014), which has been shown to be dysregulated in HD (Chiang et al., 2011). Deficits in toxin clearance by the liver have been noted in HD patients and in mouse models of HD, especially ammonia and citrulline (Chiang et al., 2011). Low protein diets has been shown to correct urea cycle deficiencies in HD mice, ameliorated the formation of mHTT aggregates and motor deficits, and showed increased BDNF expression and normalized chaperones as well as C/EBP-alpha's activity (Chiang et al., 2007).

Energy deficit due to an abnormal mitochondrial function is a key feature seen in both central and peripheral tissues in HD (Mochel and Haller, 2011). Especially the expression of PGC-1α, the transcriptional coactivator regulating mitochondrial biogenesis and respiration, has been found to be downregulated in particularly striatal neurons (Cui et al., 2006), but also peripheral tissues such as skeletal muscle and adipose tissue (Chaturvedi et al., 2009, Weydt et al., 2006). Targeting mitochondrial dysfunction by improving the function of PPARγ or overexpressing PGC-1α, both centrally and systemically, might be a beneficial way to reverse the negatively balanced energy homeostasis seen in HD (Johri et al., 2011). Many of the drugs increasing mitochondrial function has currently been shown to have promising effects in mouse models of HD normalizing an energy metabolic homeostasis and increase body weight (Chaturvedi et al., 2009, Chiang et al., 2010, Cui et al., 2006, Johri et al., 2011). Different other approaches targeting mitochondrial dysfunction have been proposed. Creatine supplement, which has antioxidant properties and stimulates mitochondrial respiration, have been found to decrease the formation of mHTT aggregates, delay brain atrophy and improve survival in R6/2 mice. Beneficial effects on body weight and motor deficits, along with delayed onset of diabetes was also found (Ferrante et al., 2000). Although beneficial effects was seen in mouse models, Phase II trials showed that creatine monohydrate was not beneficial for slowing functional decline in early symptomatic HD patients (Hersch et al., 2017).

Circulating metabolites reflect energy metabolic processes, e.g protein synthesis and catabolism, and specific amino acids, specially branched-chain amino acid (BCAA), have been shown to be lower in HD patients (Underwood et al., 2006, Mochel et al., 2011, Mochel et al., 2007), and might occur to compensate energy deficits. Triheptanoin is a triglyceride made up of fatty acid chains used for the Krebs cycle (de Almeida Rabello Oliveira et al., 2008). In a clinical study, brain energy

metabolism as well as motor function was improved using triheptanoin oil's (Adanyeguh et al., 2015). Dietary anaplerotic therapy with triheptanoin, used in clinical trials, has been shown to improve peripheral tissue energy metabolism and in particular oxidative phosphorylation in skeletal muscle, in HD patients (Mochel et al., 2010).

Weight loss is the most prominent feature seen in HD contributing to general sickness as a result of increased metabolic rate and involvement of disturbed functions of peripheral tissues. Body weight has previously been shown to slow down HD progression in patients, with improvements in functional, motor and cognitive deficits (van der Burg et al., 2017). Effects of a higher BMI on energy metabolism, as well as central and peripheral pathology in mouse models of neurodegenerative disorders, e.g. Huntington´s disease (HD)(paper I)(Sjögren et al., 2019) and Amyotrophic lateral sclerosis (ALS)(Lim et al., 2014) have been studied. A genetic approach was used to place the R6/2 mouse model of HD (paper I)(Sjögren et al., 2019) and the SOD1 mouse model of ALS (Lim et al., 2014) on leptin-deficient background, by the crossing with the obese mouse model (Ob/Ob). In addition to obesity, Ob/Ob mice are leptin-deficient, hyper-insulinemic, transient hyperglycemic and hypometabolic (Garthwaite et al., 1980, Genuth et al., 1971, Mayer et al., 1953). Leptin-deficiency in both R6/2 mice (paper I)(Sjögren et al., 2019) and SOD1 mice (Lim et al., 2014) was efficient enough to attenuate abnormal energy expenditure and increase body weight, while beneficial effects on central pathology, motor deficits and survival, suggesting a slower rate of the disease progression, was only seen in SOD1 mice (Lim et al., 2014).

In the attempt to counteract weight loss and muscle atrophy seen in HD, oral nutritional supplement of 473 kcal/day was shown to stabilize body weight and muscle mass in HD patients (Trejo et al., 2005). However, no beneficial effects were seen on motor, cognitive or functional deficits (Trejo et al., 2005).
Another approach to target whole-body energy metabolism is to activate the somatotropic axis (growth hormone (GH) / growth hormone releasing hormone (GHRH) / growth hormone secretagogues) using GHS-R1a (ghrelin receptor) agonists, e.g ghrelin and analogues (Cordido et al., 2009). The somatotropic axis exert multi-organ effects, such as bone growth and skeletal muscle anabolism and body fat distribution (Schneider et al., 2003). Ghrelin, an orexigenic peptide hormone mainly produced in the stomach (Kojima et al., 1999), stimulate appetite (Inui et al., 2004), increase body weight and adiposity (Bianchi et al., 2016, Tschop et al., 2000). In R6/2 mice, ghrelin administration was shown to rescue the catabolic profile seen in skeletal muscle, characterized by altered mRNA expression and atrophic morphology, as well as cognitive behavior deficits (Sjögren et al., 2017). GHS-R1a agonists have been shown to exert beneficial effects for many clinical problems, including muscle wasting, cancer cachexia, cognitive decline, diabetes and metabolic disorders (Reano et al., 2014, Ali et al., 2013b, McLarnon, 2012). In

addition, ghrelin analogues exert beneficial effects on brain function and cognition, increasing neuronal survival by reducing apoptosis (Li et al., 2011).

For control of glucose energy metabolism, GLP-1 analogues has been used, e.g liraglutide and exendin-4, which has an ability to decrease blood glucose levels by slowing down gastric emptying, and to enhance pancreatic insulin secretion (Martin et al., 2009). In R6/2 mice, a combination of liraglutide together with the orexigenic gut hormone ghrelin has been found to improve both systemic and brain energy metabolism (paper III)(Duarte et al., 2018).

Taken together, targeting whole-body energy metabolism may exert a beneficial effect on HD disease progression.

Pathology outside the brain

Skeletal muscle wasting

Skeletal muscle comprises around 40% of our body weight and contains between 50-75% of all body proteins (Frontera and Ochala, 2015). It allows us to perform daily activities such as physical movement and posture, and is important for vital functions such as chewing, swallowing, breathing and blood pumping (Chromiak and Antonio, 2008). Muscle mass is regulated by the balance between protein synthesis and degradation, which are sensitive to factors such as nutritional status, hormonal balance, physical activity and exercise, as well as injury and disease (Frontera and Ochala, 2015).

From a metabolic point of view, the role of skeletal muscle is to serve as storage for important substrates such as amino acids and carbohydrates, to produce heat for the maintenance of core temperature, as well as to consume oxygen and fuel during exercise (Wolfe, 2006). Metabolism of carbohydrates and fats are the two main fuels utilized by muscle cells to produce energy in the form of ATP (Romijn et al., 1993), while amino acid metabolites may contribute to a small percentage of the total energy production (Frontera and Ochala, 2015). Amino acids released from muscle are needed for the maintenance of blood glucose levels during starvation, and this release might be impaired in reduced muscle mass (Owen et al., 1998).

Skeletal muscle wasting, accompanied with gene expression alterations as well as reduced muscle mass and strength are prominent features in both HD patients and HD mouse models, progressing with severity of the disease (Busse et al., 2008, Chaturvedi et al., 2009, Farrer and Meaney, 1985, Magnusson-Lind et al., 2014, Ribchester et al., 2004, She et al., 2011, Strand et al., 2005, Trejo et al., 2004). Mechanisms underlying muscle wasting is still unclear, but it might be a direct effect

of the presence of mHTT inclusions in myocytes, shown both in in vivo and in vitro studies (Ciammola et al., 2006, Moffitt et al., 2009, Orth et al., 2003, Sathasivam et al., 1999). Myocytes cultured from premanifest and symptomatic HD patients exhibit abnormalities in myofiber morphology, enlarged mitochondria with abnormal cristae, cell differentiation defects, and apoptosis (Arenas et al., 1998, Ciammola et al., 2006).

Mutant HTT present in myocytes contributes to many different pathological findings, often in parallel to brain pathology, such as mitochondrial dysfunction (Panov et al., 2002, Parker et al., 1990, Saft et al., 2005), transcriptional dysregulation (Jones and Hughes, 2011, Strand et al., 2005) and caspase activation (Ehrnhoefer et al., 2014, She et al., 2011). Repressing the expression of PGC-1α, a transcriptional coactivator of metabolic genes, e.g. mitochondrial biogenesis and respiration, causes disruption of mitochondrial function leading to reduced ATP production (Arenas et al., 1998, Chaturvedi et al., 2009, Lodi et al., 2000, Kosinski et al., 2007, Saft et al., 2005, Turner et al., 1991). Mild myopathy with mitochondrial pathology has previously been described as a first symptom of HD in a professional marathon runner Marathon runner at risk for HD (Kosinski et al., 2007).

Alongside mitochondrial dysfunction, transcriptional dysregulation is also evident, contributing to skeletal muscle pathology in HD patients and HD mouse models (Jones and Hughes, 2011, Strand et al., 2005). Gene expression alterations observed in muscle cells in vivo and vitro are comparable to the those seen in neurons (Luthi-Carter et al., 2002), with increased expression of genes encoding for chaperone proteins, and proteins involved in the ubiquitin-proteasome system, as well as muscle-specific mRNAs (Strand et al., 2007, Luthi-Carter et al., 2002). Activation of pathways involved in apoptosis and autophagy (She et al., 2011, Magnusson-Lind et al., 2014) suggests that these mechanisms may contribute to the catabolic phenotype and muscle wasting in HD muscle. Alterations in gene expression seen in HD muscle have been shown to reflect disease progression, and could therefore be used as a valuable biomarker of the disease (Strand et al., 2005).

Presence of mHTT in HD muscle may also affect contractility and muscle strength as a result of declined resting membrane potential (Ribchester et al., 2004), and by hyper-excitability because of chloride and potassium channel dysfunction (Waters et al., 2013). These dysfunctions causes involuntary and prolonged contractions (Waters et al., 2013) that may contribute to the chorea, rigidity, and dystonia characterizing Huntington disease (Bateup et al., 2010, Graveland et al., 1985, Menalled et al., 2000, Sapp et al., 1995).

Pathogenic mechanisms seen in parallel in skeletal muscle and brain suggests that by increasing understanding of molecular dysfunctions in muscle might even provide insight in mechanisms involved in neurodegeneration in HD (Luthi-Carter et al., 2002).

Adipose tissue alterations

Adipose tissue, comprised mainly of adipocytes, plays a central role in regulating whole-body energy and glucose homeostasis (Luo and Liu, 2016, Rosen and Spiegelman, 2006). In addition to adipocytes, adipose tissue also contains pre-adipocytes, immune cells, pericytes and endothelial cells, and fibroblasts (Kershaw and Flier, 2004, Sethi and Vidal-Puig, 2007). Adipose tissue can be classified into two subtypes: white adipose tissue (WAT) and brown adipose tissue (BAT), but another type of WAT called beige or brown-like in white (brite) adipose tissue has also been found, and is stimulated by cold stress (Barbatelli et al., 2010, Bostrom et al., 2012, Petrovic et al., 2010).

WAT mainly consist of white adipocytes with variable size containing one unilocular lipid droplet, and have few mitochondria (Fasshauer and Bluher, 2015). The main function of WAT is to store energy in form of triglycerides (TG) and subsequently release energy in form of glycerol and free fatty acids through metabolic processes, e.g. lipogenesis and lipolysis, respectively (Fasshauer and Bluher, 2015, Luo and Liu, 2016, Rosen and Spiegelman, 2014, Rutkowski et al., 2015). Lipolysis is induced by several hormones, e.g. glucagon, epinephrine, norepinephrine, growth hormone, atrial natriuretic peptide, brain natriuretic peptide, and cortisol (Duncan et al., 2007, Nielsen et al., 2014). In HD mice, norepinephrine induced lipolysis has been found to be dysregulated, indication of an altered functional adipocyte capacity (Fain et al., 2001). Glycolysis is the process in which glucose is converted into pyruvic acid, and energy is released in form of the high-energy compounds adenosine triphosphate (ATP) and the reduced nicotinamide adenine dinucleotide (NADH)(Rutkowski et al., 2015). Increased glycolytic rate has been observed in platelets derived from HD patients, resulting in increased lactate/pyruvate ratio and ATP levels (Ferreira et al., 2011). In addition to storing and releasing energy, WAT have a strong endocrine activity interacting with several other organs in the body, e.g. brain, liver, skeletal muscle and pancreas (Fasshauer and Bluher, 2015), by the expression and secretion of several hormones such as leptin, adiponectin, resistin, and cytokines (Kershaw and Flier, 2004). Under normal conditions, circulating leptin levels are proportional to the amount of fat mass (Harris, 2014). In HD mouse models, despite increased fat mass at early- and mid-stage of disease, decreased circulating leptin concentration has been found (Phan et al., 2009).

BAT, on the other hand, plays a role in energy metabolism and non-shivering thermogenesis in mammals (Bartelt and Heeren, 2014). Brown adipocytes are smaller than white adipocytes in size, have multilocular morphology, and are rich in mitochondria (Bartelt and Heeren, 2014, Cannon and Nedergaard, 2004). They express uncoupling protein 1 (Ucp1), a specific marker for BAT, located in the inner mitochondrial membrane, and is using lipids to generate heat by uncoupling electron

transport from the ATP production (Bartelt and Heeren, 2014). BAT also contains more capillaries than WAT, which supply the tissue with oxygen, nutrients and distribute the produced heat throughout the body (Orava et al., 2011, Zingaretti et al., 2009). BAT from HD mice show abnormal lipid-containing vacuoles, impairment in PGC-1α levels and UCP1 activation, as well as impairment in temperature regulation (Chaturvedi et al., 2010, Weydt et al., 2006).

Beige/brite adipose tissue is derived from the same lineage as WAT (Seale et al., 2008). Hyperplasia of brown/beige cells in WAT, often referred to as "browning" of WAT, has been shown to enhance energy expenditure (Barbatelli et al., 2010, Bartelt and Heeren, 2014). Similar to BAT, beige/brite adipocytes dissipate energy in form of heat and expresses Ucp1, although the expression is relatively low in individual beige/brite adipocytes and have low metabolic activity (Luo and Liu, 2016, Wu et al., 2012). In the R6/2 mouse model, signs of "browning" of WAT has been found, which has been associated with enhanced energy metabolism, and possibly contributing the weight loss seen in these mice (McCourt et al., 2016).

Adipose tissue studies have revealed the importance of both brown and beige/brite adipose tissue in control of body weight and might be a new way to battle obesity and associated metabolic disorders, e.g. hyperglycaemia, insulin resistance and dyslipidaemia (Boss and Farmer, 2012, Cannon and Nedergaard, 2004, Lowell and Spiegelman, 2000, Ravussin and Galgani, 2011, Timmons and Pedersen, 2009, Villarroya et al., 2018). The amount of brown/beige adipocytes has been shown to be inversely correlated to body weight (Bartelt and Heeren, 2014, Cypess et al., 2009). Activation of BAT with increased UCP1 expression have been found to increase energy expenditure, and thereby induce weight loss (Carpentier et al., 2018).

Adipogenesis is a process in which pre-adipocytes differentiate into mature adipocytes, and plays an important role in the development of adipocytes and regulation of systemic energy homeostasis (Ali et al., 2013a, Lefterova and Lazar, 2009). Peroxisome proliferator-activated receptor γ (PPARγ) has been shown to act as key regulator of adipogenesis, and deficiency in PPARγ signalling and downstream targets may lead to less amount of adipocytes and disturbances in metabolic processes (Hegele et al., 2002, Rosen et al., 2000, Tontonoz et al., 1994). In HD, white adipocytes dysfunctions and defective brown adipocytes have been found to progress with disease and is found in R6/2 mice and CAG140 mice (Phan et al., 2009), accompanied by an impairment in the expression of mature adipocyte genes (Fain et al., 2001, Phan et al., 2009). Gene expression alterations found in HD mouse models, have been replicated in an HD adipocyte cell line, indicating that it might be a direct effect of mHTT (Phan et al., 2009). In addition to an altered gene expression, the expression of mHTT was also shown to affect adipocyte functions leading to an inability of triglyceride storage (Phan et al., 2009).

Studies in HD adipocytes indicate that alterations of both fat mass and gene expression levels could contribute to an altered energy metabolism homeostasis with increased energy expenditure (Fain et al., 2001, McCourt et al., 2016, Phan et al., 2009).

HD model systems

The discovery of the gene responsible for HD in 1993 (Group, 1993) led to the generation of genetic models of the disease and an increased understanding of HD pathogenesis, but also the possibility to evaluate the potential of new therapeutics (Crook and Housman, 2011, Gil and Rego, 2009, Li et al., 2005, Switonski et al., 2012).

As early as the 1970s, animal models for HD was generated using the excitotoxin kainic acid to selectively destroy the striatal medium spiny neurons (Coyle and Schwarcz, 1976, Schwarcz and Coyle, 1977), the most vulnerable neurons in HD. A few years later a selective NMDA (N-methyl-D-aspartate) receptor agonist (quinolinic acid) was used since it better replicated the neuropathology seen in HD (Schwarcz et al., 1983).

Much of what is known today about HD pathogenesis arises from studying different model systems (Ross and Tabrizi, 2011). Several animal model are used, from invertebrate models such as Drosophila melanogaster (Jackson et al., 1998, Marsh et al., 2000) or Caenorhabditis elegans (Parker et al., 2001), vertebrates including mouse and rat models, to large mammalian models such as pigs (Baxa et al., 2013), sheep (Jacobsen et al., 2010), and non-human primates (Yang et al., 2008). Cell models of induced pluripotent stem cells are derived from patients with HD (Park et al., 2008).

The majority of this thesis work focus on mouse models, which can be divided into N-terminal transgenic, full-length transgenic and knock-in mouse models (shown in Table 1).

Transgenic mouse models

N-terminal

N-terminal transgenic models are among the first HD mouse models generated, including R6/1, R6/2 and N171-82Q. R6/1 and R6/2 mice express human exon 1 fragment containing 5´end of the huntingtin gene, with originally 115 to 150 CAG repeats, respectively, under the human HTT promoter (Mangiarini et al., 1996). N171-82Q mice express a 171 amino acid mutant HTT fragment with 82 CAG

repeats under the regulation of the mouse prion promoter, which directs expression primarily in the brain (Schilling et al., 1999). It has been shown that the expression of mutant exon 1 HTT is sufficient to produce HD related phenotypes (Mangiarini et al., 1996). Like human HD (Duyao et al., 1993), both somatic and germ line instability of the CAG repeat tract has been observed in R6/2 mice, where a CAG repeat expansion >400 CAGs has been reported (Dragatsis et al., 2009, Gonitel et al., 2008, Morton et al., 2009).

The R6/2 mouse model is the most widely used with a progressive phenotype, mimicking human features. The phenotypes appear early (Carter et al., 1999), including motor, cognitive and behavioral dysfunctions, weight loss as well as a reduction in lifespan (Mangiarini et al., 1996). Weight loss is the most prominent phenotype progressing with disease, which might be a result of the hyper-catabolic state seen in R6/2 mice. Earliest motor deficits in R6/2 mice have been detected at 4.5 weeks of age with hypoactivity and reduced grip strength (Hickey et al., 2005), whereas onset of overt motor deficits appears at 7-8 weeks of age, which increases until the animals premature death around 16 weeks of age (Carter et al., 1999, Lione et al., 1999, Murphy et al., 2000). R6/2 mice also exhibit other phenotypes such as changes in the circadian rhythm, hind limb clasping, tremor, involuntary movement and seizures (Li et al., 2005, Mangiarini et al., 1996, Morton et al., 2005). Cognitive deficits appear prior to motor deficits in the R6/2 mouse model, reminiscent of HD patients, involving learning difficulties, deficits in spatial learning and impairment in sensorimotor gaiting (Aron et al., 2003, Murphy et al., 2000, Swerdlow et al., 1995). R6/2 mice exhibit brain atrophy with approximately 20% brain weight loss at 12 weeks of age (Li et al., 2005). The presence of nuclear inclusions and neuropil aggregates is also presents in the R6/2 brain, which can be detected by antibodies against ubiquitin or the n-terminal region of mHTT (Davies et al., 1997, Gutekunst et al., 1999, Li et al., 1999). The aggregation in R6/2 brains are more widespread compared to HD patients (DiFiglia et al., 1997, Gutekunst et al., 1999). Gene expression alterations in brain in parallel with peripheral tissue, e.g. skeletal muscle are found in this HD mouse model (Luthi-Carter et al., 2002). R6/2 and N171-82Q transgenic mice exhibit increased metabolic rate with elevated ghrelin levels and decreased leptin levels (Goodman et al., 2008, Martin et al., 2009, Phan et al., 2009, Underwood et al., 2006). In reminiscence to human HD, these mice also exhibit impaired glucose homeostasis with increased glucose levels accompanied with insulin resistance at early stage (Andreassen et al., 2002, Bjorkqvist et al., 2006, Hurlbert et al., 1999, Luesse et al., 2001, Martin et al., 2012, Martin et al., 2009, Weydt et al., 2006).

N171-82Q start to exhibit motor deficits around 11-12 weeks of age, involving hypo-activity, abnormal gait, and clasping, as well as tremors, which progresses until their premature death around 5-6 months of age (Andreassen et al., 2001, Schilling and Yeh, 1999, Schilling et al., 2004b). N171-82Q mice exhibit several

pathological phenotypes mimicking clinical HD (Yu et al., 2003). Brain weight loss starts at around 120 day of age (Andreassen et al., 2001, Hersch and Ferrante, 2004), with striatal volume and neuronal atrophy (Gardian et al., 2005). Neuropathological findings in N171-82Q is reminiscent of findings in HD patients, with specific localization of mHTT inclusions in striatum and cortex, and neuronal degeneration in these regions can be detected at 4-5 months of age (Yu et al., 2003). Similar to the R6/2 mouse model, N171-82Q exhibit a progressing body weight loss, starting around 90 days of age, and a shorter life span with an average survival of around 130 days (Schilling et al., 2004a), as well as hyperglycemia around 80 days of age (Andreassen et al., 2001).

Full-length

HD models expressing the full-length HTT transgene in either yeast or bacterial artificial chromosome; YAC or BAC, respectively, are also available (Gray et al., 2008, Hodgson et al., 1999, Pouladi et al., 2010). YAC and BAC mouse models do not fully recapitulate all HD features since they are resistant to germline and somatic instability (Gray et al., 2008), which is observed in the HD population (Kennedy et al., 2003). This might be a result of the mixed CAGCAA repeat in BACHD and a result of the nine interspersed CAA codons for YAC128, within the CAG repeat (Gray et al., 2008, Kennedy et al., 2003).

Unlike clinical HD and N-terminal mouse models, increased body weight is observed in YAC or BAC mouse models (Gray et al., 2008, Pouladi et al., 2012, Van Raamsdonk et al., 2006), which has been shown to reflect overexpression of full-length human HTT (Pouladi et al., 2013, Van Raamsdonk et al., 2006).

In YAC128, hyperactivity is found at 2 months of age and hypoactivity around 8-12 months of age. Motoric deficits start around 4 months and are more profound 2 months later (Graham et al., 2006, Van Raamsdonk et al., 2005b), whereas the cognitive deficits found in this model, e.g. motor learning and reversal learning deficit starts around 2 months of age (Van Raamsdonk et al., 2005c). In reminiscent to human HD, selective localization of mHTT aggregates are found in the striatum and the cortex (Van Raamsdonk et al., 2005a). Aggregation of mHTT in the striatum starts at 1-2 months of age, and at 3 months the aggregation is more profound in the striatum and is also present in the cortex and hippocampus (Van Raamsdonk et al., 2005a). Intranuclear mHTT inclusions in the striatum are not present until 18 months of age in YAC128 mice (Van Raamsdonk et al., 2005a). Loss of striatal volume are first detected around 9 months of age, which is associated with the loss of striatal neurons, and around 12 months cortical volume is also decreased (Van Raamsdonk et al., 2005a).

Like the YAC128 mouse model, BACHD mice show selective atrophy in the striatum and cortex along with progressive motor deficits, and recapitulate to some

extent the regional selectivity of adult-onset HD (Gray et al., 2008). Unlike other full-length HD mouse models, BACHD mice does not express early nuclear mHTT inclusions in striatum or cortex. Instead, large neuropil mHTT aggregates are present in cortex while few small aggregates are located in the striatum at 12 months of age (DiFiglia et al., 1997, Gutekunst et al., 1999).

Cognitive and psychiatric behavioral dysfunctions are found in BACHD mice from 2 and 6 months, respectively, which progresses with disease (Gray et al., 2008, Holmes et al., 2002, Menalled et al., 2009).

Knock-In mouse models

In knock-in mouse models, a specific number of CAG repeats are introduced into exon 1 of the mouse Htt gene. This mouse model can be either heterozygote or homozygote, making them in this sense similar to human HD. Typical knock-in mouse models include the CAG140, HdhQ150 and zQ175 models (Menalled et al., 2012). Like N-terminal transgenic mouse models, knock-in mice display CAG repeat instability, which therefore must be closely monitored to prevent the CAG repeat drift (Menalled et al., 2012).

In CAG140 mice, hyperactivity is seen around 1 month of age, which is followed by hypoactivity around 4 months of age, and around 12 months of age these mice exhibit gait abnormalities (Menalled et al., 2003). Neuropathologically, microaggregates of mHTT is present in striatal and cortical neurons. Intranuclear inclusions are present in the striatum at 4 months of age, whereas they appear in the cortex around 6 months of age. Neuropil microaggregates are also spread throughout the brain from around 2 months of age. Although this widespread aggregate formation, cell loss or brain atrophy has not been reported (Menalled and Chesselet, 2002).

HdhQ150 mice exhibit slowly progressive motor abnormalities such as hypoactivity, gait and balance abnormalities, as well as non-progressive grip strength deficits (Heng et al., 2007, Woodman et al., 2007). Striatal and cortical mHTT aggregates can be detected in HdhQ150 around 6 months of age, whereas a more widespread neuropathology is evident at 10 months of age (Tallaksen-Greene et al., 2005). Reminiscent to human HD, HdhQ150 mice exhibit reduced ligand-binding to striatal D1 and D2 dopamine receptor, and dopamine transporter (Dat), as well as significant reduction of striatal volume and neurons (Heng et al., 2007). HdhQ150 mice, hetero- and homozygote, exhibit progressive weight loss but do not have shortened lifespan (Heng et al., 2007, Woodman et al., 2007). Similar to 12 weeks old R6/2 mice, HdhQ150 mice at 22 months of age exhibit gene expression changes in the brain, involving chaperones, which was also seen at protein levels (Woodman et al., 2007).

In homozygote zQ175 mice, earliest motor deficits are found already at 4 weeks of age and progresses throughout the disease, whereas cognitive dysfunctions are found at 1 year. In heterozygote zQ175 mice, however, these alterations are found around 4.5 months of age, and especially during the dark phase of the diurnal cycle. A progressive weight loss is seen in both hetero- and homozygote zQ175 mice, whereas the survival is reduced only in zQ175 mice with a homozygote genetic background (Menalled et al., 2012, Peng et al., 2016). In zQ175 mice, mHTT aggregates has been found to be widely distributed in the brain, and that these aggregates increase with age in both the striatum and cortex (Peng et al., 2016). From 12 weeks of age, zQ175 mice exhibit decreased expression of typical striatal gene markers (Menalled et al., 2012). Brain atrophy and altered brain metabolites are also observed in the zQ175 mouse model, with a significant decrease in striatal and cortical brain volume starting around 8 months of age (Heikkinen et al., 2012).

Metabolic disturbances in both central and peripheral HD pathology have been the main focus in this thesis. Therefore, the N-terminal transgenic R6/2 mouse model has been utilized in studies on weight loss, glucose homeostasis and muscle atrophy.

Table 1. Summary of HD mouse models.

CONSTRUCT	R6/2	N171-82Q	YAC128	BACHD	HdhQ150	CAG140	zQ175
Model	Transgenic	Transgenic	Transgenic	Transgenic	Knock-in	Knock-in	Knock-in
Promoter	Human *HTT*	Murine prion	Human *HTT*	Human *HTT*	Murine Hdh	Murine Hdh	Murine Hdh
Original PolyQ repeat	144 CAG	82 CAG	128 CAA/CAG	97 CAA/CAG	150 CAG	140 CAG	188 CAG
mHTT expression	75%	20%	75%	150%	100%	100%	100%
CAG repeat stability	Unstable	Unstable	Stable	Stable	Unstable	Unstable	Stable
NEUROPATHOLOGY	Nonselective	Selective	Selective	Selective	Selective	Selective	Selective
mHTT inclusions/aggregates	4-8 weeks	16-20 weeks	12 weeks	52 weeks	20-22 weeks	16–24 weeks	8-16 weeks
mHTT neuropils	12 weeks			52 weeks			
Neuronal loss	12 weeks	16-20 weeks	1-3 months	52 weeks	100 weeks		32 weeks
Brain atrophy	12 weeks	16-20 weeks	36 weeks	52 weeks	100 weeks		32 weeks
METABOLIC PHENOTYPES							
Body weight	Loss	Loss	Gain	Gain	Loss	Loss	Loss
Muscle wasting	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hyperglycemia	8 weeks	12-13 weeks		+			
Insulin resistance	8 weeks	12-13 weeks		+			
Motor impairements	4-8 weeks	11-12 weeks	16-17 weeks	8-9 weeks	24-30 weeks	16 weeks	4 weeks
Cognitive deficits	4 weeks		8-9 weeks	8-9 weeks	24 weeks	39 weeks	52 weeks
Study durationl	12-14 weeks	24 weeks	52 weeks	52 weeks	100 weeks	30 weeks	90 weeks
References	Hickey et al., 2005, Mangiarini et al., 1996, Menalled et al., 2009	Andreassen et al., 2001, Schilling et al., 1999	Slow et al., 2003, Van Rammsdonk et al., 2007	Gray et al., 2008	Heng et al., 2007, Lin et al., 2001, Woodman et al., 2007	Menalled et al., 2003	Heikkinen et al., 2012, Menalled et al., 2012

Aims of the thesis

The aim of this thesis was to investigate whether targeting energy metabolism in the R6/2 mouse model of HD could ameliorate phenotype alterations, but also the mechanisms behind these alterations. The specific aims were as follows:

Paper I

Is it possible to reverse the hyper-catabolic state and prevent weight loss in leptin-deficient R6/2 mice? Does higher BMI have a beneficial effect on central and peripheral pathology, generating a slower disease progression?

Paper II

Does ghrelin treatment have beneficial effects on metabolic aspects in R6/2 mouse model of Huntington´s Disease?

Paper III

Is it possible to normalize energy metabolism in periphery and brain using administration of liraglutide and ghrelin in R6/2 mice?

Paper IV

Does mutant HTT affect myogenic processes of cultured satellite cells from R6/2 mice? Is it possible to promote increased proliferation and differentiation processes using ghrelin administration?

Summary of key results

Paper I: Leptin deficiency reverses high metabolic state and weight loss without affecting central pathology in the R6/2 mouse model of Huntington´s disease

In paper I, a genetic approach was used to generate a leptin-deficient R6/2 mouse model (R6/2;Ob/Ob), by crossing the transgenic R6/2 mouse model with the leptin-deficient mouse model (Ob/Ob). The CAG-repeat lengths in the R6/2 mice from two different strains used in this study ranged either between 345-352 or 242-257. The aim in this study was to assess whether leptin-deficiency could reverse the high metabolic state and thereby prevent weight loss and affect central pathology seen in R6/2 mice.

Leptin-deficiency in R6/2 mice leads to decreased energy expenditure, increased body weight and fat mass

In line with previous findings, significantly lower body weight was found in male R6/2$^{(CAG\ 345\text{-}352)}$ mice at 14.5 weeks of age **(Figure 5A)**, and in female R6/2$^{(CAG\ 345\text{-}352)}$ mice at 14 weeks of age (*data shown in the paper*) compared to WT littermates. Similarly, significantly lower body weight was seen in male R6/2$^{(CAG\ 242\text{-}257)}$ mice at 9.5 weeks **(Figure 5B)**, and at 12 weeks of age in female R6/2$^{(CAG\ 242\text{-}257)}$ mice (*data shown in the paper*) compared to WT mice. Male and female leptin-deficient R6/2 mice with CAG repeat lengths 345-352 and 242-257 had a dramatically increased body weight compared to R6/2 and WT littermates throughout the study **(Figure 5A and 5B)**.

An altered body composition has previously been shown in R6/2 mice (She et al., 2011). Therefore, we assessed body composition using a DEXA. Ob/Ob mice as well as R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice exhibited dramatically increased fat mass compared to both WT and R6/2$^{(CAG\ 345\text{-}352)}$ mice **(Figure 5C)**. Although fat mass was increased in R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice to R6/2$^{(CAG\ 345\text{-}352)}$ mice and WT mice, male R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice demonstrated significantly lower fat mass compared to Ob/Ob mice **(Figure 5C)**.

Body weight loss has been associated with high oxygen consumption in R6/2 mice (van der Burg et al., 2008). We therefore evaluated whether leptin-deficiency could restore the high energy expenditure state seen in R6/2$^{(CAG\ 345\text{-}352)}$ mice, utilizing the Pheno-master system. Here in this study we found that R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob males exhibit a significant and profound reduction in respiratory exchange rate compared to R6/2$^{(CAG\ 345\text{-}352)}$ littermates **(Figure 5D)**. The difference in respiratory exchange rate could not be explained by altered food or water consumption, as this was unaltered across the four groups assessed (*data shown in the paper*).

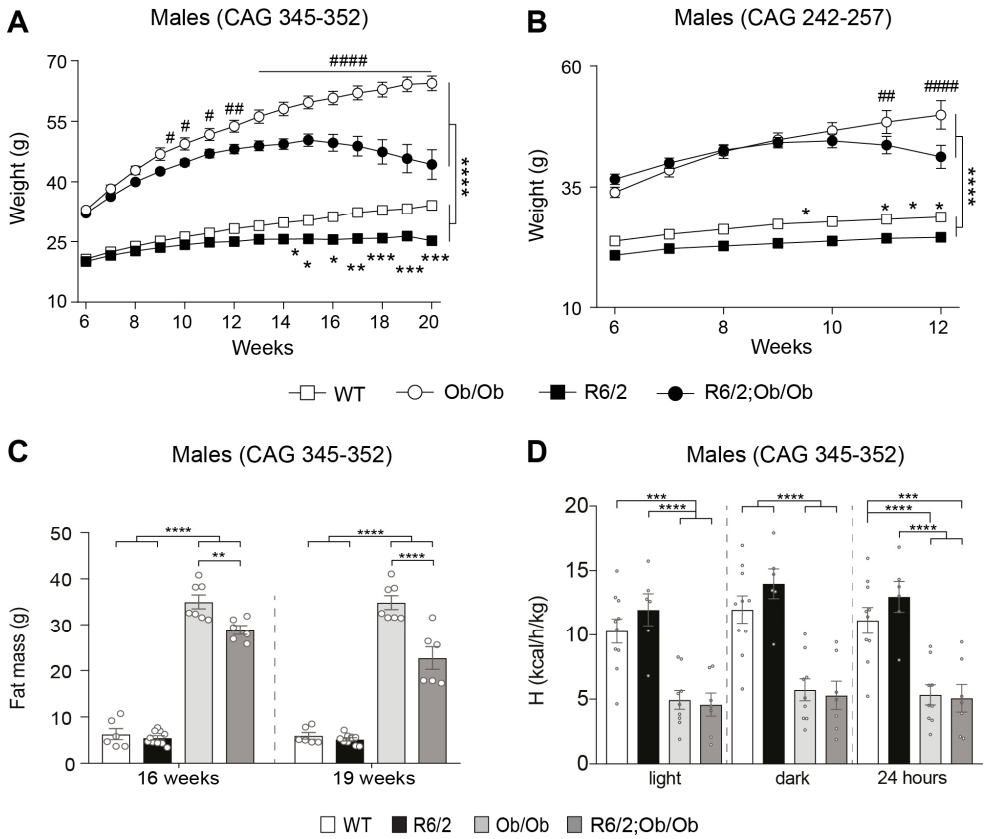

Figure 5. Leptin-deficient R6/2 mice exhibit decreased respiratory exchange rate, which rescues body weight loss and increase fat mass. Body weight was weekly monitored up to 20 weeks in the R6/2$^{(CAG\ 345\text{-}352)}$ cohort **(A)** and up to 12 weeks in the R6/2$^{(CAG\ 242\text{-}257)}$ cohort **(B)**. Significantly lower body weight was seen in male R6/2$^{(CAG\ 345–352)}$ mice at 14.5 weeks of age **(A)**, and in male R6/2$^{(CAG\ 242\text{-}257)}$ mice at 9.5 weeks of age **(B)** compared to WT littermates. R6/2 mice from both strains on a leptin-deficient genetic background have a significantly higher body weight compared to R6/2 littermates **(A, B)**. Assessment of body fat composition using DEXA scan in males at 16 and 19 weeks **(C)**. There was a significant increase in fat mass in R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob males **(C)** compared to R6/2$^{(CAG\ 345\text{-}352)}$ littermates. Indirect Gas Calorimetry was measured over 24 hours using the PhenoMaster/ LabMaster Home cage System in males group at 15 weeks of age. R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob males exhibit reduced energy expenditure during light and dark phase **(D)** compared to R6/2$^{(CAG\ 345\text{-}352)}$ littermates.

R6/2$^{(CAG\ 345\text{-}352)}$ mouse leptin-deficiency is not sufficient to improve neuropathological measures

Neuropathological changes of the striatum and cortex are seen in both HD patients and mouse models of HD (Graveland et al., 1985, Vonsattel et al., 1985). One of the hallmarks of the HD is inclusion formation and it is primarily associated with cell death (Davies et al., 1997, DiFiglia et al., 1997). Here in this study we evaluated the effect of leptin-deficiency and higher body weight on central pathological features in R6/2 and R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice.

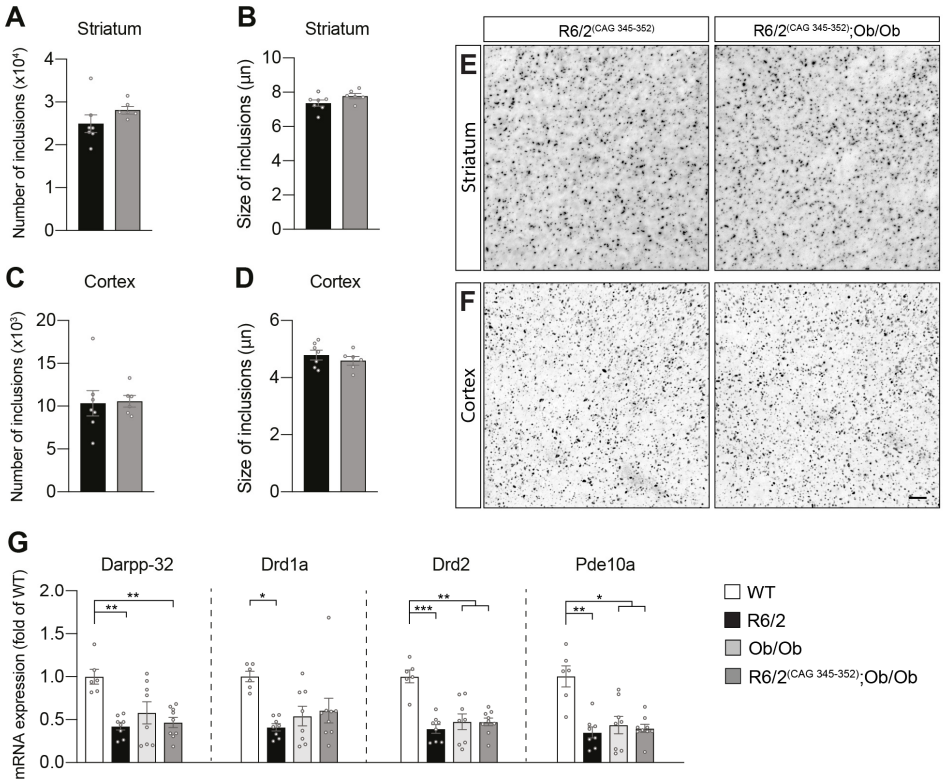

Figure 6. Leptin-deficiency and increased body weight have no effect on striatal mRNA dysregulation in R6/2 mice. The number and size of inclusions was assessed in R6/2 and R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice on EM48 (anti-HTT) strained brain sections. The number **(A)** and the size **(B)** of EM48 positive inclusions in the striatum. Assessment of number **(C)** and size **(D)** of inclusions in the cortex. Representative images illustrate HTT inclusions in the striatum **(E)** and cortex **(F)** of R6/2 and the R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice. No effect was found on striatal or cortical inclusion formation **(A-F)**. Scale bar represents 50 µm. Striatum mRNA levels of *Darpp-32*, *Drd1a*, *Drd2* and *Pde10a* in 20 weeks old mice **(G)**. The downregulation of striatal gene expression levels was not ameliorated R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice **(G)**.

The R6/2 mouse model featured these HD intranuclear and neuropil inclusions appearing earliest in the cortex and striatum (Meade et al., 2002). Therefore, number and size of inclusions were assessed in in R6/2 and R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice in both brain areas. There was no effect on the cortical and striatal inclusion formation **(Figure 6A-F)**.

Transcriptional dysregulation has previously been shown in HD mouse models (Desplats et al., 2006, Menalled et al., 2014). To further investigate the influence of increased body weight on R6/2 neuropathology, we assessed mRNA levels of well-characterized transcriptionally dysregulated genes in HD striata (Desplats et al., 2006, Menalled et al., 2014). Downregulation of striatal *Darpp-32*, *Drd1a*, *Drd2*, and *Pda10a* mRNA levels was not ameliorated in R6/2$^{(CAG\ 345\text{-}352)}$;Ob/Ob mice **(Figure 6G)**.

Paper II: Ghrelin rescues skeletal muscle catabolic profile in the R6/2 mouse model of Huntington's disease

In paper II, we targeted energy metabolism in R6/2 mice using ghrelin administration (subcutaneous 150 μg/kg daily injections) for 2, 4 or 6 weeks. The main aim, presented in this thesis, was to evaluate the effect of ghrelin administration on muscle atrophy and behavioral deficits seen in R6/2 mice.

Ghrelin reverse catabolic profile in R6/2 skeletal muscle

Muscle atrophy with altered gene expression is a prominent feature in HD (Ribchester et al., 2004, Trejo et al., 2004, Strand et al., 2005, Magnusson-Lind et al., 2014). Therefore, we evaluated gene expression changes related to muscle damage and cachexia in skeletal muscle (gastrocnemius) and studied the effect of 2 and 4 weeks of ghrelin administration **(Figure 7A and 7B)**. An increased expression in apoptotic (*Caspase 3* and *Caspase 8*) and NFκ-B (*Smad3* and *Traf 5*) pathways was found in R6/2 skeletal muscle compared to WT littermates. Also, increased expression of *Creb 1*, which is activated during muscle injury (Stewart et al., 2011), was found. Notably, already after 2 weeks of ghrelin treatment, normalized skeletal muscle mRNA expression of *Caspase 8*, *Traf 5*, and *Creb 1* was found in R6/2 mice compared to WT mice **(Figure 7B)**.

Alongside gene expression alterations seen in R6/2 skeletal muscle (Magnusson-Lind et al., 2014, Strand et al., 2005), possible skeletal muscle morphological alterations were evaluated in hematoxylin-eosin stained femoris skeletal muscle. R6/2 mouse skeletal muscle exhibits signs of atrophy compared to WT littermates

(Figure 7D). Morphological changes are illustrated by convoluted shaped fibers and signs of non-muscular infiltration **(Fig 7D)**. Ghrelin administration for 4 weeks normalized R6/2 skeletal muscle morphology **(Figure 7E)**. For the assessment of morphological changes seen in R6/2 muscle, a morphological score from 0 to 4 was given for shape and grade of non-muscular tissue infiltration. We found that R6/2 mice exhibit significantly higher score compared to WT mice, which was decreased to score levels comparable to WT mice after ghrelin administration **(Figure 7F)**.

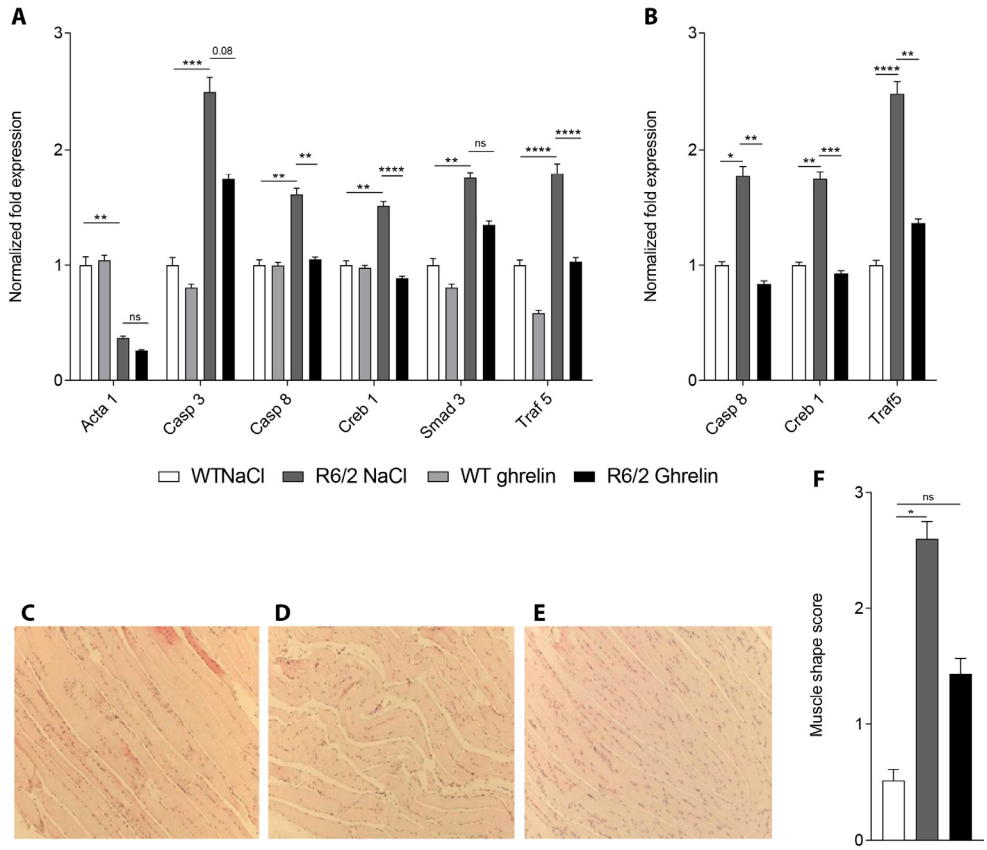

Figure 7. Ghrelin reverse the catabolic profile in R6/2 skeletal muscle. Normalized skeletal muscle (gastrocnemius) gene expression related to muscle contractility and atrophy after 2 and 4 weeks of ghrelin administration **(A and B)**. Increased expression of *Casp 3*, *Casp 8*, *Creb 1*, *Smad 3* and *Traf 5* were found in R6/2 muscle compared to WT mice at 14 weeks, while the expression of Acta1 was decreased **(A)**. *Casp 8*, *Creb 1* and *Traf-5* mRNA levels were normalized after both 2 and 4 weeks of ghrelin administration **(A and B)**. Skeletal muscle (femoris) morphology was evaluated in R6/2 mice after ghrelin or vehicle administration for 4 weeks and compared to WT littermates **(C-F)**. H&E stained muscle sections are represented in **(C-E)**, illustrating longitudinal skeletal muscle from WT treated with vehicle **(C)**, R6/2 treated with vehicle **(D)**, and R6/2 treated with ghrelin **(E)**. R6/2 skeletal muscle morphology exhibit a convoluted shape, and non-muscular infiltrations were found **(E)**, instead of the parallel aligned fibers seen in vehicle treated WT littermates **(D)**. An improvement was seen after 4 weeks of ghrelin administration in R6/2 mice, and the morphology was comparable to WT littermates **(E)**. A morphology score (0-4) **(F)** was given to longitudinal skeletal muscle (femoris) indicating presence of convoluted shape, muscle fiber degeneration and grade of non-muscular infiltrations. We found a significant increase in score in vehicle treated R6/2 mice compared to WT mice **(F)**, which was normalized after 4 weeks of ghrelin administration **(F)**. The scale bar represents 50 µm.

Ghrelin administration rescues nest building deficits in R6/2 mice

Nest building is a spontaneous but also a complex behavior seen in mice, which requires a high degree planning, organization, problem solving and social ability (Deacon, 2012), as well as motoric skills for carrying, pulling and bedding of nest material (Gaskill et al., 2012). Disease progression in neurodegenerative diseases can be monitored by testing the ability to build a nest, and it might also be a valuable way to test the effect of treatments on motoric and cognitive deficits (Deacon et al., 2008, Guenther et al., 2001, Paumier et al., 2013). Here in this study we assessed the nest building ability in R6/2 mice after 2 or 6 weeks of ghrelin or vehicle administration and was compared to WT littermates. We found that a deficit in nest building was evident in vehicle treated R6/2 mice after 2 and 6 weeks of treatment, which was rescued with ghrelin administration **(Figure 8)**.

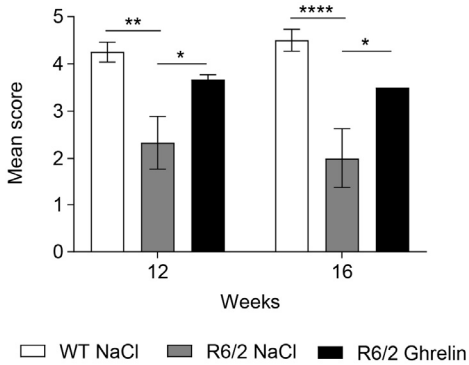

Figure 8. Ghrelin administration rescues nest building deficits in R6/2 mice. Deficits in the nest building behavior were seen at 12 and 16 weeks of age in vehicle treated R6/2 mice compared to wild-type mice. Ghrelin was here administrated once daily for either 2 or 6 weeks starting at 10 weeks. Ghrelin administration rescued deficits in the nesting behavior seen in R6/2 mice already after 2 weeks. Nesting material was introduced into mice home-cage (mice assessed in pairs), and the morning after, nest quality was scored as 1–5 (representative figures in material and methods). Score 1 represents an almost untouched nestlet, for score 3: >50% was shredded but not into a specific nest site, and score 5 represents an almost perfect nest were >90% of the nestlet was used to build the nest as a crater and the walls were higher than the mouse body height.

Paper III: Dual Therapy with Liraglutide and Ghrelin Promotes Brain and Peripheral Energy Metabolism in the R6/2 Mouse Model of Huntington's Disease

In paper III, we targeted brain and peripheral energy metabolism in R6/2 mice using subcutaneous daily injections of liraglutide (0.2 mg/kg) and ghrelin (150 μg/kg) for 2 weeks. We evaluated the effects of liraglutide alone or together with ghrelin on peripheral glucose homeostasis, as well as brain cortical hormone-mediated intracellular signaling pathways, metabolic and apoptotic markers.

Liraglutide alone or in combination with ghrelin normalize peripheral glucose homeostasis in R6/2 mice

Both liraglutide and ghrelin have been shown to affect glucose homeostasis (Ronveaux et al., 2015). We, therefore, evaluated the effects of the administration of liraglutide and ghrelin on glucose homeostasis parameters in 12-week old R6/2 mice and WT littermates **(Table 2)**. In this study, we found increased serum glucose levels in R6/2 mice, in line with previous findings (Bjorkqvist et al., 2005). Interestingly, both liraglutide alone and together with ghrelin normalized serum glucose levels in R6/2 mice. HOMA-IR and HOMA-β indexes (homeostasis models to assess insulin resistance and β-cell function, respectively) (Matthews et al., 1985, Wallace et al., 2004) were also calculated **(Table 2)**. HOMA-β was significantly decreased in R6/2 mice compared to WT littermates, which was rescued after treatment with liraglutide alone and in combination with ghrelin **(Table 2)**. HOMA-IR was significantly increased in R6/2 mice compared to WT littermates, which was normalized after treatment with liraglutide alone and in combination with ghrelin **(Table 2)**.

Table 2.
Effect of liraglutide and ghrelin on blood biochemical features in 12-week old R6/2 mice.

	WT	R6/2		
	Vehicle	Vehicle	Liraglutide	Liraglutide/Ghrelin
Serum glucose levels (mM, n=10)	9.3 ± 0.47	15.11 ± 1.27**	8.72 ± 0.89^{ff}	9.89 ± 1.6^{f}
Serum insulin levels (mg/L, n=8-10)	0.079 ± 0.0003	9.3 ± 0.47	15.11 ± 1.27**	0.081 ± 0.0011
HOMA-IR (n=10)	0.81 ± 0.04	1.33 ± 0.11**	0.80 ± 0.095^{ff}	0.99 ± 0.15^{f}
HOMA-β (n=9)	660.8 ± 36.8	339.8 ± 31.1*	927.1 ± 180.6^{f}	1202 ± 357.6^{ff}

Effects of administration of liraglutide in combination with ghrelin on brain cortical hormone-mediated intracellular signaling pathways, metabolic and apoptotic markers

Increased insulin levels and insulin signaling have been suggested to act as a compensatory mediator to normalize systemic insulin resistance and contribute to neurodegeneration in HD (White, 2014, Sadagurski et al., 2011). Therefore, we evaluated possible effects of liraglutide and ghrelin on brain cortical signaling.

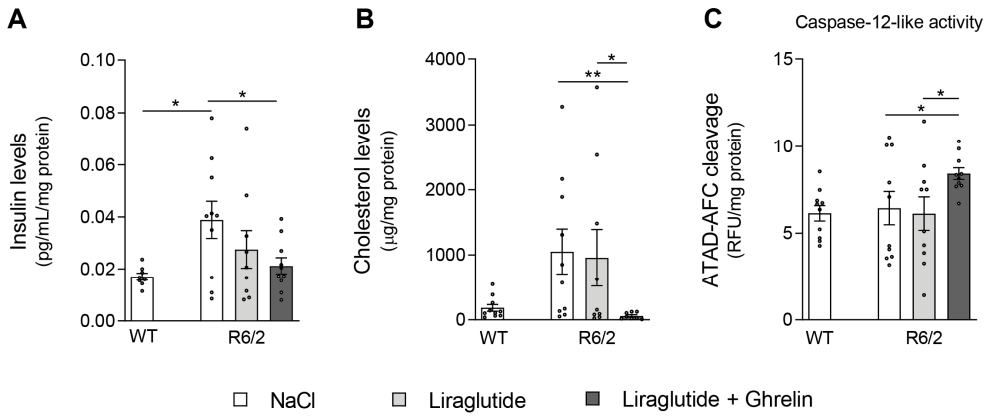

Figure 9. Effects of administration of liraglutide in combination with ghrelin on brain cortical hormone-mediated intracellular signaling pathways, metabolic and apoptotic markers.
Brain cortical levels of insulin **(A)**, cholesterol **(B)** and activity of caspase-like-12 **(C)** after 2 weeks of liraglutide and ghrelin administration. The increased cortical insulin levels found in R6/2 mice compared to WT littermates were reduced after liraglutide and ghrelin administration **(A)**. Co-administration of liraglutide and ghrelin decreases cholesterol levels in R6/2 mice, while liraglutide alone decreases cholesterol levels close to WT mice levels (although not significant)**(B)**. Brain cortical activities of caspases-like-12 was increased in R6/2 mice after liraglutide and ghrelin administration **(C)** compared to saline treated R6/2 mice.

We here found increased cortical insulin levels in R6/2 mice compared to WT littermates, which was reduced after administration of liraglutide in combination with ghrelin **(Figure 9A)**.

Progressive dysfunction of the cholesterol biosynthesis has been found in R6/2 brains (Valenza et al., 2005). We therefore evaluated brain cholesterol levels; as well as brain triglyceride and free fatty acids (data shown in paper) levels in R6/2 mice.

Regarding the possible use of triglycerides, free fatty acids (FFA) and cholesterol as alternative brain metabolic substrates, we observed that, although not significant, there was a trend towards a change in brain cortical cholesterol (and triglyceride, and FFA; in paper) levels. Cortical cholesterol was found decreased upon liraglutide and ghrelin administration **(Figure 9B)**. Liraglutide per se normalized cholesterol levels to nearly those of WT mice **(Figure 9B)**. These results suggest that liraglutide and ghrelin administration may attenuate the use of cholesterol as brain alternative metabolites.

Low levels of brain caspase activity exert beneficial effects on axonal function and cognitive deficits (Hyman and Yuan, 2012, Li et al., 2010), whereas increased caspase activity may lead to inhibition of autophagy (Hara et al., 2006, Lamy et al., 2013). Therefore, we assessed possible neuroprotective effects by the administration of liraglutide and ghrelin, evaluating caspase activity in R6/2 and WT brain cortex.

Co-administration of liraglutide and ghrelin resulted in increased Caspase-12-like activity in R6/2 mice compared to saline treated R6/2 mice **(Figure 9C)**.

Paper IV: Satellite progenitor cell dysfunction in the R6/2 mouse model of Huntington disease

Since we found beneficial effect of ghrelin administration on the catabolic profile in R6/2 skeletal muscle (paper II)(Sjögren et al., 2017), we therefore wanted to understand underlying mechanisms involved using both *in vitro* and *in vivo* studies. The underlying mechanisms of muscle wasting in HD are still not known, but it may be a direct consequence of the presence of mutant huntingtin in myocytes (Orth et al., 2003, van der Burg et al., 2009). Satellite cells, progenitors of muscle cells (Mauro, 1961, Katz, 1961) could potentially also be affected in HD. In addition, the effect of ghrelin administration on muscle growth and differentiation was evaluated.

Satellite cells from R6/2$^{(CAG\ 242\text{-}257)}$ mice exhibit reduced myofiber diameter and altered gene and protein expression *in vitro*

R6/2 muscle display muscle cell shrinkage and atrophy (Sathasivam et al., 1999). Therefore, in paper IV, we isolated satellite cells from two R6/2 colonies expressing mHTT with CAG repeat length of either 266-328 or 242-257 to evaluate their differentiation potentials.

First, stereological measurements of the myofiber diameter on R6/2$^{(CAG\ 242\text{-}257)}$, R6/2$^{(CAG\ 266–328)}$, and their WT littermates was assessed *in vitro* **(Figure 10A)**. Here, we found a significant decrease in myofiber diameter in R6/2$^{(CAG\ 242\text{-}257)}$ mice compared to WT mice and R6/2$^{(CAG\ 266–328)}$ mice **(Figure 10A)**. We found no difference between R6/2$^{(CAG\ 266–328)}$ mice and WT littermates **(Figure 10A)**. An explanation might be, as previously described by Morton and co-workers in 2009 that the longer CAG repeat size results in a slower disease progression (Morton et al., 2009).

Next, we analyzed the morphology and fiber formation at the end of the differentiation process *in vitro*. We observed thick and branching WT myofibers, whereas myofibers derived from R6/2$^{(CAG\ 242\text{-}257)}$ mice were non-branching and did not represent the same thickness **(Figure 10B)**. Some of the myofibers from R6/2$^{(CAG\ 242\text{-}257)}$ mice revealed bulky unstructured fiber formation with vacuoles, which were absent in WT myofibers **(Figure 10B)**.

Alterations in skeletal muscle gene expression profile has been found in R6/2 mice (Strand et al., 2005, Magnusson-Lind et al., 2014), including activation of apoptotic

and HDAC4-Dach2-myogenin axis (Mielcarek et al., 2015, She et al., 2011). Here, we found significant increases in mRNA levels of *Irs2* (insulin receptor substrate 2) the gene involved in regulation of muscle growth and metabolism; *MyoG* (myogenic factor 4) the transcription factor involved in myogenesis and repair; and *Sirt1* (Sirtuin 1) the transcription factor and co-regulator of several genes **(Figure 10C)**. In line with previous studies (Magnusson-Lind et al., 2014), *Myh2* (Myosin Heavy Chain 2) gene expression level was significantly decreased in R6/2$^{(CAG\ 242\text{-}257)}$ group *in vitro* **(Figure 10C)**.

We next determined early and late-stage markers of myogenesis at protein levels; such as Desmin, MyoD1, as well as the fast-twitch and late-stage marker MYH2 (Chal and Pourquie, 2017). Protein levels of these markers were evaluated in differentiated satellite cells from R6/2$^{(CAG\ 242\text{-}257)}$ and their WT littermates, *in vitro* **(Figure 10D)**. No change was seen in early-stage marker Desmin, which is also essential for the structural integrity and function of muscle (Paulin and Li, 2004). The late-stage and fast-twitch marker MYH2 protein level were unchanged in differentiated satellite cells from R6/2$^{(CAG\ 242\text{-}257)}$ compared to the WT group **(Figure 10D)**. However, MyoD1 protein level, which is involved in muscle commitment and differentiation, was decreased in myotubes from R6/2$^{(CAG\ 242\text{-}257)}$ compared with WT **(Figure 10D)**.

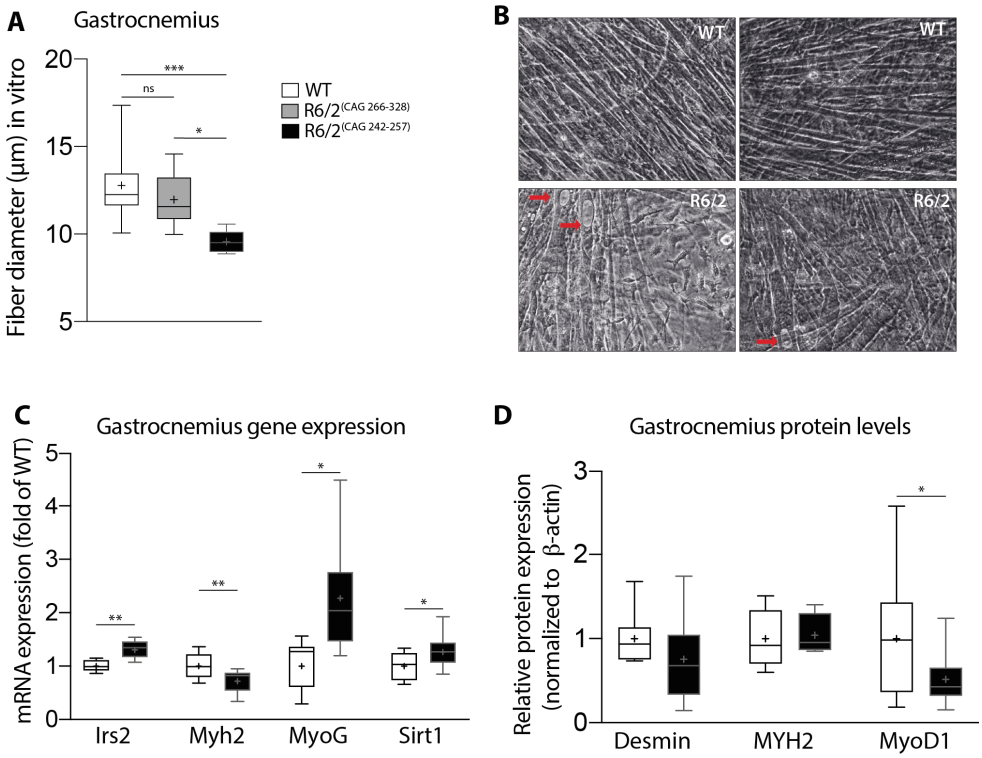

Figure 10. Satellite cells from R6/2$^{(CAG\ 242-257)}$ mice exhibit reduced myofiber diameter and altered gene and protein expression *in vitro*.

Fiber diameter of cultured satellite cells from R6/2$^{(CAG\ 242–257)}$, R6/2$^{(CAG\ 266–328)}$ and WT mice was assessed after 7 days of differentiation **(A)**. A reduction in fiber diameter in R6/2$^{(CAG\ 242-257)}$ mice was found compared to WT mice and R6/2$^{(CAG\ 266–328)}$ mice **(A)**. No difference was found in differentiated satellite cells from R6/2$^{(CAG\ 266–328)}$ mice compared to WT mice **(A)**. Representative figures of gastrocnemius muscle in cultured satellite cells from R6/2$^{(CAG\ 242–257)}$ mice **(B)**. We observed thick and branching WT myofibers, whereas R6/2$^{(CAG\ 242–257)}$ myofibers were non-branching and did not represent the same thickness **(B)**. Gene expression levels in gastrocnemius of cultured satellite cells from R6/2$^{(CAG\ 242–257)}$ mice were analyzed **(C)**. Here, we found significant increases in *Irs2*, *MyoG* and *Sirt1* mRNA levels **(C)**. Next, protein levels of early and late stage markers of differentiation in differentiated satellite cells from R6/2$^{(CAG\ 242-257)}$ and their WT littermates was assessed **(D)**. MyoD1 protein level was decreased in myotubes from R6/2$^{(CAG\ 242-257)}$ compared with WT **(D)**, while no change were seen in Desmin or MYH2 **(D)**.

Ghrelin treatment leads to an increase in skeletal muscle fiber size in both R6/2$^{(CAG\ 242-257)}$ and WT *in vitro*

The orexigenic hormone ghrelin have protective effects in the state of muscle atrophy and promotive effects in the state of attenuating the myotube formation *in vitro* (Filigheddu et al., 2007, Porporato, 2016). Based on this, we assessed the effects of ghrelin on proliferated satellite cells from WT and R6/2$^{(CAG\ 242-257)}$ skeletal muscle gastrocnemius *in vitro*. We treated mononuclear cells either with saline or 100 nM ghrelin for 96 hours, and we consequently assessed myofiber diameter at the end of 96 hours differentiation period. Reduced fiber diameter was seen in

saline-treated R6/2$^{(CAG\ 242-257)}$ group compared with WT, which was restored by ghrelin administration **(Figure 11A)**. The ghrelin treatment led to ~13% increase in fiber diameter of R6/2$^{(CAG\ 242-257)}$ mice compared to saline-treated R6/2$^{(CAG\ 242-257)}$ mice. In line with previous studies (Filigheddu et al., 2007) the ghrelin treatment in WT group also resulted in an increase in fiber diameter compared to saline-treated WT group. As we found a significant reduction in MyoD1 protein levels in R6/2$^{(CAG\ 242-257)}$ crude muscle tissue **(Figure 10D)**, we next investigated the protein levels of MyoD1 in saline- and ghrelin-treated (10, 100 nM) cells *in vitro*. We detected an increase in MyoD1 protein levels in both 10 nM ghrelin treated R6/2$^{(CAG\ 242-257)}$ and WT groups compared with their saline-treated controls **(Figure 11B)**.

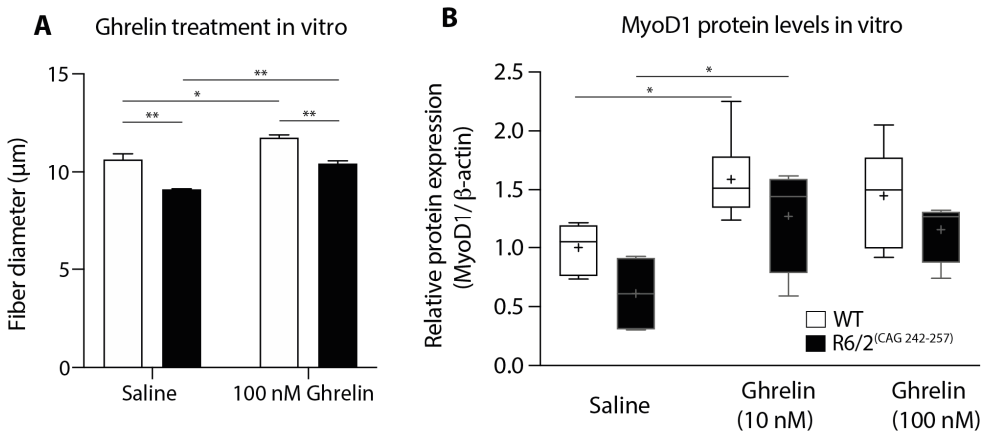

Figure 11. Ghrelin treatment leads to an increase in skeletal muscle fiber size in both R6/2$^{(CAG\ 242-257)}$ and WT in vitro.

Stereological measurements of fiber diameter in ghrelin treated (100 nM) cultured satellite cells from R6/2$^{(CAG\ 242–257)}$ mice **(A)**. Myofiber diameter was assessed at the end of 96 hours differentiation period. The significant reduction in fiber diameter of saline-treated R6/2$^{(CAG\ 242-257)}$ group compared with WT was restored in R6/2$^{(CAG\ 242-257)}$ mice after ghrelin administration. Ghrelin treatment of WT cells also resulted in an increase in fiber diameter compared to saline-treated WT cells **(A)**. We next investigated protein levels of MyoD1 in saline- and ghrelin treated cells in vitro **(B)**. We detected an increase in MyoD1 protein levels in both 10 nM ghrelin treated R6/2$^{(CAG\ 242-257)}$ and WT groups compared to their saline-treated controls **(B)**.

Discussion and future perspective

General discussion

In this thesis, results demonstrate beneficial effects on both central and peripheral pathology by targeting whole-body energy metabolism in the R6/2 mouse model of HD. In vitro studies have provided new important information regarding muscle stem cell dysfunction in HD.

HD is one of the most common inherited neurodegenerative disorders worldwide, and research in this field has over the years mostly been focusing on brain pathology (Ross et al., 2014, Vonsattel et al., 1985). A reason for this focus might be the direct link between core features of HD e.g. psychiatric problems, as well as motor and cognitive deficits, and the degeneration of striatal and cortical neurons (Rosas et al., 2008, Vonsattel et al., 1985). However, strong evidence suggest HD to be a whole-body disorder since the causative mutant huntingtin is widely expressed throughout the body, affecting both neuronal and non-neuronal cells (Hoogeveen et al., 1993, Li et al., 1993b, Strong et al., 1993b, Trottier et al., 1995). Accumulating evidence shows that, alongside neurodegeneration, HD is considered to be a metabolic disorder with a hyper-catabolic state, in which small changes in oxygen consumption lasting over a long period of time might have an impact in altered body weight (Bjorkqvist et al., 2006, Carroll et al., 2015, Sathasivam et al., 1999, van der Burg et al., 2009, van der Burg et al., 2011). In both clinical HD and in mouse models of HD, an increased metabolic rate with weight loss and muscle wasting is prominent, as well as body composition and endocrine alterations (Bjorkqvist et al., 2006, Carroll et al., 2015, Sathasivam et al., 1999, van der Burg et al., 2009, van der Burg et al., 2011). It has been suggested that energy homeostasis alterations seen in HD (Carroll et al., 2015, van der Burg et al., 2009, van der Burg et al., 2011), might be due to either central pathology, especially the hypothalamus (Petersen and Bjorkqvist, 2006, Petersen and Gabery, 2012) or peripheral tissue alterations (McCourt et al., 2016, Strand et al., 2005, van der Burg et al., 2009, van der Burg et al., 2011), or by a combination (Carroll et al., 2015). It was recently shown that a higher BMI in HD patients is associated with slower rate of functional, motor, and cognitive dysfunctions, suggesting that systemic metabolism could influence clinical progression in HD (van der Burg et al., 2017). However, it was not shown

whether the beneficial effects seen with a higher BMI was an effect of increased fat mass or lean mass, or a combination of both (van der Burg et al., 2017).

In paper I, we therefore aimed to evaluate the effect of an altered metabolism and increased body weight on R6/2 mice disease features. We demonstrated that by using a genetic approach it was possible to increase body weight and fat mass, and decrease whole-body energy metabolism in the transgenic R6/2 mouse model of HD. R6/2 mice was placed on a leptin-deficient (Ob/Ob) background and thereby creating a novel mouse model (R6/2;Ob/Ob) exhibiting a hypometabolic state leading to increased body weight and fat mass. A similar approach was used by Lim et al in 2014, targeting energy metabolism in amyotrophic lateral sclerosis (ALS)(Lim et al., 2014). Increased metabolic rate resulting in weight loss is, similar to HD, a prominent feature in ALS, and an association with disease progression has been demonstrated (Dupuis et al., 2011). By placing the G93A mutant SOD1 mice, a well-established amyotrophic lateral sclerosis (ALS) mouse model, on a leptin-deficient genetic background they were able to reverse the catabolic state seen in the ALS mouse (Lim et al., 2014).

The mouse model homozygote for the obese mutation (Ob/Ob) was first developed in an outbred mouse colony with a C57BL background at the Jackson Laboratory, Bar Harbor, Maine in 1949 (Ingalls et al., 1996). In addition to obesity, Ob/Ob mice are leptin-deficient and hypometabolic 1949 (Coleman, 1978, Garthwaite et al., 1980, Ingalls et al., 1996, Mayer et al., 1953). Body weight in R6/2;Ob/Ob mice was dramatically increased compared to both WT and R6/2 mice and demonstrated a weight gain curve comparable to Ob/Ob mice until 10 weeks of age. The weight gain in both Ob/Ob and R6/2;Ob/Ob mice might be as a result of increased food intake, which has repeatedly been demonstrated in Ob/Ob mice (Szczypka et al., 2000). The increased body weight might also due to increased fat mass as we observed a dramatic increase in fat mass in both Ob/Ob and R6/2;Ob/Ob mice.

The body mass index (BMI), which is measured with respect to height and weight, does not distinguish between fat and lean mass, and a higher BMI has been shown in clinical HD to correlate with slower disease progression (van der Burg et al., 2017). In this study we wanted to investigate whether the higher BMI was neuroprotective in R6/2 mice. Although there was no significant change between the groups, we found a trend toward increased number of inclusions in R6/2;Ob/Ob mice compared to R6/2 mice. The increased fat mass might interfere with inclusion clearance, since it has previously been shown that an obese phenotype is associated with increased ER stress, which then prevents degradation of misfolded proteins (Pagliassotti et al., 2016). It is not yet fully known whether mHTT inclusions are toxic or protective (Arrasate et al., 2004, Kim et al., 1999, Sakahira et al., 2002). However, strong evidence indicate that the inclusions causes transcriptional dysregulation and cellular trafficking (Li et al., 2001, Passani et al., 2000). It is

possible that inclusion formation is also involved in the striatal transcriptional changes found in our study. We found that R6/2;Ob/Ob mice exhibit similar striatal gene expression alterations as R6/2 mice, which indicates that increased body fat and lower metabolic rate in R6/2 mice do not have a neuroprotective role in the progression of HD. A possible reason might be the ongoing brain pathology seen in Ob/Ob mice (Bereiter and Jeanrenaud, 1979, Sena et al., 1985), which can aggravate HD pathology, and thereby cover the beneficial effects of a reduced energy metabolism and higher body weight.

In contrast to our results indicating no effect on neuropathology, Lim et al found beneficial effects on cognition, motor deficits and survival, suggesting a slower disease progression using the same genetic approach (Lim et al., 2014). Although we found beneficial effects on body weight and fat mass in leptin-deficient R6/2 mice by lowering their metabolic rate, we only saw marginal effects on lean mass. In contrast to the study by Lim et al. in 2014, the increase in weight and fat mass was not sufficient to generate any neuroprotection in our novel leptin-deficient R6/2 mouse model.

Future strategies focusing on restoring energy metabolism by targeting lean mass could lead to a better understanding on the relevance of body composition and a higher BMI in HD progression. To follow up, we therefore in paper II targeted peripheral metabolic disturbances seen in R6/2 mice using ghrelin administration. In this study, the primary aim was to delay weight loss and reduce skeletal muscle atrophy. Ghrelin, a hormone peptide secreted mainly from the stomach, and other GHS-R1a agonists have been shown to have beneficial effects for many clinical problems, including muscle wasting, cancer cachexia, cognitive decline, diabetes and metabolic disorders (Ali et al., 2013b, McLarnon, 2012, Reano et al., 2014).

Weight loss and skeletal muscle atrophy are prominent features in HD, which progress with disease and affect the quality of life (Strand et al., 2005, van der Burg et al., 2011, Ribchester et al., 2004, Trejo et al., 2004). Alterations in muscle gene expression levels, triggered by mHTT inclusions present in muscle cells, are found in both clinical HD (Ciammola et al., 2006) and mouse models of HD (Luthi-Carter et al., 2002, Moffitt et al., 2009, Sathasivam et al., 1999, Strand et al., 2005). In line with previous studies, we found an increase in apoptotic (*Caspase 3*, *Caspase 8*) and NFκ-B pathway transcripts (*Smad 3* and *Traf 5*) (Magnusson-Lind et al., 2014, She et al., 2011), and an increase in *Creb 1*, which is activated during muscle injury (Stewart et al., 2011). Although we could not find any dramatic effect on body weight or fat mass (data shown in paper), we found that administration of ghrelin for 2 and 4 weeks had beneficial effects on catabolic features seen in both gene expression (*Caspase 8*, *Creb 1* and *Traf 5*) levels as well as in muscle morphology. Our results are in line with previous studies where ghrelin inhibited doxorubicin-

induced apoptosis in skeletal muscle (Yu et al., 2014). A possible mechanism of ghrelin's action is presented in Figure 12.

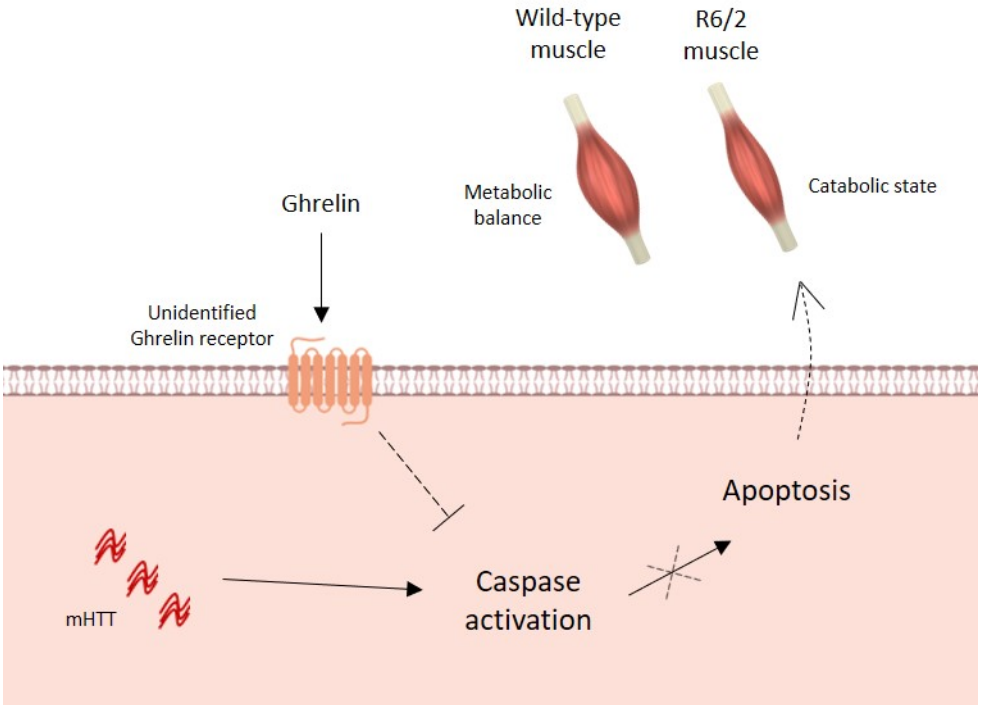

Figure 12. Possible action of ghrelin in skeletal muscle.
Skeletal muscle atrophy is a prominent feature in HD (Ribchester et al., 2004, Trejo et al., 2004, Strand et al., 2005, Magnusson-Lind et al., 2014). In paper II, we found that typical gene expression levels associated with apoptotic features were altered in R6/2 muscle, which was normalized after ghrelin administration. A possible action of ghrelin is through its influence on e.g caspase activation and apoptosis, and thereby also have an impact on muscle mass and morphology.

In HD, motoric dysfunction is accompanied with a reduced ability to perform everyday tasks. Nest-building behavior, a test to study fine motoric skills, as well as cognitive function (Deacon, 2012), have been found to be affected in both Alzheimer´s disease (Deacon et al., 2008) and Parkinson´s disease (Paumier et al., 2013). Here, we found that R6/2 mice exhibit nest-building deficits, which was robustly improved after ghrelin administration, suggesting a beneficial effect of ghrelin on behavioral deficits.

These results suggest that targeting and rescuing the catabolic profile seen in skeletal muscle in R6/2 mice might have beneficial effects on cognitive behavior seen in HD. However, further studies on the underlying mechanisms involved in both the improvement of skeletal muscle wasting phenotype and cognition are warranted.

In paper III, both peripheral and central energy metabolic alterations were targeted in R6/2 mice using co-administration of liraglutide together with ghrelin.

Since weight loss is a common feature in HD (van der Burg et al., 2008, van der Burg et al., 2011), and liraglutide have anorectic effect alongside the anti-diabetic (Vilsboll et al., 2008) and neuroprotective role (McClean et al., 2011), we therefore hypothesize that dual combination of liraglutide and ghrelin might maintain body weight, and in addition, normalize peripheral glucose levels and affect metabolic and apoptotic markers in R6/2 brain.

Hyperglycemia along with reduced levels of insulin has previously been shown in R6/2 mice resulting in diabetes (Bjorkqvist et al., 2005). Activation of the GLP-1 receptor by GLP-1 and different analogues, e.g. liraglutide and exendin-4 have been shown to exert anti-diabetic effects by improving pancreatic β-cell function and glucose homeostasis in HD mouse models (Martin et al., 2009, Vilsboll et al., 2008). Ghrelin administration, on the other hand, has given contradictory results on β-cell insulin secretion in rodents and in vitro models, with both inhibitory (Wierup et al., 2004) and beneficial effects (Lee et al., 2002). We found that both pancreatic β-cell function and insulin resistance, presented as HOMA-β and HOMA-IR respectively was improved with the dual therapy with liraglutide and ghrelin. Although glucose levels were elevated in our study, insulin levels maintained normal. A possible reason might be that at the time-points chosen for our study the R6/2 colony presents an early to middle stage disease phenotype in which insulin production has not been dramatically affected yet. Maintaining a stabilized systemic glucose metabolism is important since alterations could influence whole-body metabolism as well as possibly influencing brain pathology contributing to neurodegeneration and motor deficits (Martin et al., 2008, Verdile et al., 2015).

An increased brain insulin level has previously been observed, and is suggested to have compensatory effects to overcome systemic insulin resistance in R6/2 mice (White, 2014). Another study has also shown that elevated insulin signaling in the brain could contribute to neurodegeneration in HD due to mitochondrial dysfunction and oxidative stress (Sadagurski et al., 2011). Alongside the observed normalization of peripheral insulin resistance seen in our R6/2 mice, elevated brain insulin levels were here normalized with co-administration of liraglutide and ghrelin. This suggests that peripheral pathology contributes to brain dysfunctions in HD (Chiang et al., 2007, Martin et al., 2009), and by normalizing systemic insulin resistance might exert beneficial effect on brain energy metabolism. Neuroprotective effects by liraglutide and exendin-4 are also found in mouse models of different neurodegenerative disorders such as Alzheimer's disease (AD)(McClean et al., 2011), Parkinson's disease (PD)(Bertilsson et al., 2008), and Huntington's disease (HD)(Martin et al., 2009), but also in clinical studies of AD and PD (Holscher, 2014). Caspase activation has an important role in both apoptosis and autophagy,

turnover of protein aggregates and eliminating damaged cells (Wu et al., 2014). Low levels of caspase activity in the brain have been shown to have beneficial effects on axonal function and cognition (Hyman and Yuan, 2012, Li et al., 2010), while increased levels might lead to autophagy inhibition (Hara et al., 2006, Lamy et al., 2013). Caspase-12-like activity was here found to be stimulated due to administration of liraglutide in combination with ghrelin, suggesting a neuroprotective role in HD.

Our findings in paper III targeting peripheral and brain energy metabolism might exert beneficial effects on HD progression, and therefore support further studies.

In paper II, we found beneficial effects in the catabolic profile in R6/2 skeletal muscle by targeting energy metabolism with ghrelin administration. To follow up on this study, possible dysfunctions in satellite cells, progenitors of muscle cells (Mauro, 1961, Katz, 1961), as well as the effect of ghrelin administration were evaluated *in vitro* and *in vivo* studies in paper IV.

In this study, our results suggest that pathogenic effects of mHTT is involved in development disturbances of skeletal muscle tissue, i.e. muscle atrophy at the neonatal stage exemplified by reduced skeletal muscle fiber diameter and abnormal muscle morphology. These alterations in R6/2$^{(266\text{-}328)}$ mice suggest that a delay in the developmental process might underlie reduced muscle strength and atrophy in adulthood. Inclusions of mHTT is observed in both neuronal (Graveland et al., 1985, Vonsattel et al., 1985) and non-neuronal tissues in both clinical HD (Ciammola et al., 2006) and in HD mouse models (Moffitt et al., 2009, Sathasivam et al., 1999). In line with previous studies (Sathasivam et al., 1999), intranuclear mHTT inclusions were detected in skeletal myofibers in 12 weeks old R6/2$^{(CAG\ 242\text{-}257)}$ mice and signs of muscle atrophy were evident in R6/2 mice (Shown in paper IV). We found that a fraction of R6/2$^{(CAG\ 242\text{-}257)}$ myofiber nuclei was positive for mHTT inclusions, and this inhomogeneous distribution of inclusions might be a consequence of high muscle turnover, or a ubiquitin-proteasome system and autophagic activity of muscle fibers (Orth et al., 2003). Further studies on whether myotube nuclei or quiescent satellite cells are positive for inclusion bodies are needed.

Muscle atrophy is a prominent feature in both mouse models and in clinical HD (Luthi-Carter et al., 2002, Ribchester et al., 2004, Strand et al., 2005, Trejo et al., 2004), and could be the direct consequence of the presence of mutant huntingtin in satellite cells (Orth et al., 2003). Satellite cells are essential for proliferation, differentiation, and repair of muscle tissue during the regeneration process in response to muscle injury or exercise (Dumont et al., 2015). Activated satellite cells (characterized by expression of: Pax7^{+}MyoD^{+}), start to proliferate, which is further differentiated into mature muscle fibers (characterized by expression of: Pax7^{-} MyoG^{+}MYH2^{+}), and thereby repair the injured muscle (Almada and Wagers, 2016).

Dysfunctions in satellite cell capacity has been observed in several muscular dystrophies, e.g. Duchenne muscular dystrophy (Ervasti et al., 1990), which lead to severe muscle wasting by causing myofiber instability and impaired regeneration (Chang et al., 2016).

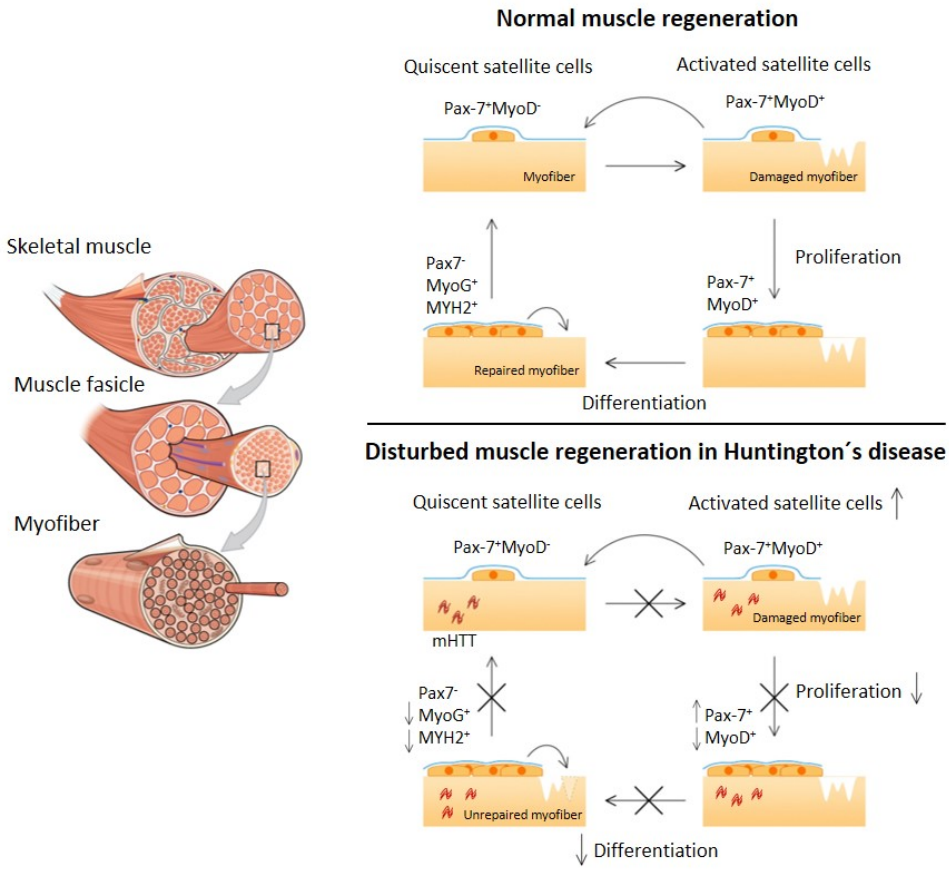

Figure 13. Hypothesis of possible muscle regeneration in Huntington´s disease.
Under normal conditions, satellite cells become activated upon injury or during exercise for the repairment of the effected myofibers. In HD, this process might be dysfunctional as a direct effect of mHTT, leading to decreased protliferation, differentiation and less repaired myofibers.

Our hypothesis in paper IV, is that the satellite cell regeneration process is disturbed in the HD mouse models R6/2 **(Figure 13)**, leading to decreased proliferation and differentiation as a direct effect of mHTT inclusions (Orth et al., 2003). Although an increased activation of satellite cells might be seen, proliferation and differentiation processes are decreased resulting in decreased myofibers, as well as gene expression and protein level alterations.

To further test this, we next evaluated the fiber formation at the end of the differentiation process *in vitro* using two different R6/2 colonies containing CAG repeat length between CAG 242-257 and CAG 266–328. Like human HD (Duyao et al., 1993), both somatic and germ line instability of the CAG repeat tract has been observed in R6/2 mice, where a CAG repeat expansion >400 CAGs has been reported (Dragatsis et al., 2009, Gonitel et al., 2008, Morton et al., 2009). The R6/2 colonies used in this thesis have longer CAG repeat length compared to the initial CAG repeat length of 150 CAG repeats, resulting in a slower progressing phenotype (Morton et al., 2009). Here we found that R6/2$^{(242\text{-}257)}$ mice exhibit more severe HD phenotype compared to R6/2$^{(CAG\ 266\text{–}328)}$ mice. We observed that the myofibers derived from R6/2$^{(242\text{-}257)}$ mice exhibit decreased diameter compared to WT mice, which was not seen in R6/2$^{(CAG\ 266\text{–}328)}$ mice. Furthermore, vacuole formation was observed in R6/2 muscles, a common feature in muscle dystrophy diseases and a sign of necrosis (Vattemi et al., 2014), This suggests that the HD pathology seen in R6/2 myofibers exacerbates due to CAG repeat size.

Mitochondrial dysfunction, with impaired PGC-1α leading to energy deprivation has been suggested to be a key player in skeletal muscle pathology in HD (Chaturvedi et al., 2009). Increased expression of *Sirt1*, which acts as a sensor of energy deprivation, as well as increased *Irs2* expression, possibly indicates that energy deprivation alongside decreased regeneration ability occur in our *in vitro* study. Differentiation capacity has been shown to be decreased in MyoD knock-out skeletal muscle, in which elevated quiescent satellite cells and lower number of differentiated myofibers are present. In our study, we found that MyoD1 protein levels were reduced in differentiated satellite cells from R6/2$^{(242\text{-}257)}$ mice. Our findings, with thinner fiber diameter, and alterations in gene expression and protein levels involved in differentiation suggest that R6/2 muscle cells distinguish disturbed differentiation and muscle growth. However, further studies in order to better understand the developmental effects of mutant HTT expression in skeletal muscle, the satellite cell renewal, and myogenic commitment processes should be further investigated at the gene expression and protein levels.

Ghrelin and des-Acyl ghrelin has previously been shown to promote differentiation and fusion of C2C12 skeletal muscle cells (Filigheddu et al., 2007). Ghrelin administration showed in our study beneficial effects on muscle fiber diameter both *in vivo* and *in vitro*, as well as protein levels of the differentiation marker MyoD1,

in cultured satellite cells from both WT mice and R6/2 mice. These findings suggest that ghrelin might increase differentiation of muscle cells, but also have an anabolic effect inducing muscle growth.

Future perspective

Although a lot of research has been done and more is known about HD pathogenesis, there are still no curable treatments for HD. Different approaches treating HD has emerged over time, where the most promising currently has been by targeting huntingtin (Pagani et al.), neuro-inflammation and synaptic transmission in central pathology (Caron et al., 2018). Due to ubiquitous expression of mHTT throughout the body, it is likely that truly effective treatments will need to address both central and peripheral pathology of HD. Abnormalities in the periphery are suggested not to necessarily arise from degeneration of the brain, but might instead be a consequence of mHTT inclusions located in peripheral tissues (Sathasivam et al., 1999, Strong et al., 1993b). Targeting only CNS pathology by silencing mutant HTT might therefore not be sufficient to ameliorate all HD symptoms, since it has been suggested that peripheral pathology might even contribute to brain pathology (Chiang et al., 2007, Martin et al., 2009).

Studying abnormalities in peripheral tissues has contributed to insights of underlying mechanisms leading to a disturbed metabolism with a catabolic state. This information could contribute to the development of new biomarkers in HD leading to novel therapeutic treatments in the future. Although most of these insights have derived from animal studies, it has become clearer that these abnormalities are also evident in clinical HD. Weight loss and muscle atrophy are two prominent features progressing with disease and affecting these features in HD with therapeutically approaches might ease the cachexia-like symptoms. Successful treatment of muscle atrophy and strength deficits might have important effects on mobility and strength impairments. It is still unknown to what extent weight loss influence disease progression. It has been suggested as a result of metabolic changes (Goodman et al., 2008, Mochel et al., 2007, van der Burg et al., 2008), but reduced absorption of nutrients due to altered digestive tract may also play a role (van der Burg et al., 2011). A recent study found that higher baseline BMI was associated with a slower rate of HD progression with reduced motor, cognitive and functional impairments in HD patients, independent of CAG repeat size and disease stage (van der Burg et al., 2017).

Our findings support that by using the approach targeting peripheral tissue and energy metabolism and normalizing the hyper-catabolic state might delay the onset and progression of the disease, and thereby increase the quality of a daily life.

Conclusions

Paper I

Increased BMI has been shown to slow disease progression in clinical Huntington's disease.

We here show that leptin-deficient R6/2 mice display increased body weight and increased fat mass.

Leptin-deficient R6/2 mice display reduced energy expenditure accompanied by white adipose tissue characteristics, contrary to R6/2 mice.

Leptin-deficient R6/2 mice exhibit no neuropathological improvement.

Paper II

Ghrelin administration rescues the catabolic profile in R6/2 muscle, with reversed gene expression of *Caspase 3*, *Creb 1* and *Traf 5*, and normalized muscle morphology.

Beneficial effects are found in body weight after ghrelin administration.

Ghrelin treated R6/2 mice exhibit no improvement in body composition or in glucose metabolism.

Cognitive deficits (nesting behavior deficits) are rescued after ghrelin administration.

Paper III

Liraglutide and ghrelin administration normalizes peripheral glucose homeostatic features in the R6/2 mouse, e.g. glucose levels, insulin resistance and pancreatic β-cell function, without substantially affecting body weight or body composition.

We here show that liraglutide administration decreased brain cortical active GLP-1 and IGF-1 levels in R6/2 mice, alongside higher ADP levels.

Liraglutide and ghrelin administration decreased brain insulin, lactate, AMP and cholesterol levels in R6/2 mice.

Paper IV

R6/2$^{(242\text{-}257)}$ muscle (gastrocnemius) express intranuclear em48 (anti-mHTT) positive inclusions.

Histopathological signs of skeletal muscle degeneration are observed in the R6/2 mouse model.

Satellite cells from R6/2$^{(CAG\ 242\text{-}257)}$ mice exhibit reduced myofiber diameter and altered gene and protein expression *in vitro*.

We here show that ghrelin treatment leads to increased skeletal muscle fiber size and MyoD1 protein level in both R6/2$^{(CAG\ 242\text{-}257)}$ and WT *in vitro*.

Material and Methods

In this section, I will give an overview on materials and methods used in this thesis, and more detailed descriptions can be found in respective paper.

Animals

All experimental procedures performed on mice were carried out in accordance with the approved guidelines in the ethical permit approved by The Malmö/Lund Animal Welfare and Ethics Committee. All mice were housed in groups of 2-5 in universal Innocage mouse cages under standard conditions (12 h light/dark cycle, 22°C), and with ad libitum access to chow food, and water, and cages were enriched with nesting material.

Experimental models

In paper I, a two-step breeding strategy was used to generate a leptin-deficient R6/2 mouse model (R6/2;Ob/Ob), by crossing the transgenic R6/2 mouse (CAG 242-257 or CAG 345-352) with the obese mouse model (Ob).

In paper II, III and IV, male transgenic R6/2 HD mice (CAG 266-328) and their WT littermates were utilized, obtained by crossing heterozygous R6/2 males with WT females on a CBAxC57BL/6 J background.

In paper IV, *in vitro* studies were conducted using satellite cells from 7-9 days old transgenic R6/2 mice (CAG 242-257 or CAG 266-328) and their WT littermates on a CBAxC57BL/6 J background.

Mice used in this thesis have a slower disease progression than the original R6/2 mouse line with 150 CAG repeats, as described by Morton and co-workers in 2009 (Morton et al., 2009).

Metabolic measurements

Alterations in energy metabolism with a hyper-catabolic state are seen in clinical HD and mouse models of HD (Carroll et al., 2015, van der Burg et al., 2009, van der Burg et al., 2011). Therefore, different methods were used to evaluate the metabolic state of the mice used in paper I-III.

Body composition (DEXA)

In paper I, II, and III, fat and lean mass was measured using the Lunar PIXImus whole-body DEXA scanning (GE Lunar Corp., Madison, WI) in isofluorane-anesthetized (Apoteksbolaget, Lund, Sweden) mice. Obtained dataset for each mouse included: body fat (%), lean and fat mass (g); bone mineral density (g/cm^2) and bone mineral content (mg), but only fat and lean mass (g) was reported in paper I-III. Image analysis of the scanning was performed with PIXImus2 2.10 software (Lunar Corporation).

Indirect gas calorimetry

In paper I, whole-body energy metabolism, locomotor activity, and food and water intake were measured using the PhenoMaster Home Cage System (TSE-systems, Bad Homburg, Germany).

The animals were habituated to the drinking bottles one-day prior for acclimatization. Animals were weighed, single-housed and acclimatized to the calorimeter chambers for one day prior to the experiments. Food and water were available ad libitum. All parameters were recorded every 15 min for 24 hrs, and reported as light phase, dark phase and 24 hours. Metabolic rates were calculated using a correction for body weight. Obtained parameter for each mouse included: energy expenditure, oxygen consumption, CO_2 production, respiratory exchange rate, food and water intake, and distance moved.

Behavioral testing

Mice were handled prior to testing. Mice were transported in their home cages to the behavioral testing room and could acclimate to the room for at least one hour before testing.

Marble burying test

Marble burying test was used in paper I to evaluate anxiety and repetitive behavior in rodents (Deacon, 2012). Twelve glass marbles (1.5 cm in diameter) were placed, evenly spaced (3x4 rows), on a flat layer of 3 cm corn cob in the home

cage (37.3 x 23.4 x 14.0 cm). A mouse was placed single caged and left for 30 min after which the number of marbles buried 2/3 were counted.

Nest building test

Nest building is a spontaneous and complex behavior of mice, requiring a high degree of organization, planning, problem solving, social ability (Deacon, 2012), as well as motoric skills for pulling, carrying, and bedding of nest material (Deacon, 2012).

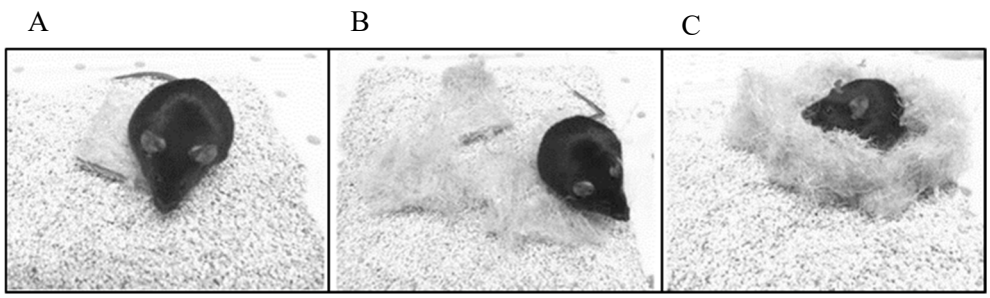

Figure 14. Nest building scores.
Figures represent different scores for the nesting behaviour testing. An almost untouched nestlet gives the score 1 (a), a score of 3 is given when more than 50% of the nestlet is shredded but no identified nest is found (b), and a score of 5 is given to an almost perfect nest where the nest walls are higher than the mouse body and have a form of a crater (c).

In paper I and II, nest building behavior was assessed to evaluate cognitive and motoric abilities. Mice were single caged overnight, with access to food and water, but with no environmental enrichment. Approximately 1.5 g of nesting material was placed in the cage, and torn material was weighed the next morning. Nesting ability was assessed on a point scale as previously described (Deacon, 2006), from 1 to 5,

analyzing both touched nesting material and shape of the nest. A score 1 was given when the nestlet was mainly untouched (>90% was intact), score 3 was given when the nestlet was mostly shredded but no identifiable nest site was seen, and score 5 was given to an almost perfect nest, where >90% of the nestlet was torn up into a crater where the walls were higher than the body height (As shown in figure 14).

Open Field test

Open field behavior testing is a way to assess the locomotor and behavioral activity in rodents (Gould et al., 2009), which has been shown to be affected in the HD mouse model R6/2 (Hickey et al., 2005).

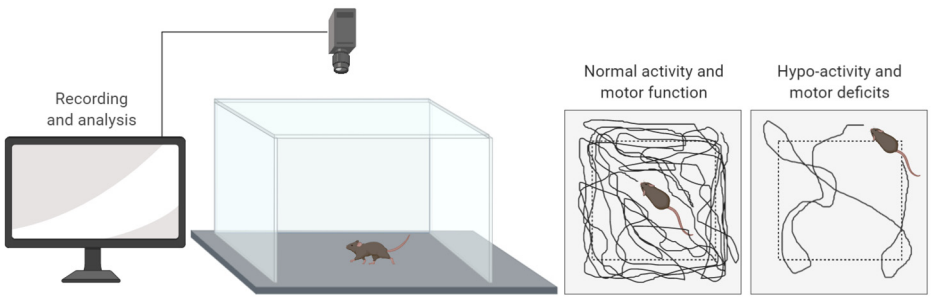

Figure 15. Illustration of behavior test for the assessment of locomotoric activity using an open field arena. (Created with BioRender.com)

In paper I and III, locomotor activity was assessed. Distance travelled was evaluated in paper I during dark phase; and distance travelled, mean speed, mean time mobile, number of line crossings and rearing in paper III during light cycle. An open field arena was used with open-topped Plexiglas boxes of 50 × 50 cm, and the Stoelting ANY-MAZE video tracking system (Dublin, Ireland), detecting position of the animal's head, body and tail (Shown in Figure 15). Mice were placed individually in the corner of the open field arena and were recorded for a 30-min period (paper III) or 60-min period (paper I). Data was collected for every 5 minutes.

Paw clasping test

In paper III, paw clasping test was assessed. Clasping is a behavior test that has previously been evaluated in R6/2 mice to study the disease progression, showing a clasping phenotype (Li et al., 2005, Mangiarini et al., 1996). Mice were suspended by the tail for 180 s and scored as previously described (Hansson et al., 2003), from 0 to 2; where 0 represents no clasping behavior, 1 means that the hind or paws clasp for at least 1 s, and a score of 2 means that the paws clasp for more than 5 s.

Satellite cell culture

Cell culture

Satellite cells was isolated from gastrocnemius muscle from 7-9 days old R6/2 and WT mice to evaluate the effect of mHTT in functional capacity of the proliferation and differentiation process (paper IV). The cells were cultured as mononuclear cells in Matrigel-coated 12-well plates (~25 000 cells/well). The cells were left for proliferation for 3 days with 1 ml of proliferation media containing Ham's 12, 20% FBS, 20mM L-glutamine, 1% penicilin/streptomycin and 1% amphotericin B. The plates were kept in a 5% CO2 incubator at 37°C. To induce differentiation of satellite cells, growth media was replaced with differentiation media containing Dulbecco's Modified Eagle Medium (DMEM), 2% horse serum, 1% penicillin/streptomycin and 1% amphotericin B. The differentiation media was refreshed every day and incubated for 7 days in total.

Ghrelin treatment

In paper IV, harvested mononuclear cells (~25 000 cells/well) from gastrocnemius muscle were incubated with proliferation media (Ham's 12, 20% FBS, 20mM L-glutamine, 1%penicilin/streptomycin +1% amphotericin B) for 3 days. Following the proliferation state, at the differentiation state, cells from WT and R6/2$^{(CAG\ 242\text{-}257)}$ mice were treated with either ghrelin (10nM or 100 nM; Rat, mouse; Phoenix Pharmaceuticals, Belmont) or sterile saline for 96 hrs to investigate the effects of ghrelin on fiber diameter, gene expression and protein levels. Differentiation media containing treatment was refreshed every day. On the last day of differentiation, 25 pictures at magnification 20x were taken randomly at 5 locations for each well and fiber diameter was assessed.

qPCR analysis

RNA extraction and cDNA synthesis

Gene expression alterations in brain cortical tissue, skeletal muscle (gastrocnemius), WAT and in differentiated satellite cells, were evaluated using qPCR analysis. Total RNA was extracted using the E.Z.N.A. Total RNA Kit II (Omega bio-tek, Norcross, GA, USA) before complementary DNA (cDNA) was synthesized using iScript cDNA Synthesis Kit (Bio-Rad Laboratories, CA, USA). RNA concentration and

purity were measured by a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

Real-time quantitative PCR

SsoAdvanced Universal SYBR Green Supermix from Bio-Rad Laboratories was used for RT-qPCR and performed following manufacturer´s protocol. All RT-qPCR plates were run on a CFX96 touch real-time PCR detection system (Bio-Rad, Hercules, CA, USA).

Primers utilized for RT-qPCR validations were designed using either QuantPrime69 or PrimerQuest from Integrated DNA Technologies (http://eu.idtdna.com/PrimerQuest). The efficiency of each primer pair was tested before use by performing a standard curve, and the efficiency criteria for using a primer pair was 90%<E<110%, with an R^2 cut-off >0.990. Changes in gene expression were calculated using the CFX manager software program (Bio-Rad, Hercules, CA, USA), using the ΔΔCt method with a fold change cut-off at ≥ 1.5 and $p<0.05$ considered significant. All samples were run in triplicate and relevant positive and negative controls were run on each plate. Housekeeping genes used to normalize brain cortical tissue, skeletal muscle (gastrocnemius) and WAT qPCR in paper I-IV are listed in Table 3.

Table 3.
Overview of housekeeping genes used to normalize brain cortical tissue, skeletal muscle (gastrocnemius) and WAT qPCR.

Housekeeping genes	Forward Sequence (5´→ 3´)	Reverse Sequence (5´→ 3´)	Paper
18S	ACCGCAGCTAGGAATAATGGA	GCCTCAGTTCCGAAAACCA	I, II
Actb	GCTGTGCTATGTTGCTCTA	TCGTTGCCAATAGTGATGA	I, IV
Atp5b	GGCACAATGCAGGAAAGG	TCAGCAGGCACATAGATAGCC	I, III, IV
Canx	AGCTGTTGAGGCTCATGATGGAC	CTGGAGCTTTGTAGGTGACCTTTG	III
Gusb	CCGACTTCATGACGAACCAGTCAC	TGTCTCTGGCGAGTGAAGATCC	II
Hsp90ab1	ATGATTAAACTAGGCCTGGGCATC	GCTTTAATCCACCTCTTCCATGCG	II
Ppia	GGGTTCCTCCTTTCACAGAA	GATGCCAGGACCTGTATGCT	I
Rpl13a	CCAAAGGTTCCTTAGGCACTGCTC	TGCGCTGTCAGCTCTCTAATGTC	III
Tbp	TCTGAGAGCTCTGGAATTGTACCG	TGATGACTGCAGCAAATCGCTTG	I, II

Western blotting

In paper II and IV, immunoblotting (WB) was used to analyze skeletal muscle protein alterations *in vivo* and *in vitro* and evaluate the effect of ghrelin administration. Protein were extracted from approximately 30 mg of skeletal muscle gastrocnemius and approximately 25 000 differentiated satellite cells in a lysis buffer supplemented with protease inhibitors (Complete, Roche, Basel, Switzerland) and phosphatase inhibitors (PhosSTOP, Roche, Basel, Switzerland). After protein extraction, the concentration was measured using Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA). In paper II, ~20µg protein were loaded on Mini-PROTEAN TGX Stain-Free Precast Gels (Bio-Rad, CA, USA) and blotted on 0.2 µm PVDF membrane (BioRad CA, USA). In paper IV, ~20µg protein was loaded on 4-15% Mini-PROTEAN TGX Precast Gels (Bio-Rad, CA, USA), and blotted on 0.2 µm Trans-Blot Turbo Midi Nitrocellulose membrane (BioRad CA, USA). Membranes were blocked using 5% non-fat dry milk in TBS-T, and incubated in primary antibody (shown in Table 4) overnight at +4°C, and incubated with a secondary Donkey Anti-Rabbit IgG H&L (Abcam; ab16284; 1:10000) or Donkey Anti-Mouse IgG H&L (Abcam; ab6820; 1:10000) in RT for 2 hrs. Signal was visualized using Western Blotting Luminol Reagent (Santa Cruz, TX, USA) and imaged using a ChemiDoc MP Imaging System (Bio-Rad, CA, USA). *In paper II*, stain-free technology was used as loading control and for protein normalization using Image Lab Software 5.2.1 (Bio-Rad, CA, USA). *In paper IV*, β-actin was used as loading control and for protein normalization using ImageJ 1.50 software (National Institutes of Health, Bethesda, MD).

Table 4.
Overview of primary antibodies used for immunoblotting (WB) and immunofluorescence (IF) in paper II and paper IV.

Primary antibody	Application	Dilution	Target species	Company (ref)	Paper
β-Actin HRP conjugated	WB	1:10 000	-	Sigma Aldrich (A3854)	IV
Creb	WB	1:1000	Rabbit	BioNordika (9197S)	II
Phospho-Creb	WB	1:1000	Rabbit	BioNordika (9198S)	II
Desmin	WB	1:500	Rabbit	Abcam (ab15200)	IV
anti-HTT; clone mEM48	IF	1:400	Mouse	Millipore Sigma (MAB5374)	IV
Laminin	IF	1:500	Rabbit	Abcam (ab11575)	IV
MY-32 (MYH2)	WB	1:500	Mouse	Abcam (ab7784)	IV
MyoD1	WB	1:500	Mouse	Abcam (ab16148)	IV
Traf-5	WB	1:750	Rabbit	Santa Cruz Bio. (sc-7220)	II

Histological analysis

Perfusion and tissue preparation

Mice were perfused transcardially, under terminal sodium pentobarbital anesthesia (Apoteksbolaget, Lund, Sweden), with saline and subsequently with ice-cold 4% paraformaldehyde (PFA) for 10 min (at the rate of 10 ml/min)(shown in Figure 16) to rapidly and uniformly preserve tissue in a life-like state utilizing the circulatory system. The thoracic cavity was opened to expose the heart, and a small incision was made at the tip of the left ventricle. The perfusion needle (12-gauge) was inserted through the cut ventricle into the ascending aorta and stabilizes with a hemostat. A small incision on right atrium was made to allow the exit of blood and perfusates. The vessels were first rinsed at room temperature with saline solution to prevent high background staining in immunohistochemistry due to hydrogen peroxidase activity in the blood, until the saline solution was running clear. The flow was switched to freshly prepared ice-cold 4% PFA, and the same perfusion rate was used. The PFA was circulated for an additional 8 minutes to ensure adequate fixation of the whole body. An indicator of a good perfusion is the clearing of the liver, and full forelimb and tail extension. Brain, skeletal muscle, BAT and WAT were promptly isolated by placing them in 4% PFA for 24 hours at 4°C for post-fixation. Brains were then transferred to 30% sucrose solution complemented with sodium azide at 4 °C until use. Skeletal muscle, WAT and BAT were transferred to 70% ethanol solution and stored at 4°C before paraffin embedding.

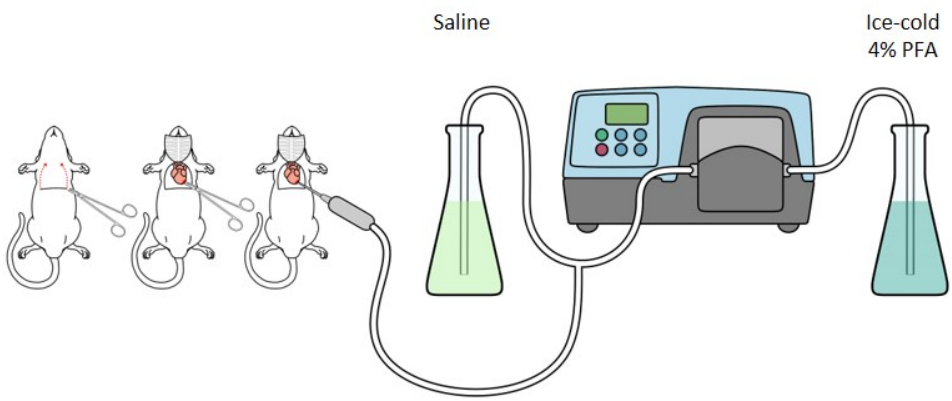

Figure perfusion 16. Illustated figure on whole-body perfusion.
The thoracic cavity was opened and a small incision was done in the tip of the left venticle, in which the perfusion needled was inserted into the ascending aorta. Before starting the perfusion, a small incision was done in the right atrium to allow the exit of blood, and perfusates. Saline was first used in the perfusion to rinse the vessels, which was then switch to ice-cold 4% PFA for fixation and preservation of the whole-body.

Immunohistochemistry (IHC)

IHC is a common technique for morphological characterization using antibodies to detect and analyze protein expression while the composition, cellular characteristics and structure are maintained.

In paper I, free-floating brain sections (30 μm sections, six series per animal) were used for immunohistochemistry (IHC) staining. Free-floating brain sections were rinsed with 0.01M phosphate buffered saline (PBS) 3 times for 10 minutes. Next, the sections were incubated in 0.01M citrate buffer, pH 8.5 at 80°C for 40 minutes for the antigen retrieval. Sections were left in PBS containing 3% H2O2 and 10% methanol for quenching. The sections were preincubated for 1hr at room temperature (RT) with blocking solution containing 5% normal donkey serum and 1% bovine serum albumin (BSA) in 0.3% Triton X/PBS, followed by incubation with primary antibody (Merck Millipore; MAB5374; 1:400) in blocking solution overnight on a shaker at 4°C. The sections were washed 3 times for 10 minutes in PBS then incubated with secondary (biotinylated horse anti-mouse; 1:200 dilutions) antibody. Sections were rinsed with PBS and incubated with the avidin-biotinperoxidase complex solution (VECASTAIN ABC Kit; Elite PK 6100 – Standard, Vector Laboratories Inc., Burlingame, CA USA) for 1h at RT. The sections were rinsed with PBS 3x10min and then lastly incubated with 3'3 – Diaminobenidine (DAB) and H2O2 (DAB Kit SK-4100, Vector Laboratories Inc., Burlingame, CA USA) to visualize the aggregates. Finally, the sections are mounted on gelatin covered slides, dried overnight and dehydrated through a graded alcohol series to xylene and coverslipped with DPX mountant (Sigma Aldrich, Saint Louis, MO, USA). For the assessment of striatal volume, cortex and corpus callosum thickness, cresyl violet (Nissl) staining was performed. First, free-floating brain sections were rinsed 3 times for 10 minutes with TBS to remove the antifreeze solution. The brain sections were mounted on gelatin-coated glass slides and air-dried at room temperature. The slides were passed through xylene and a series of decreasing ethanol solutions for 1 minute each (100%, 95%, 70% ethanol). After dehydrating in distilled water for 1 minute, the sections were left in 0.5% cresyl violet solution for 30 seconds to 1 minute. The sections were dehydrated in increasing ethanol solutions (70%, 95%, 100%) and cleared in xylene for 5 minutes. Lastly, the glass slides were covered using DPX (Sigma-Aldrich, Saint Louis, MO, USA).

Immunofluorescence (IF)

IF is a method used to reveal the localization and analyze the expression levels of the protein of interest using a fluorescent tagged primary or secondary antibody. IF is a powerful tool, since multiple fluorescent with different colors can be used within the same sample to investigate changes and co-localization of proteins.

In paper IV, paraffin embedded sections (7µm) of skeletal muscle gastrocnemius from 12 weeks old WT and R6/2$^{(CAG\ 242\text{-}257)}$ male mice were stained for HTT (em48), Laminin and DAPI, in order to visualize mHTT inclusions in skeletal muscle fibers. Deparaffinized sections went through antigen retrieval using 0.01M citrate buffer for 20 min at 95°C before pre-incubation with 5% NDS, 0.25% Tx, 1% BSA and Tris HCl ph 6 for 1 hr at RT. The sections were incubated overnight at 4°C in pre-incubation solution containing mouse monoclonal em48 (Merck Millipore; MAB5374; 1:400) and rabbit polyclonal Laminin (Abcam; ab11575; 1:500) antibodies. After washing several times with PBS, sections were incubated for 2 hrs in pre-incubation solution containing secondary Donkey AF488 anti-mouse (Jackson ImmunoResearch; 715-545-150; 1:500) or Donkey Cy3 anti-rabbit (Jackson ImmunoResearch; 711-165-152; 1:500) antibody and DAPI.

Hematoxylin & Eosin (H&E) staining

H&E staining is a method used to study histological changes in tissue. Hematoxylin stains the cell nuclei blue, while eosin stains the extracellular matrix and cytoplasm pink. In paper I, II and IV, we used this method to stain skeletal muscle (gastrocnemius, femoris and soleus) and WAT. Paraffin embedded tissue were sectioned (7µm), and H&E stained. Digital images of the stained sections were used to identify the morphological features of the muscle and WAT. A bright-light microscope (Olympus U-HSCBM, Olympus, Tokyo, Japan) with a 20x magnification objective, digital camera and image capture software (cellSens Dimensions 1.11 software; Olympus, Tokyo, Japan) were used.

Stereological measurements

To assess the diversity in samples from HD mouse models and compared to WT mice, stereological measurements were carried out under the blinded conditions to genotypes and treatment.

In paper I, volume of striatum, cortex, corpus callosum thickness, and number and size of mHTT inclusions were assessed on cresyl violet stained coronal sections. First, cresyl violet strained brain sections were scanned using an automated digital microscope (Zeiss, Axio Zoom.V16), and then dorsal striatum volume (bregma 1.70 mm to 0.38 mm), medial corpus callosum thickness (bregma 1.10 mm to 0.38 mm), and primary motor area Bregma (1.70 mm to 0.14. mm) were measured (Franklin and Paxinos, 2008) using Zeiss Zen (Blue edition) software. Quantification for mHTT inclusions in the striatum and cortex were implemented on brain sections stained for EM48 antibody using DAB immunohistochemistry. For the quantification of striatal inclusions, a total of 16 z-stack images collected per animal (4 Z-stack images from 4 consecutive brain sections between the bregma levels of anterior-posterior: 1.10mm to 0.38 mm). The cortical inclusion quantifications were performed on the insular cortex and the sampling was performed between the bregma levels of anterior-posterior: 1.54 mm to 0.62 mm and 4 z-stack images were collected per animal (1 z-stack image from 4 consecutive sections of insular cortex). The z-stack images were processed using ImageJ/FijiJ (National Institutes of Health, USA).

In paper IV, fiber diameters were assessed on H&E stained gastrocnemius and soleus muscles from R6/2$^{(CAG\ 266\text{-}328)}$ and WT mice.

Stereological measurements were performed using Stereo Investigator, and a Leitz DMRBE (Leica, Kista, Sweden) microscope with a 20x magnification objective was used.

Statistical analysis

Statistical analyses were performed using GraphPad Prism GraphPad software Inc. San Diego, CA, USA). Results are presented as means ± SEM. Differences with a $p<0.05$ were considered statistically significant.

In paper I, Shapiro-Wilk normality test was used to determine a Gaussian distribution. One-way or two-way factor analysis of variance (ANOVA) with Holm-Sidak post-hoc test, or Kruskal-Wallis with Dunn´s post-hoc test was used for multiple comparisons. Student´s unpaired t-test with Welch's correction was used analyzing number and size of inclusions in striatum and cortex.

In paper II, one-way or two-way factor analysis of variance (ANOVA), with Bonferroni post-hoc test, were used for multiple comparisons.

In paper III, after the identification of outliers with the ROUT test and the Kolmogorov-Smirnov normality test, statistical significance was determined using one-way factor analysis of variance (ANOVA), with Bonferroni, Tukey, Sidak or unprotected Fisher's LSD post-hoc tests (for a Gaussian distribution), or Kruskal-Wallis test, with Dunn post-test (non-Gaussian distribution) for multiple comparisons.

In paper IV, Shapiro-Wilk normality test was used to determine a Gaussian distribution. One-way or two-way factor analysis of variance (ANOVA) with Holm-Sidak's post hoc test was used for multiple comparisons, Kruskal-Wallis with Dunn´s post-hoc test or two-tailed Student's t-test was used for comparisons.

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Appendix (*Paper I-IV*)

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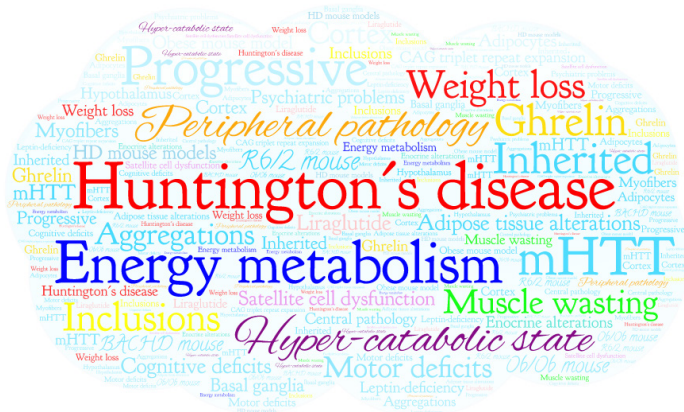

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