β-cell dysfunction in Huntington's disease

Bacos, Karl

2009

β-cell dysfunction in Huntington's disease

Karl Bacos

Unit of Molecular Metabolism
Department of Clinical Sciences in Malmö

LUND UNIVERSITY
Faculty of Medicine

With approval of the Lund University Faculty of Medicine,
this thesis will be defended on May 8 2009, at 13.00
in the Segerfalk lecture hall, Wallenberg Neuroscience Center, Lund.

Faculty opponent:
Professor Neil Aronin
Department of Medicine
University of Massachusetts Medical School

Organization	Document name
LUND UNIVERSITY Molecular Metabolism CRC 91:11 205 02 Malmö	DOCTORAL DISSERTATION
	Date of issue May 8, 2009
	Sponsoring organization
Author(s) Karl Bacos	

Title and subtitle

β-cell dysfunction in Huntington's disease

Abstract

Huntington's disease (HD), a fatal neurodegenerative disorder, is associated with an increased risk of diabetes mellitus. The reason for this is unknown, but considering the functional similarities of neurons and the insulin secreting pancreatic β-cell, pathological mechanisms may be shared by both cell types and account for neuronal as well as endocrine dysfunction. Hence, finding the mechanisms behind β-cell dysfunction in HD could identify potential therapeutic targets for the neuronal disease.

Upon characterization of the R6/2 mouse model we found that mutant huntingtin renders β-cells replication-deficient. This results in a reduced β-cell mass in R6/2 compared to WT mice. In addition, islet insulin content is reduced and a dramatic degranulation of β-cells is evident. As a consequence, insulin secretion is severely blunted and R6/2 mice become glucose intolerant. In our present studies we could, however, not find any morphological alterations in pancreatic sections from HD patients. Thus, what pathogenetic defects that underlie the secretory deficiency evident in HD patients remain to be resolved. In an effort to identify such defects we created an in vitro model of the HD β-cell and found that glucose-stimulated insulin secretion is significantly blunted by mutant huntingtin. Because metabolic perturbations have been identified in HD and are a possible cause of the secretory defect, we investigated cellular metabolism. However, we found glucose oxidation and mitochondrial respiration, as well as expression levels of metabolic enzymes, unaltered. This suggests that metabolic aberrations do not contribute to cell dysfunction in our model. Next we analyzed vesicular trafficking as this has been found aberrant in HD and is vital for sustained insulin release. These studies showed that the transport of insulin granules along microtubule filaments is perturbed. Furthermore, we found that mutant huntingtin interacts aberrantly with β-tubulin. Therefore we hypothesize that mutant huntingtin acts as a physical block for vesicular transport. This mechanism is likely not specific for β-cells and might therefore contribute to the trafficking defects seen in neurons. Thus, attenuating the huntingtin/β-tubulin interaction may have beneficial effects in HD patients.

Key words: Insulin, secretion, trafficking, R6/2 mouse, granule, exocytosis, huntingtin, neurodegeneration, microtubule, metabolism

Classification system and/or index termes (if any):

Supplementary bibliographical information:		Language English
ISSN and key title: 1652-8220		ISBN 978-91-86253-37-0
Recipient's notes	Number of pages 140	Price
	Security classification	

β-cell dysfunction in Huntington's disease

Karl Bacos

Unit of Molecular Metabolism
Department of Clinical Sciences in Malmö

LUND UNIVERSITY
Faculty of Medicine

ISSN 1652-8220
ISBN 978-91-86253-37-0
Lund University, Faculty of Medicine Doctoral Dissertation Series 2009:49

Table of contents

Articles and manuscripts included in this thesis

This thesis is based on the following articles, referred to in the text by their roman numerals.

I: Björkqvist M, Fex M, Renström E, Petersén Å, Wierup N, Gil J, **Bacos K**, Popovic N, Li J.-Y, Sundler F, Brundin P, Mulder H. **The transgenic R6/2 mouse model of Huntington's disease develops diabetes due to deficient β-cell mass and impaired exocytosis.** Hum Mol Genetics 14:565-574, 2005.

II: **Bacos K**, Björkqvist M, Petersén Å, Luts L, Maat-Schieman MM, Roos RA, Sundler F, Brundin P, Mulder H, Wierup N. **Islet β-cell area and hormone expression are unaltered in Huntington's disease.** Histochem Cell Biol 129: 623-9, 2008.

III: **Bacos K**, Malmgren S, Kotova O, Smith R, Mulder H. **Perturbation of mitochondrial metabolism does not underlie early β-cell dysfunction in a model of Huntington's disease.** Manuscript

IV: Smith R*, **Bacos K***, Fedele V, Soulet D, Walz H, Obermuller S, Lindqvist A, Björkqvist M, Klein P, Önnerfjord P, Brundin P, Mulder H, Li JY. **Mutant huntingtin interacts with β-tubulin and disrupts vesicular transport and insulin secretion.** Manuscript
* Equal contribution

Published articles not included in this thesis

Fex M, Olofsson CS, Fransson U, **Bacos K**, Lindvall H, Sörhede-Winzell M, Rorsman P, Holm C, Mulder H. **Hormone-sensitive lipase deficiency in mouse islets abolishes neutral cholesterol ester hydrolase activity but leaves lipolysis, acylglycerides, fat oxidation, and insulin secretion intact.** Endocrinology. 2004 Aug;145(8):3746-53.

Björkqvist M, Petersén A, **Bacos K**, Isaacs J, Norlén P, Gil J, Popovic N, Sundler F, Bates GP, Tabrizi SJ, Brundin P, Mulder H. **Progressive alterations in the hypothalamic-pituitary-adrenal axis in the R6/2 transgenic mouse model of Huntington's disease.** Hum Mol Genet. 2006 May 15;15(10):1713-21.

Anderson K, Rusterholz C, Månsson R, Jensen CT, **Bacos K**, Zandi S, Sasaki Y, Nerlov C, Sigvardsson M, Jacobsen SE. **Ectopic expression of PAX5 promotes maintenance of biphenotypic myeloid progenitors coexpressing myeloid and B-cell lineage-associated genes.** Blood. 2007 May 1;109(9):3697-705.

van der Burg JM, **Bacos K**, Wood NI, Lindqvist A, Wierup N, Woodman B, Wamsteeker JI, Smith R, Deierborg T, Kuhar MJ, Bates GP, Mulder H, Erlanson-Albertsson C, Morton AJ, Brundin P, Petersén A, Björkqvist M. **Increased metabolism in the R6/2 mouse model of Huntington's disease.** Neurobiol Dis. 2008 Jan;29(1):41-51.

Abbreviations

AD	Alzheimer's disease
ATP	adenosine triphosphate
ADP	adenosine diphosphate
BDNF	brain-derived neurotrophic factor
cAMP	cyclic adenosine monophosphate
CPT1	carnitine palmitoyl-transferase 1
eGFP	enhanced green fluorescent protein
ER	endoplasmic reticulum
ETC	electron transport chain
$FADH_2$	flavin adenine dinucleotide
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GK	glucokinase
GLP-1	glucagon-like peptide-1
GLUT	glucose transporter
GRX	glutaredoxin
GSIS	glucose-stimulated insulin secretion
GTP	guanosine triphosphate
GTT	glucose tolerance test
HAP1	huntingtin-associated protein 1
HD	Huntington's disease
HEK	human embryonic kidney
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
IAPP	islet amyloid polypeptide
(i)FRAP	(inverse) fluorescence recovery after photobleaching
IGT	impaired glucose tolerance
ITT	insulin tolerance test
JNK	c-Jun N-terminal kinase
K_{ATP}-channel	ATP-sensitive potassium channel
kDa	kilodalton
αKDH	α-ketoglutarate dehydrogenase
KHC	kinesin heavy chain
KLC	kinesin light chain

LDH	lactate dehydrogenase
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
MCS	multiple cloning site
MFN2	mitofusin 2
MPT	mitochondrial permeability transition
MS	mass spectrometry
MTS	methyl-tetrazolium
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartic acid
NSF	N-ethylmaleimide-sensitive factor
PC	pyruvate carboxylase
PDH	pyruvate dehydrogenase
PFK	phosphofructokinase
PGC-1α	peroxisome proliferator-activated receptor gamma 1α
Phogrin	phosphatase on the granule of insulinoma cells
PKA	protein kinase A
PLA	proximity ligation assay
(Q)-PCR	(quantitative) polymerase chain reaction
ROS	reactive oxygen species
RRP	readily releasable pool
SBMA	spinal and bulbar muscular atrophy
siRNA	short interfering RNA
SDH	succinate dehydrogenase
(α-)SNAP	soluble NSF attachment protein
SNAP-25	synaptosome-associated protein of 25kDa
SNARE	soluble NSF attachment protein receptor
T2D	type 2 diabetes
TCA	tricarboxylic acid cycle
VAMP2	vesicle-associated membrane protein 2
VSVG	vesicular stomatitis virus G protein
WT	wild-type
YFP	yellow fluorescent protein

Introduction

This thesis covers an area between two research fields; experimental neurology and endocrinology. They overlap as several of the neurodegenerative diseases, including Huntington's disease, are associated with an increased risk of diabetes. Why this is the case remains unknown, but considering the functional similarity of neurons and β-cells, it is not unlikely that pathological alterations in one cell type are reflected by similar changes in the other. The first half of this introduction is focused on the β-cell and its function. The second half covers Huntington's disease and its pathogenesis.

The pancreatic β-cell

The pancreas contains both exocrine and endocrine tissue. The exocrine pancreas, which constitutes the vast majority of the organ, secretes digestive enzymes into the duodenum, thereby facilitating food digestion and nutrient uptake. The endocrine part consists of the islets of Langerhans, small clusters of cells dispersed throughout the pancreas. These islets contain glucagon producing α-cells, somatostatin producing δ-cells, pancreatic polypeptide producing PP-cells, ghrelin producing cells and insulin producing β-cells[1, 2]. The latter cell type comprises 60-80% of the cells in a human islet and has one major function: controlling blood glucose levels by secreting insulin.

Glucose-stimulated insulin secretion (GSIS)

Secretion of insulin is a tightly regulated multi step process that can be divided into a triggering and an amplifying pathway[3] (figure 1). Both pathways start with glucose transporter (GLUT) mediated uptake of glucose into the cell by a low affinity, high capacity transporter[4]. Glucose is then phosphorylated by the β-cell hexokinase, glucokinase (GK)[5], and metabolized through glycolysis into two molecules of pyruvate. In addition, glycolysis also produces two molecules of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH). Pyruvate enters the mitochondrion where it is converted into acetyl-CoA by pyruvate dehydrogenase (PDH), or into oxaloacetate by pyruvate carboxylase (PC). These molecules are oxidized in the tricarboxylic acid (TCA) cycle. In the process, six additional NADH molecules, two reduced flavin adenine dinucleotide ($FADH_2$) and two guanosine

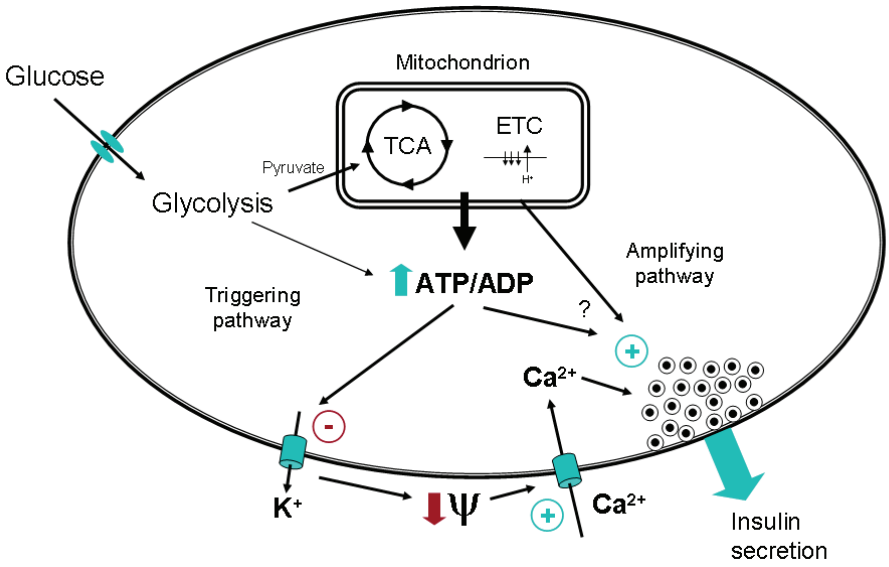

Figure 1. Schematic view of the triggering and amplifying pathways of GSIS. Details are provided in the text.

triphospate (GTP) molecules are produced per original glucose molecule. The cytosolic NADH produced by glycolysis are funneled into the mitochondria by mitochondrial shuttles[6] and, together with NADH and $FADH_2$ produced by the TCA cycle, activate the electron transport chain (ETC). Situated in, or attached to, the inner mitochondrial membrane, the four complexes of the ETC are NADH dehydrogenase (complex I), succinate dehydrogenase (SDH, complex II), coenzyme Q-cytochrome c reductase (complex III) and cytochrome c oxidase (complex IV). Electrons, from reducing equivalents produced by glycolytic and TCA cycle metabolism, are passed from complex to complex, via the ubiquinone and cytochrome c electron carriers. In the process, hydrogen ions are pumped out from the mitochondrial matrix into the intermembraneous space. This builds up an electrochemical proton gradient across the inner mitochondrial membrane that is used by the ATP synthase, sometimes referred to as complex V, to produce ATP[7]. ATP is transported into the cytosol, resulting in an increase of the cytosolic ATP/ADP ratio and closure of the ATP sensitive K^+ (K_{ATP})-channels[8]. Because the current of K^+ ions out of the β-cell ceases, the plasma membrane will depolarize[9] and voltage-sensitive Ca^{2+} channels will open[10]. Due to the electrical gradient, Ca^{2+} rushes into the cell and triggers insulin secretion. While the triggering pathway thus has reached completion, insulin secretion can be augmented further by the so called amplifying pathway. This became evident almost 20 years ago when Gembal et al. showed

that glucose metabolism could further enhance insulin secretion even when the K_{ATP} channels were circumvented and Ca^{2+} influx was not further increased[11]. Over the years that have passed since the original discovery, many factors have been suggested to contribute to the amplifying pathway (as reviewed by Wollheim and Maechler[12]); some of them will be reviewed briefly here.

ATP not only affects the K_{ATP} channel, it is also involved in priming insulin granules for exocytosis and can further enhance insulin secretion at stimulatory Ca^{2+} levels[13]. In addition, reducing the ATP/ADP ratio by inhibiting mitochondrial function reduces insulin secretion even though cytosolic Ca^{2+} levels are kept elevated[14].

GTP levels also increase during glucose metabolism[15] and short interfering (si)RNA mediated knock down of the GTP-producing isoform of succinyl-CoA-synthetase, resulted in lower levels of cellular GTP and a reduction of insulin secretion. Knocking down the ATP producing isoform instead had the opposite effects[16].

NADPH has long been thought to enhance insulin secretion[17]. More recently it was shown that mitochondrial shuttles activated by glucose metabolism cause an increase in the $NADPH/NADP^+$ ratio[18]. This may in turn augment insulin secretion through the redox protein glutaredoxin (GRX), possibly by direct effects on insulin granules[19].

Glutamate was shown to be increased upon glucose stimulation of β-cells and stimulate insulin secretion independently of mitochondrial metabolism[20]. As insulin granules are loaded with glutamate, it was suggested to have a direct effect on the vesicles[21]. However, the role of glutamate in the amplifying pathway remains controversial as it has been refuted as a coupling factor in insulin secretion in some studies[22, 23].

Malonyl-CoA is increased by glucose metabolism. This molecule inhibits the carnitine palmitoyl-transferease (CPT1). As a result, acyl-CoA transport into the mitochondria and β-oxidation are reduced. Thus, acyl-CoA may accumulate in the cytoplasm and act as a coupling factor stimulating insulin secretion[24]. Again, however, studies arguing against this hypothesis have been published[25].

Whether cyclic AMP (cAMP) is produced in response to increased glucose metabolism or not is uncertain[26-28], but it is an important potentiator of insulin secretion. Via protein kinase A (PKA) activation, cAMP increases Ca^{2+} influx[29] and accelerates the refilling of the so called readily releasable pool[30] (RRP, see next section) of insulin granules. In addition, cAMP also increases exocytosis of insulin granules independently of PKA by activating the cAMP-GEFII/Rim2 complex[31] and thereby vesicle acidification and priming[32].

Insulin secretion is biphasic

A mouse β-cell contains ~10 000 insulin granules[33]. Of these, ~600 are docked to the plasma membrane and contain a subset (~10%) of granules that constitute the RRP. These granules are primed for exocytosis and can fuse with the plasma membrane immediately upon Ca^{2+} triggering[33, 34]. The granules in the RRP are secreted during the first phase of insulin secretion, a rapid and transient (~5-10min) response to glucose stimulation characterized by a high frequency of secretory events[34] (figure 2). After this initial burst of granule secretion, the RRP has to be replenished for insulin secretion to continue with its second phase. This occurs both by priming of granules in the remainder of docked granules and by translocation and priming of granules from the interior of the cell out to the plasma membrane. This is a relatively slow process causing exocytotic events to occur with lower frequency. However, the second phase will be sustained as long as the β-cell is exposed to stimulatory glucose levels.

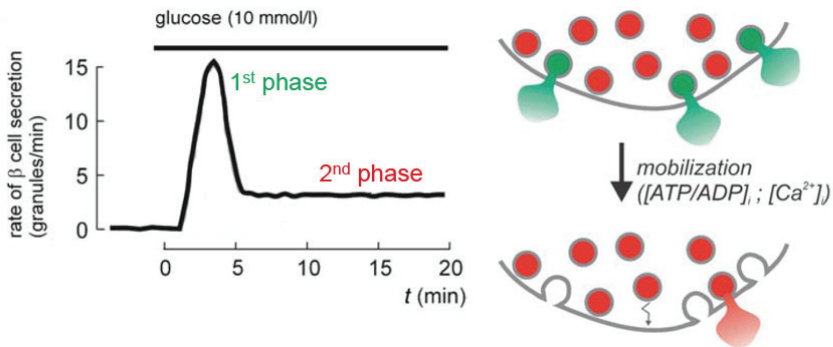

Figure 2. Insulin secretion is biphasic. Docked and primed vesicles (green) are released during the first phase. The second phase of secretion is dependent on release of both previously docked and newly recruited granules (red). Figure adapted from Rorsman and Renström[34].

Granule translocation

Granules in the interior of the β-cell have to be translocated to the plasma membrane to be secreted. This process is ATP-dependent and relies on the motor proteins kinesin and myosin and their transport along cytoskeletal tubulin and actin filaments respectively. Kinesins are heterotetramers built up by two heavy (KHC) and two light (KLC) chains. The N-terminal globular heads of KHC bind to microtubules and move the complex towards the plus end of the filament while hydrolyzing ATP. The elongated coiled-coil domain is in-

volved in dimerization. The C-terminal end of KHC, together with KLC, binds to the cargo to be transported[35, 36]. The involvement of kinesin in insulin secretion was shown by reducing its expression[37, 38] or introducing a dominant negative kinesin mutant[39], as this leads to reduced granule movement and a blunted insulin secretion in response to glucose. The increase in cytosolic Ca^{2+} in response to elevated extracellular glucose activates the phosphatase calcineurin. In turn, calcineurin dephosphorylates and activates kinesin[40]. Pharmacological inhibition of the phosphatase decreases the amount of insulin released during the second phase by ~50%[40]. Disrupting the microtubule network also decreased insulin release by half, but reduced the number of directed granule translocations by as much as 85%[41]. This suggests that diffusion of insulin granules maintains secretion to some degree. The fact that ~2000 insulin granules are situated within one granule diameter of the plasma membrane[33] supports this assumption. Interestingly, Ivarsson et al. also found that the number of kinesin-mediated translocations along microtubular strands depends on random diffusive movements by the vesicle, probably through increasing the likelihood of a kinesin/vesicle complex to come in contact with a microtubule[41]. Although kinesins are vital, they do not transport the vesicles all the way to the plasma membrane and are therefore not enough to sustain stimulated insulin release at a high level. Along the actin filaments located immediately beneath the plasma membrane, insulin granules are instead transported by Myosin Va. Disrupting this motor protein causes a reduction in granule recruitment and insulin release[42,43]. Dynein motors, moving towards the minus end of microtubular strands, are involved in retrieving insulin granules after kiss-and-run exocytosis[38].

Granule priming and exocytosis

The acquisition of release competence, a process called priming, is a prerequisite for the exocytosis of an insulin granule. How priming occurs is not yet completely known, however, the process is dependent on ATP[13] and granular acidification[44]. Exocytosis of granules requires the assembly of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complexes, as do all membrane fusion events in the secretory pathway[45, 46]. The SNARE complex involved in insulin granule release consists of syntaxin 1 and SNAP-25 (synaptosome-associated protein of 25kDa), situated in and in association with the plasma membrane, and synaptobrevin (also called vesicle-associated membrane protein, VAMP2) situated in the granule membrane. Together, the three SNARE proteins form a helical bundle tightly binding the granule in close proximity of the plasma membrane. According to the most widely supported model, energy released during the formation of this structure mediates the fusion of the plasma and vesicular membranes. The importance of a functional

SNARE complex for insulin release has been shown in numerous studies and disturbing any of the three components leads to secretory defects[47-49]. The formation, function and dissociation of the SNARE complex is regulated by a plethora of other proteins[45, 46]. Sec1/Munc18-related proteins are believed to be involved in the formation of SNARE complexes. Munc13-1 interacts with syntaxin 1 and may facilitate the conversion from a closed to an open state, thereby making it possible for syntaxin 1 to associate with SNAP-25 and synaptobrevin[50]. An opposite function of Munc18-1 has been proposed. Recent data, however, suggests that Munc18-1 in association with the granular protein granuphilin also plays a positive role in SNARE-complex formation[51]. Synaptotagmins are probably the Ca^{2+} sensors coupling the inflow of ions to the initiation of secretion. There are several different isoforms of synaptotagmins and the expression pattern differs from cell type to cell type. In β-cells synaptotagmin VII[52, 53] and IX[54] are important for insulin release. After mediating fusion of the granular and plasma membranes, SNARE complexes have to be dissociated and recycled for reuse. If not, secretion will be disrupted. The process is ATP-dependent and mediated by NSF (N-ethylmaleimide-sensitive factor), which is activated by and binds to the SNARE complex through α-SNAP (α-NSF attachment protein)[45, 55, 56]. Several SNARE and SNARE-modulating proteins are downregulated in type 2 diabetes (T2D)[57] indicating that exocytotic defects may contribute to the development of this disease.

β-cell dysfunction in T2D

In the face of an augmented insulin demand due to insulin resistance, β-cell mass and insulin secretory capacity increase to maintain blood glucose and whole body metabolism regulated. When this fails, due to metabolic overload, oxidative stress, increased apoptosis and down-regulation of components in the secretory pathway, diabetes develops[58]. Apart from *in vivo* metabolic analyses, e.g. glucose tolerance tests showing secretory defects in individuals with insulin resistance or T2D, studies on human islets are limited. However, reduced β-cell mass as a result of increased apoptosis[59] and reduction in secretory capacity due to mitochondrial perturbations[60, 61] are evident in T2D. An abundance of studies on animal and cell models of T2D have been published and these have identified other pathological processes. For example, augmented metabolism driven by a surplus of nutrients results in elevated production of reactive oxygen species (ROS). β-cells express low levels of antioxidant enzymes[62], suggesting that they are more sensitive to these reactive molecules. In fact, studies have shown that elevating levels of ROS scavenging enzymes protect rodents from oxidative stress induced diabetes[63]. Unfolded protein response and endoplasmic reticulum (ER) stress might also contribute[64]. Proteins involved in these processes are upregulated in patients and rodent

models of T2D[65]. Furthermore, saturated lipids induce a stress response by depleting the ER of Ca^{2+} and thereby interfering with protein folding[66] and also elevated glucose levels can induce ER stress[67].

Huntington's disease

Huntington's disease (HD), first described in the second half of the 19th century by George Huntington[68], is an autosomal dominant neurodegenerative disorder. Apart from the characteristic symptoms emanating from cell dysfunction and death within the central nervous system, HD patients also develop and suffer from peripheral pathologies. Among these, impaired glucose tolerance is the focus of this thesis.

Genetics

HD affects 4-8 out of every 100 000 individuals in populations of European descent, while the frequency is approximately one order of magnitude lower in Asian and African populations[69]. The disease develops due to an expansion of a CAG triplet repeat in the *IT-15* gene on the short arm of chromosome 4[70]. Alleles with >40 repeats invariably leads to disease development while alleles with 36-39 repeats are associated with a reduced penetrance[71, 72]. Alleles with 27-35 repeats do not cause HD, but are associated with an increased risk of expansion during transmission, especially paternal[72]. Repeat length also correlates with the age of onset of the disease with longer repeat length causing earlier onset[73]. As a result, repeat sizes of ~60 and upwards are associated with juvenile (21 or younger) onset[74]. The correlation is strongest for repeat sizes above 50 and, in total, repeat size accounts for ~65% of the variation of age at onset[72]. The remaining variation is accounted for by modifier genes and environment[75]. The gene alteration is dominant and homozygosity does not affect age of onset, however, it leads to a more rapid disease progression[76].

Pathology of the central nervous system

The literature on neuronal degeneration and dysfunction and the resulting symptoms of HD is abundant. The scope of this thesis allows only a very brief summary of the topic and the reader is referred to other sources for more information[77, 78].

The striatum is the most severely affected region of the brain with the caudate nucleus being involved before the putamen[79]. The neuronal subtypes in the striatum are differentially affected; medium-sized spiny neurons degenerate first while aspiny interneurons are affected later in the disease. Apart from striatal degeneration, also cortex[80], thalamus[81], hypothalamus[82], cerebellum[83], hippocampus[84] and brainstem[85] degenerate.

Although varying somewhat from case to case, symptoms of HD develop gradually and usually start with personality changes and involuntary movements of smaller distal muscles. Eventually, patients develop the characteristic dance-like (choreatic) movements due to involvement of larger muscle groups. In end stage patients, this is replaced by rigidity, dystonia and hypokinesis. Concurrent with the development of locomotor disturbance, cognitive function, mood disorder and dementia worsen. The disease progresses for ~15-20 years and invariably leads to death. The most common causes of death are pneumonia and cardiovascular disease[86].

Glucose intolerance in HD

Several neurodegenerative diseases are associated with impairments of glucose metabolism[87]. In some, diabetes is a typical component while others are associated with an increased risk of diabetes or milder defects in glucose tolerance. HD belongs to the latter group. However, the degree of association of HD and diabetes is somewhat tentative due to four facts. First, the functional studies performed have identified highly variable frequencies of alterations in glucose tolerance, ranging from 0 to 60%. Second, most studies have been small, making frequency estimates uncertain. Third, individual data were not presented in all studies, making it possible that alterations in just one or a few patients are averaged out by normoglycemic patients. Finally, subjects in some studies are incompletely characterized with regards to treatment, disease severity etc. The published functional studies are discussed in the next section and summarized in table 1.

Podolsky and coauthors found that 50-60% of HD patients exhibited impaired glucose tolerance (IGT)[88-90]. Fasting plasma insulin levels were slightly elevated and insulin secretion in response to an oral glucose tolerance test (GTT) was augmented, suggesting insulin resistance. Schubotz et al. presented similar data in a study where 32% of subjects had abnormal GTTs[91]. In the study from Kremer et al., one out of nine patients exhibited IGT[92]. In a follow up 2.5 years later of six of the patients, clinical deterioration was apparent but only the

Table 1. Summary of GTTs performed on HD patients

Study	IGT[a]	Insulin	Reference
Podolsky et. al	7/14 and 6/10	+	88-90
Schubotz et. al	8/25	+	91
Kremer et. al	1/9	+/-	92
Davidson et. al	2/11	+/-	93
Phillipson et. al	No[b]	+/-	94
Keogh et. al	No[b]	+/-	95
Lalic et. al	Yes[b]	-	97

[a] Number of HD patients with IGT [b] No individual data presented
+ increase, - decrease, +/- no change

same one patient exhibited impaired glucose tolerance. Two out of eleven patients were determined to be glucose intolerant in a study by Davidson and collaborators[93]. In addition, one subject was normoglycemic but exhibited severely elevated insulin levels during the GTT, indicating insulin resistance. Phillipson et al. reported normoglycemia in a group of nine patients[94]. However, plasma insulin levels were not measured in this study. Furthermore, insulin tolerance tests (ITT) were performed and shown to be normal in two studies[95,96]. Keogh and coauthors also performed a GTT in five patients, but no differences were reported[95]. The largest and most recently published functional study on HD patients is the one by Lalic et al[97]. They investigated 29 untreated non-diabetic HD patients and found that hepatic and peripheral insulin sensitivity was decreased and that insulin secretion was reduced. In summary, the outcome of glucose tolerance tests in HD probably depends on the patients included in the study. A more severe disease, longer duration of disease and metabolic state of the patients probably increases the chance of detecting differences. For example, in the study by Podolsky et al.[90] HD patients could be divided into two clearly distinct groups, glucose tolerant and glucose intolerant. The mean duration of disease in these groups were 4.3 and 8.2 years respectively. In addition to the functional studies, an epidemiological study showed that 10.5% of HD patients in the National HD Research Roster had diabetes[98]. Diabetes was more prevalent in HD patients compared to the control population at all age intervals, with differences being largest at young ages.

Mouse models of HD also develop abnormalities in glucose homeostasis. Elevated glucose levels have been found in several colonies of R6/2 mice[99-108] (see Models and Methods part for more information on the R6/2 mouse). In one study, seven week old R6/2 mice were

found to have elevated plasma insulin levels despite being normoglycemic[100]. These changes were likely due to corticosterone-induced insulin resistance. At end stage glucose homeostasis deteriorated indicating a failure of the compensatory hypersecretion of insulin. In other studies, however, mice did not exhibit elevated insulin levels[109] or responded normally to the secretagogue glibenclamide, but not to the insulin sensitizers rosiglitazone[102] and metformin[105], indicating that insulin resistance was not a factor. Instead, reduced levels of transcription factors PDX1 and p300 and a resulting decline of insulin content and secretion[109] was proposed to be the cause of glucose intolerance. Studies using a metabolism enhancing drug or dietary supplement also hint towards a secretory defect. Reversing PDH inactivation with dichloroacetate normalized glucose levels in twelve week old R6/2 mice[99]. Although neither circulating insulin levels nor release was measured, such an effect could possibly be attributed to an increased metabolism in the β-cells, but liver may also be a target. Creatine supplement increased ATP production and insulin secretion *in vitro*[110] and also improved glucose tolerance in R6/2 mice[111]. Again, insulin was not measured and results on the effect of creatine on glucose homeostasis in humans are conflicting. Therefore, predicting the outcome of creatine supplements on glucose homeostasis in HD patients is difficult. The R6/1 mouse, a "sister model" of the R6/2 mouse, is not as well studied. Although some colonies have been reported as being normoglycemic[112, 113], also R6/1 mice show impairments in glucose homeostasis when challenged with a GTT[114]. The mice were normosensitive to insulin but exhibited reduced β-cell mass and insulin secretion. Finally, also the N171-82Q mouse is hyperglycemic[115-117] due to severe insulin resistance and reduced β-cell mass[117]. Treatment with Exendin-4, a long lasting glucagon like peptide (GLP)-1 receptor agonist, reversed the reduction in islet area and improved insulin sensitivity and blood glucose levels[117].

One last important issue has to be addressed and that is the possible contribution of diabetes to the development of HD. Insulin signalling has been shown to affect amyloid formation in models of Alzheimer's disease (AD) and diabetes is, although somewhat controversial, associated with an increased risk of AD[118]. Insulin signalling is likewise important for autophagocytic clearing of huntingtin aggregates[119] implying that diabetes might also affect the risk and/or development of HD. However, studies in mice argue against this. First of all, not all mice have defective glucose homeostasis. Those mice who do develop a diabetes-like state do not exhibit exaggerated neurological phenotypes or have shorter life spans[104]. In addition, DNA-vaccination against mutant huntingtin partially reversed the diabetic phenotype but had no effect on neurological findings[106].

Huntingtin

Huntingtin, a 350kDA protein encoded by the *IT-15* gene, is widely expressed both at the mRNA[120, 121] and protein[122-124] level. At the subcellular level, it is mainly localized in the cytoplasm in association with for example vesicles[125, 126], microtubule[126, 127] and mitochondria[128, 129]. Although the function of huntingtin is still debated, it is clear that abolishing its expression is not compatible with life, as knock-out embryos die in utero around embryonic day 8[130-132].

Huntingtin is processed by cellular proteases[133]. Several caspase and calpain cleavage sites have been identified in the vicinity of the polyQ domain. Cleavage at these sites produces N-terminal fragments that are suggested to be toxic. Accordingly, inhibiting these enzymes attenuate cell death and slows progression in HD models[133]. Caspase-6 mediated cleavage at amino acid 586 may be of special importance, as eliminating this site in HD mice hinders neurodegeneration[134]. N-terminal fragments also heavily contribute to the huntingtin aggregates that are formed in HD[135-137]. In HD patients aggregates have so far been found in brain[136] and muscle[138], while being more widespread in mouse models[139]. Aggregates were originally proposed to be the toxic species underlying neuronal death. However, during the last decade this viewpoint has been challenged[140, 141] and a study even suggests that aggregates may be protective[142]. There is no consensus on how the expanded polyQ domain causes cell dysfunction and death. However, several pathological mechanisms have been proposed and a few of them will be summarized in the following sections.

Mitochondrial dysfunction

Metabolic derangement in general, and mitochondrial dysfunction in particular, has long been hypothesized to cause or contribute to the pathogenesis of HD[143]. This assumption was based on a number of findings. For example, HD patients suffer from severe wasting despite normal or even increased caloric intake[144, 145] and patients with higher body mass index exhibit slower rate of disease progression[146]. Furthermore, glucose metabolism is reduced[147] and lactate levels increased[148] in both presymptomatic and symptomatic HD patient brain. Finally, chemical inhibition of complex II of the mitochondrial ETC produces functional and histological effects that bear some resemblance to those seen in HD[149].

Although a few studies indicate otherwise, most evidence points towards impaired cellular energetics in HD (summarized in figure 3). First of all, mitochondrial ultrastructural changes[150-154] and increased frequency of mitochondrial DNA deletions in leukocytes[155] and

brain[156] of HD patients have been reported. However, the deletion frequency was normal in striatum[156] and the authors suggested that this might be due to enhanced sensitivity and death of neurons with deletion in this brain region. Similarly, in a very small study (three patients and three controls), mitochondrial DNA deletions were found to be less frequent in HD striatum[157]. Extensive alterations of mitochondrial enzymes have also been reported, most frequently for the complexes of the ETC. Expression and/or activity of complex I[150, 158, 159], II/III[158, 160-166] and IV[158, 161, 163, 167] have been shown to be reduced in HD. Although not exclusive to the striatum, changes seem exacerbated in this region. Similar changes have also been identified in cell and animal models of HD[160, 168-172]. Alterations in ETC complex activity are, however, likely not a primary pathogenetic mechanism of HD since no changes can be seen in presymptomatic or grade 1 HD patients or before the onset of neuronal degeneration in HD mice[158]. Similarly, a cell line established from knock-in striata ($STHdh^{Q111/Q111}$) exhibit reduced ATP production without impairment of ETC complex activity, indicating that other factors contribute to the energetic defect[173]. These could be changes in metabolic enzymes upstream of the ETC. SDH, discussed above as complex II, is also a part of the TCA cycle and activities of α-ketoglutarate dehydrogenase[174] (α-KDH) and aconitase[166,172,175], also involved in this process, are reduced. In addition, lactate dehydrogenase[176] (LDH) and PDH[99, 162, 177, 178] activity is diminished indicating that the lactate/pyruvate conversion and the entry of the latter into the TCA might be perturbed.

Reduced level of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) is a possible cause of the reductions in mitochondrial enzymes. PGC-1α is a transcriptional coactivator regulating expression of for example proteins involved in oxidative phosphorylation. Mutant huntingtin binds to the PGC-1α promoter, causing lower transcript levels[179]. Consequently, transcription of PGC-1α target genes is reduced in HD[180]. Recently, sequence variation in the PGC-1α gene has been found to modify the age of onset of HD[181, 182]. There is also evidence for oxidative stress in the mitochondrial pathogenesis, for example increased oxidation of metabolic enzymes and mitochondrial DNA[183]. Together, the mitochondrial changes mentioned here contribute to the reduced basal ATP levels[154, 184-186] and production[138, 173, 185] seen in HD and cell models thereof.

Mitochondrial Ca^{2+} handling is also perturbed in HD. Mitochondria from HD patients and models have reduced calcium retention capacity. Therefore, increasing Ca^{2+} concentration, by direct addition of the ion to mitochondrial suspensions or activation of N-methyl-D-aspartic acid (NMDA) receptors on intact cells, leads to increases in cytosolic Ca^{2+}, reductions of mitochondrial membrane potential and respiration[128, 170, 187-192]. Inhibiting the mito-

chondrial permeability transition (MPT) pore can reverse these changes[128, 187, 188]. Interestingly these effects can, at least in part, be attributed to immediate consequences of mutant huntingtin. When added to healthy mitochondria, mutant huntingtin interacts with the outer mitochondrial membrane and induces Ca^{2+} deregulation and loss of mitochondrial membrane potential[128, 191, 192]. Ca^{2+} handling defects are evident before phenotypic onset in a mouse model[192], suggesting that they could be central in disease development. More indirect effects seem to be mediated by the tumor suppressor protein p53. Mutant huntingtin interacts with and enhances the expression of this protein, resulting in mitochondrial depolarization[168]. One possible explanation for the effect of p53 is through its target genes, Bax and Puma, which could depolarize the mitochondria. Furthermore, ATP production is vital for mitochondrial Ca^{2+} homeostasis[189] and other mitochondrial abnormalities could therefore exacerbate these defects at later stages of the disease. The deregulation of mitochondrial Ca^{2+} handling and opening of the MPT pore is associated with cytochrome c release[128] and caspase activation[193], lending further support to the hypothesis that excitotoxicity underlies cell death in HD[194]. One should keep in mind that many of these studies have been performed on isolated mitochondria and as these respond differently than mitochondria *in situ* the experimental results may be somewhat misleading[190].

A recent study implicated aberrant mitochondrial fusion and fission in HD pathogenesis[154]. This study reports increased mitochondrial fragmentation in a cell model of HD, possibly through aberrant interaction between mutant huntingtin and the mitochondrial fusion protein mitofusin 2 (Mfn2). Overexpressing this protein, or inhibiting mitochondrial fission, reversed mitochondrial fragmentation and normalized levels of ATP and cell death. Furthermore, locomotor activity in a Caenorhabditis elegans model of HD was enhanced by Mfn2 expression.

The above mentioned studies all point to mitochondrial dysfunction in HD. However, there are also reports suggesting that metabolic alterations outside the mitochondria contribute. An unbiased gene expression analysis revealed a significant suppression of glycolytic gene expression in striatal cells from a knock-in mouse model[195]. In addition, the activity of the glycolytic enzymes enolase[177], phosphofructokinase[196] (PFK) and glyceraldehyde 3-phosphate dehydrogenase[197] (GAPDH) have been found to be reduced. Moreover, an *in vivo* study showed that while cerebral oxygen metabolism is unchanged, glycolytic activity is lower in HD subjects than healthy controls[198].

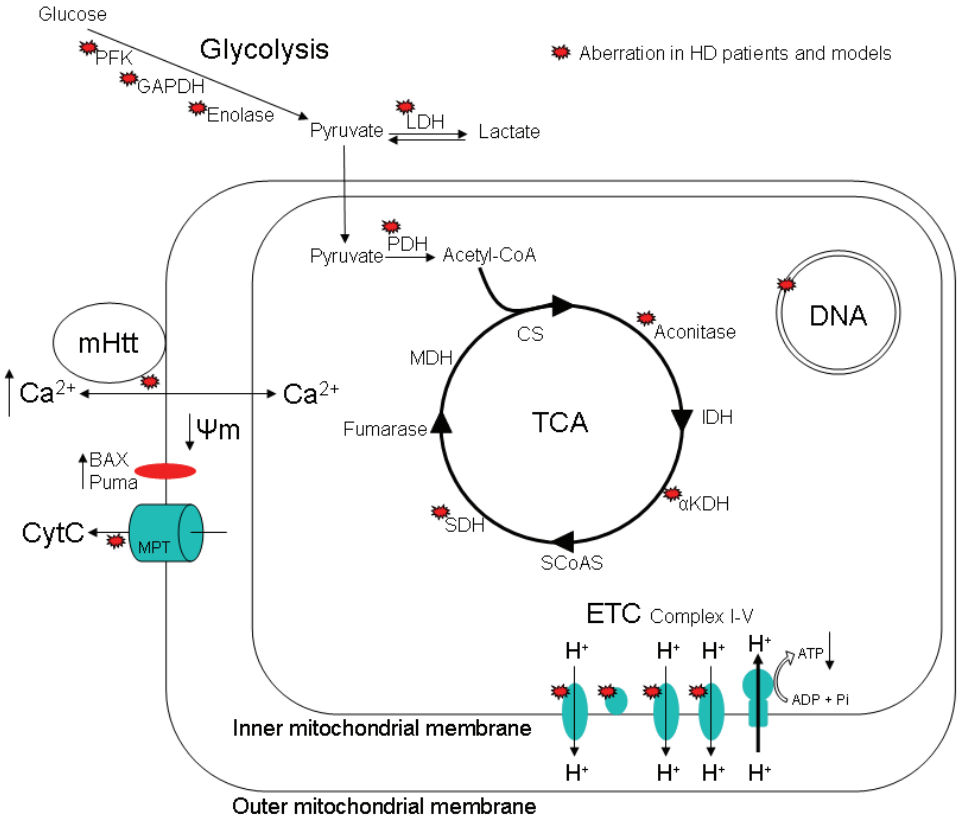

Figure 3. Schematic view of cellular energetics and aberrations thereof identified in HD. Details are provided in the text.

Microtubular trafficking

A role for huntingtin in intracellular trafficking was first hypothesised 15 years ago, when huntingtin was found to associate with vesicles[125, 126] and microtubules[126]. Since then, biochemical and functional studies have provided strong support for this theory. Huntingtin is transported with vesicles[199] and seems to be a part of the motor protein complexes that move cargos along microtubular strands. Evidence for this include the interaction with the motor protein dynein[200] and huntingtin-associated protein (HAP)-1[201]. Through the latter, huntingtin also interacts with the p150^{Glued} subunit of dynactin[202, 203], involved in binding dynein motors to vesicles, and KLC[204]/KHC[200, 205]. Elevating the huntingtin levels results in increased vesicular transport, both in the retrograde (dynein mediated) and anterograde (kinesin mediated) direction[205-207]. Furthermore, siRNA-mediated knock down[205-207] or anti-

body mediated inhibition[200] of huntingtin results in reduced trafficking. The phosphorylation state of serine 421 of huntingtin influences the directionality of cargo transport as unphosphorylated and phosphorylated huntingtin enhances retrograde and anterograde transport, respectively. Precisely how this effect is mediated is uncertain but phopsphorylation of huntingtin enhances the p150^{Glued}/KHC interaction and kinesin mediated transport[205]. Also HAP1 phosphorylation might play a role as it reduces the interaction of HAP1 with KLC[208].

Expanding the polyQ stretch of huntingtin leads to disruption of microtubule mediated transport of vesicles and mitochondria[207, 209-211]. Several mechanisms have been proposed to underlie these defects (figure 4). (1) Huntingtin aggregates can grow so large they more or less completely cover the entire diameter of axons and dendrites, causing a physical block of transport[212, 213]. (2) Levels of soluble p150^{Glued} and KLC are reduced[214, 215], possibly through recruitment into huntingtin aggregates[215]. (3) The polyQ expansion enhances the HAP1/huntingtin interaction[201] and thereby hampers the binding of the motor complex to microtubules[206]. (4) Tubulin acetylation is decreased in HD patient brain and reversing this pharmacologically in a cell model results in enhanced binding of KHC, p150^{Glued} and dynein to microtubular strands and improves trafficking efficiency[216]. (5) N-terminal huntingtin fragments associate with mitochondria and reduces the binding of motor complex proteins to the organelle. As a result, mitochondrial trafficking to and from the cell body is reduced and dysfunctional mitochondria accumulate in nerve terminals[217]. (6) Del Toro and coworkers found that mutant huntingtin disrupted the huntingtin/optineurin/Rab8 complex and suggested that this caused reduced association of vesicles with their motor proteins after exciting the Golgi apparatus[218]. (7) Finally, an expanded polyQ domain in the androgen receptor, the genetic alteration causing spinal and bulbar muscular atrophy (SBMA), activates c-Jun N-terminal kinase (JNK). This results in the phosphorylation of KHC and inhibition of binding of the protein to microtubules[219]. Mutant huntingtin possibly has the same effect.

Interestingly, the trafficking defects are, at least *in vitro*, an early event. Szebenyi and coworkers perfused mutant huntingtin into squid axoplasm and detected negative effects on trafficking within minutes[211].

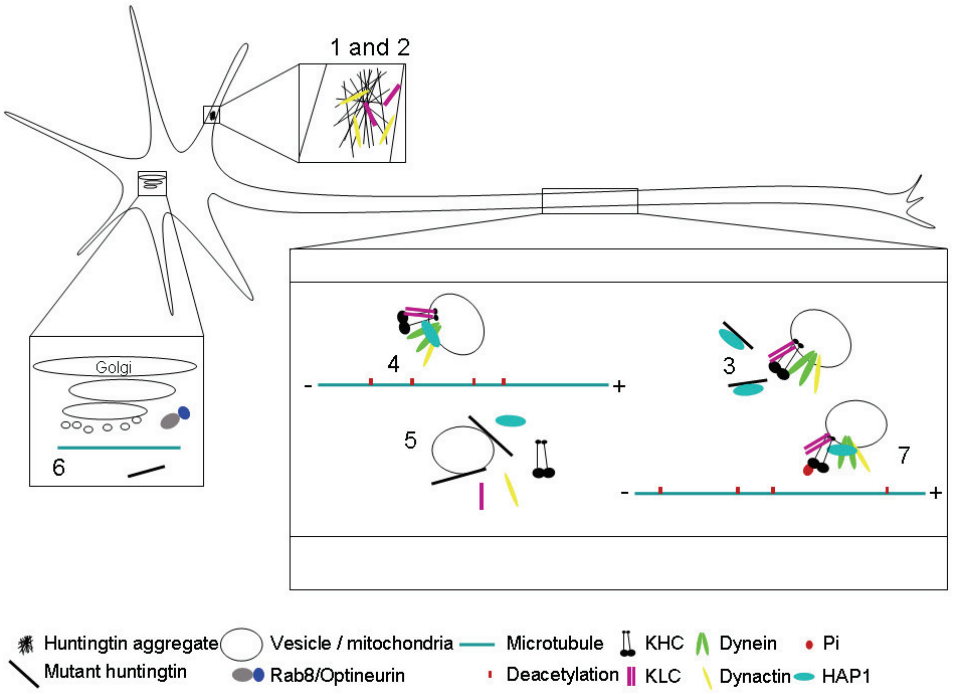

Figure 4. The expression of mutant huntingtin induces defects in intracellular trafficking. Numbers refer to suggested mechanisms discussed in the text.

The defects summarized above result in the reduced release of brain derived neurotrophic factor (BDNF)[206] and most probably also other factors important for neuronal well being. Reduced mitochondrial trafficking cause an accumulation of degenerated mitochondria in nerve terminals[217]. This results in lower ATP levels in the synaptosomal fraction[217]. Furthermore, components of the different cellular compartments are produced in the cell body and thereafter transported to their correct locations. Hampering this process could therefore contribute to defects in many locations, for example synaptic dysfunction[220].

Models and Methods

I will not describe standard methods used in biomedical research here. Information on these can be found in the original papers included at the end of this thesis. Instead, I will focus on giving more details on the "non standard" models and methods on which this thesis is based.

R6/2 mouse

Since the gene alteration causing HD was identified, several animal models have been created. The most widely used and best characterized is the R6/2 mouse[221]. This model was produced by microinjecting a ~2kb DNA fragment containing promoter sequences, the first exon and part of the first intron of human huntingtin into mouse embryos. The only resulting transgenic mouse was subsequently mated to wild type (WT) CBAxC57BL/6 mice producing five different transgenic lines, differing in the repeat length and expression levels of the transgene. Originally, the R6/2 line had ~150 CAG repeats, but due to genetic instability, this number varies both within and between colonies. Mice used in paper I and IV of this thesis had 160-180 repeats. The expression level of the transgene is 75% of that of endogenous huntingtin. R6/2 mice have a phenotype in many ways similar to HD. For example, they develop progressive motor dysfunction from four weeks of age, cognitive decline, loss of skeletal muscle tissue, weight loss and brain atrophy[221]. They also exhibit the characteristic huntingtin inclusions[135] as early at post-natal day one[222] in striatum. As the mice grow older, aggregates become more numerous and also develop in peripheral tissues[109, 139]. The major demerit of the R6/2 mouse is the lack of extensive neuronal cell loss until late in the disease[222].

832/13 INS-1 cell line

The original INS-1 cell line was in 1992 generated from an X-ray induced rat insulinoma[223]. Although an improvement compared to preexisting cell lines, the INS-1 cells still exhibited one big draw back making it a rather poor model of the *in vivo* β-cell: the secretory response to glucose was diminutive in comparison to freshly isolated pancreatic islets. To improve on this, Hohmeier et al. isolated single cell clones of INS-1 cells transfected with the cDNA for human insulin[224]. One of these clones, 832/13, was found to be functionally stable over

many passages and secreted insulin in response to glucose at levels more comparable to fresh islets, albeit with a left-shifted dose-response curve. Before functional assays, cells are seeded in multi-well plates and allowed to reach confluence to maximize the secretory response.

Adenoviral vectors

In our lab we use adenoviral vectors of serotype five to introduce and investigate the function(s) of proteins. To accommodate exogenous DNA in the viral genome, parts of the so called early genes, involved in triggering viral replication, have been removed. This deletion makes the virus replication deficient and viral propagation has to be performed in a cell line, human embryonic kidney (HEK) 293, which has been stably transfected with the deleted adenoviral genes. In our system, viral vectors are produced by co-transfection of the pJM17 plasmid containing the gene deleted viral genome and a shuttle plasmid into HEK 293 cells[225]. The latter plasmid contains a multiple cloning site (MCS) into which a gene-of-interest can be inserted. Surrounding the MCS are viral sequences, allowing for homologous recombination between the two plasmids to occur. Upon successful recombination, functional virions will be produced, lyse its host cell and infect the remaining cells of the culture dish. This yields a cell lysate containing one or more viral clones. Plaque purification is then used to isolate single working clones of each virus; a mix of media and melted agarose is poured onto HEK 293 cells infected with the original lysate. When this mix solidifies, the spread of viral particles produced by a single infected cell is limited to its neighbors, eventually producing an empty plaque in the monolayer due to cell detachment and lysis. By picking pieces of agarose immediately above these plaques, clones originating from one viral particle can therefore be isolated. These isolated clones can then be expanded by infection of large amounts of HEK 293 cells producing cell lysates from which a high titer viral stock can be purified by cesium chloride gradient centrifugation. The titer of these stocks is determined by serial dilution and counting of plaques formed in a plaque assay[225, 226]. In this thesis I generated two vectors, Ad-17Q and Ad-69Q, to confer the expression of hemagglutinin-tagged sequences encoding exon 1 of normal and mutant huntingtin respectively.

Patch clamp

By utilizing the patch clamp technique one can study single cell electrophysiology, including changes in membrane capacitance. As the capacitance depends on the membrane area it reflects exo- and endocytosis of vesicles. In paper I, we used the standard whole-cell configuration, meaning that capacitance changes in the whole membrane are recorded. A glass

micropipette is attached to the cell by gentle suction force, resulting in the formation of a high resistance seal between the pipette tip and the membrane. Further suction disrupts the membrane inside the pipette tip resulting in access to the interior of the cell. In this set up, soluble constituents of the cytoplasm is replaced by the pipette solution making it possible to introduce compounds into the cell interior. By depolarizing the patched β-cell, thereby triggering Ca^{2+}-influx, and measuring the capacitance increase resulting from the fusion of insulin granules to the plasma membrane, one can deduce the number of exocytotic events. When performing patch clamp on islet cells, one can not visually distinguish between the different islet cell types. However, β-cells can be identified by measuring the Na^{+}-currents during depolarization, as these are inactivated in β-cells when the membrane potential is lower than -70mV.

Micro fluidic card

The micro fluidic card, a low density microarray from Applied Biosystems, allows you to simultaneously study the expression of up to 380 genes of your choice in a single sample by quantitative polymerase chain reaction (Q-PCR). Although not as extensive as a microarray, using tens of thousands of probes, it gives more reproducible results. Our laboratory has designed a micro fluidic card for the investigation of 46 genes involved in glycolysis, TCA cycle metabolism and oxidative phosphorylation, in four parallel samples. An assay for ribosomal RNA 18S is included as an obligatory standard from the manufacturer and we have also included an assay for hypoxanthine-guanine phosphoribosyltransferase (HPRT) as an additional control. Although not complete, this card yields a rather extensive overview of expression levels of enzymes involved in the metabolic processes controlling insulin secretion.

VSVG-trafficking

Vesicular stomatitis virus G protein (VSVG) traffics to the plasma membrane through the secretory pathway. One feature of VSVG that makes it a good tool to measure trafficking is that it can be synchronized. VSVG misfolds at 39°C and will therefore be accumulated in the ER. Upon transfer to 33°C, the protein will fold correctly and be transported through the Golgi apparatus and towards the plasma membrane by vesicular trafficking[227]. It is therefore possible to detect alterations in both ER to Golgi and Golgi to plasma membrane transport by measuring the relative intensities in the different compartments. In paper IV, we co-

transfected a yellow fluorescent protein (YFP) tagged VSVG construct with plasmids for the 17Q or 69Q huntingtin to investigate trafficking in 832/13 INS-1 cells.

CD4-phogrin

Phosphatase on the granule of insulinoma cells (phogrin) is a dense-core secretory granule membrane glycoprotein[228]. Replacing the luminal domain of phogrin with the extracellular domain of human CD4 does not change the subcellular localization[229] and the CD4 domain will therefore be exposed on the cell surface after exocytosis (figure 5). By transfecting 832/13 INS-1 cells with the CD4-phogrin fusion construct and stimulating them with glucose in the presence of CD4 antibodies, one can immunocytochemically investigate the levels of exocytosis and ensuing endocytosis. A lower number of stained granules within the cell, in the absence of CD4 build up in the cell membrane, implies a defective exocytosis, possibly in combination with perturbed endocytosis.

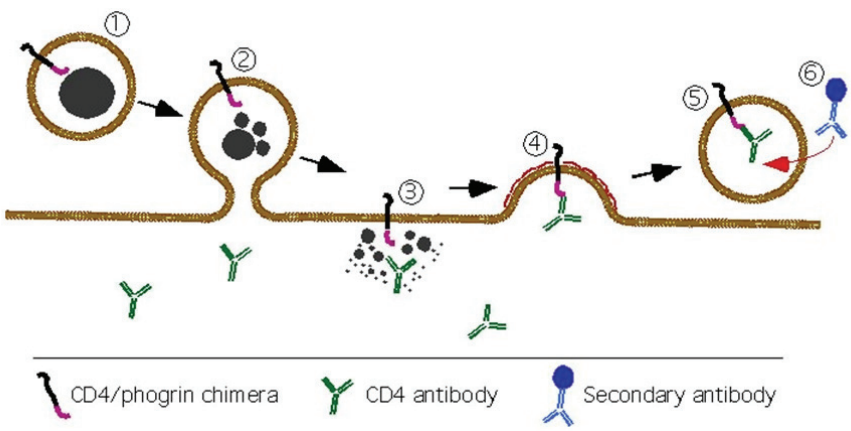

Figure 5. The CD4 domain of the fusion protein is exposed on the cell surface upon granule fusion to the plasma membrane. Detection of intracellular CD4-phogrin can therefore give an indirect measurement of exo- and endocytotic activity. Figure by Ruben Smith.

FRAP / iFRAP

In addition to the above mentioned VSVG-trafficking experiment, we also performed fluorescent recovery after photo bleaching (FRAP) and inverse FRAP (iFRAP) experiments to investigate insulin granule trafficking more specifically. In these experiments, we infected

Ad-17Q and Ad-69Q transduced cells with a virus conferring expression of enhanced green flurorescent protein (eGFP) tagged islet amyloid poly peptide (IAPP), an insulin granule protein[230]. In the FRAP experiment we analyzed ER to Golgi transport by bleaching the Golgi with maximum laser power in a confocal microscope. We acquired images of cells incubated in a live cell chamber and calculated the fluorescence recovery of the Golgi apparatus after normalization for prebleaching fluorescence and continuous bleaching due to repeated laser scanning[209, 231].

We investigated transport from the Golgi by iFRAP. In this experiment, we bleached everything but the Golgi, making it possible to analyze the fluorescence loss from this organelle. Before initiating the experiment, one has to preincubate the cells at room temperature for 2h. This allows for the build up of fluorescent protein in the Golgi apparatus. In addition, cycloheximide is included in the medium to stop protein translation and therefore inhibiting the refilling of the Golgi apparatus with newly synthesized proteins. We analyzed measures of fluorescence in acquired confocal images and again normalized for initial fluorescence and bleaching[209, 232].

Velocity of vesicular transport

We used the eGFP-IAPP virus also to measure the velocity of vesicular movements through the cytoplasm. By acquiring high speed confocal scans it is possible to track individual vesicle movements for significant distances. Analyzing the vesicle dislocation from frame to frame allows for the calculation of distance, average speed and peak velocities of each vesicle. However, vesicles moving along the z-axis will be missed or underestimated.

Proximity ligation assay (PLA)

PLA is a novel method for identifying protein-protein interactions[233]. The two proteins being investigated are bound by regular primary antibodies produced in different host species. Secondary antibodies with attached DNA oligonucleotides are then applied followed by addition of two linear DNA connector oligonucleotides. If the two proteins are in close proximity, the connector oligonucleotides will be able to pair up with the two oligonucleotides on the secondary antibodies forming a circular structure that can be covalently joined by enzymatic ligation. This circular DNA then act as a template for rolling circle PCR amplification, yielding a transcript with many repeated sequences covalently linked to one of the secondary antibodies. These repetitive structures are detected with fluorescent DNA probes (figure 6).

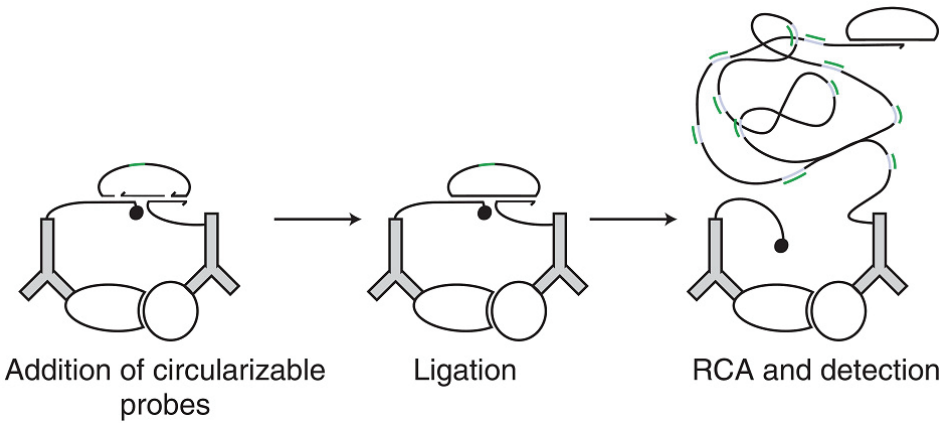

Figure 6. Proximity ligation assay is a sensitive method for detection of protein-protein interaction. Details are provided in the text. Figure adapted from Soderberg et. al[232].

Depending on the quality of the primary antibodies used, PLA can be a very specific method. In addition, due to the rolling circle transcription, up to a 1000 copies of detectable sequences can be produced making it a very sensitive method.

Mass spectrometry (MS)

In paper IV we used matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS[234] to identify single proteins isolated by excision from an SDS-PAGE gel. For MALDI-TOF detection, the protein is digested by a protease, for example trypsin, yielding a unique set of peptides (peptide mass fingerprint) by which the protein can be identified. A small volume of digested protein is mixed with a suitable acidic matrix molecule solution and pipetted onto a target plate. When exposed to laser light, the matrix molecules will be ionized and transfer this ionization to the peptides. The matrix is included to protect peptides from direct laser application, which would result in further fragmentation and hamper subsequent identification. The ionized peptides are accelerated in an electrical field (15-25kV) towards a detector. The fingerprint of masses detected can then be compared to available databases to identify the isolated protein. Trypsin peptides will also be detected as they invariably contaminate the peptide mix. These can be used for calibration and will not affect identification if manually removed from the data.

Aims

This thesis aims at elucidating the pathogenetic mechanisms underlying diabetes and β-cell dysfunction in HD. Thereby we hope to identify cellular processes of potential importance also for neurodegeneration.

More specifically, the aims are:

- To further characterize glucose intolerance in R6/2 mice (paper I)

- To investigate whether there are any histological alterations in HD patient pancreas that can underlie glucose intolerance (paper II)

- To study the potential involvement of metabolic derangements in β-cell dysfunction in HD (paper III)

- To assess the contribution of trafficking aberrations in β-cell dysfunction in HD (paper IV)

Summary and discussion of articles in this thesis

Paper I

For a decade it has been known that R6/2 mice are unable to properly control their blood glucose levels[103]. Andreassen and coauthors suggested that this was caused by a reduced expression of the transcription factor PDX-1 and a resulting loss of insulin secretion[109]. Using a spectrum of imaging, biochemical and functional assays, we set out to further expand on these findings.

Results

We found that the proportion of pancreatic β-cells that display huntingtin aggregates increases with age in R6/2 mice. We observed aggregates in 19 and >95% of β-cells at 7 and 12 weeks of age, respectively. While there were no aggregates in other islet cell types at 7 weeks, 24 and 6% of α- and δ-cells respectively had aggregates at 12 weeks. At this stage, R6/2 mice were hyperglycemic and hypoinsulinemic, indicating insulin-deficient diabetes. This notion was further strengthened by measurements of *in vitro* insulin secretion from isolated islets. We found that islets from 7 week old R6/2 mice responded normally to both glucose and potassium chloride. However, at 12 weeks the response to both stimuli was severely blunted (figure 7A). Concomitant with these changes, we found that β-cell area increase between 7 and 12 weeks in WT mice. This increase was totally absent in R6/2 mice resulting in a significantly lower β-cell area at 12 weeks of age (figure 7B). By performing cell death and proliferation assays we showed that this was due to diminished β-cell replication as

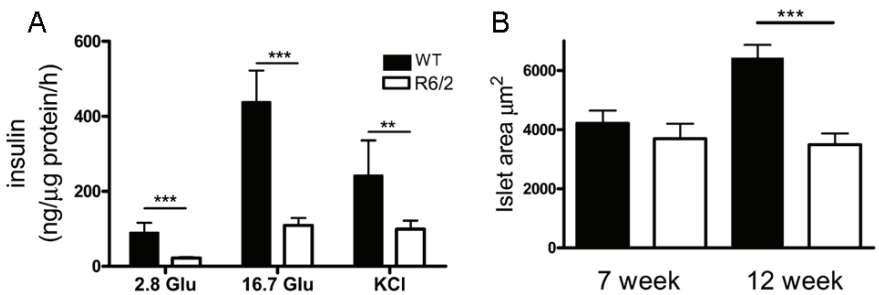

Figure 7. Insulin secretion (A) and β-cell area (B) are reduced in 12 week old R6/2 mice when compared to WT mice.

indicated by a 6-fold reduction in the number of bromodeoxyuridine-positive cells in R6/2 islets. Upon measurement of hormone content, we found that insulin levels in R6/2 mice were only one sixth of those in WT pancreata. In an effort to further characterize the secretory defect we utilized patch clamp to measure depolarization-induced exocytosis. The response of β-cells from R6/2 mice at 8 weeks of age was largely unaffected while being almost completely abolished in cells from 12-week animals. By ultrastructural examination of the islets we found a likely cause of this exocytotic defect; as can be seen in figure 8, R6/2 β-cells were dramatically degranulated.

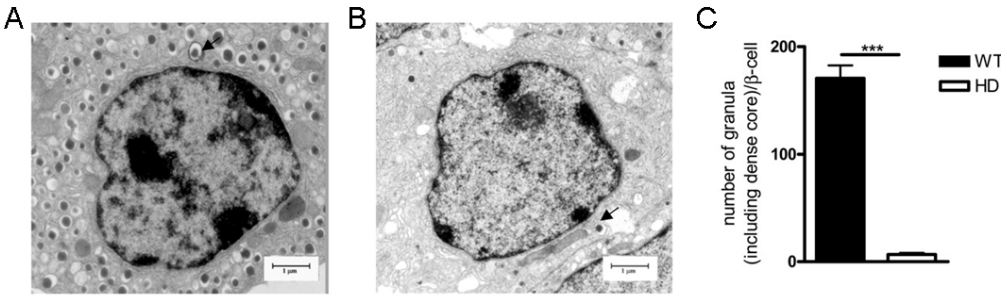

Figure 8. Electron micrographs show that R6/2 β-cells (B) are dramatically degranulated when compared to WT cells (A). (C) Quantification of β-cell granules.

Discussion

The mice in our R6/2 colony are insulin resistant. Up until a certain age the pancreatic β-cells compensate for this by hypersecretion of insulin so that normoglycemia is achieved. However, as the mice grow older, this fails and hyperglycemia develops[100]. The data presented in this paper suggest that a reduced capacity to increase β-cell mass and a severe degranulation underlie the secretory defect and elevation of blood glucose in R6/2 mice.

While the number of insulin granules is reduced ~25 fold, islet insulin content is "only" reduced ~6 fold. Thus, total cellular insulin content is most likely less reduced than granular insulin content. Where does the remaining insulin go? One possible explanation is defective granulogenesis and a higher than normal fraction of insulin localized to the Golgi apparatus. In support of this, we revealed an accumulation of insulin/proinsulin signal in the Golgi by immunohistochemistry (see paper IV). The mechanism for the degranulation is unknown to us, but some of the proteins involved in vesicle formation at the trans Golgi network[235] interact directly or indirectly with huntingtin and/or are altered in HD[236-243], suggesting that this process may be disturbed. Del Toro and coauthors suggested that the disruption of the huntingtin/Rab8/optineurin-complex seen in mutant huntingtin cells perturbed the assem-

bly of clathrin-coated vesicles with their motor proteins and this resulted in an accumulation of vesicles in the close proximity of the Golgi apparatus[218]. However, we could not observe similar changes.

Paper II

Although several reports concerning glucose homeostasis in HD patients have been published[90, 92, 97], no histological studies on pancreatic tissues from HD patients have previously been performed. In this paper we used immunohistochemistry and *in situ* hybridization to investigate the morphology of pancreatic islets in a set of pancreatic sections from grade II-IV HD patients.

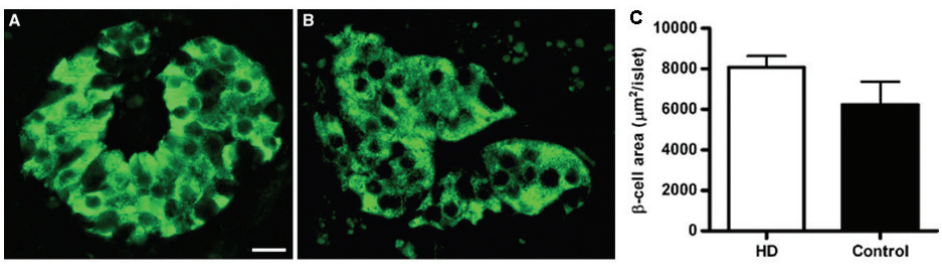

Figure 9. Pancreatic islets from HD patient (A) and control (B) stained for insulin. (C) No difference of β-cell area could be identified upon morphometric analysis of insulin stained area.

Results

Using immunohistochemistry with antibodies against islet hormones, we did not reveal any changes in islet morphology or β-cell area (figure 9). Moreover, we found islet distribution and number to be normal and hormone expression levels unaltered. As equal fluorescence does not necessarily mean that there is no difference of expression, we measured levels of insulin mRNA with in situ hybridization. Again, we could not identify any differences between HD patients and controls. We also stained sections with the EM48 antibody to detect possible huntingtin aggregates but found no such structures in any of the sections.

Discussion

We show that pathology of the pancreatic islets, if any, in the general HD population is milder than that seen in the brain. Whether the islets of diabetic HD patients are more affected remains to be seen, as only one of our subjects were known to be diabetic. It is inter-

esting to note, however, that this patient had the lowest level of insulin mRNA expression of all subjects and the smallest β-cell area in the HD group. We argue against the insulin resistance suggested by the initial studies with GTTs[90] as HD patients generally are underweight[145] and we could not see a compensatory increase of the β-cell mass. Since the publication of our paper, Lalic et al. found that a group of non-diabetic HD patients, despite having an average body mass index of just 20.4, indeed were insulin resistant and unable to compensate for this with increased insulin secretion in response to a glucose challenge[97]. It is possible that this is similar to the situation in the R6/2 mouse, which exhibit replication-deficient β-cells resulting in the lack of compensation for insulin resistance (paper I and Björkqvist et al.[100]). A more likely reason for reduced insulin secretion is β-cell dysfunction without morphologic changes. Several of the processes implicated in HD pathogenesis, e.g., mitochondrial metabolism, vesicular trafficking and exocytosis, are all vital for normal insulin release and could therefore underlie the secretory defect observed by Lalic et. al. A possible cause for insulin resistance in HD is the increased cortisol levels[100, 244, 245]. Elevated cortisol levels can reduce peripheral blood flow and GLUT4 translocation to the plasma membrane, thus inhibiting glucose uptake[246], and potentially increase glucose production from the liver. Another possibility is downregulation of GLUT expression. This is seen in HD brain[247], but no published studies examining peripheral tissues exist. Alterations in the insulin signaling cascade could also contribute. For example, activation of Akt, a pro-survival kinase protective in HD models and acting downstream of the insulin receptor, is hampered in HD brain and peripheral cells[248].

Paper III

Many studies have implicated aberrations in cellular metabolism in the pathogenesis of HD[143]. As glucose metabolism is the key regulator of insulin release[12], such perturbations could very well affect β-cell function. Therefore, we studied these processes *in vitro*.

Results

We found that expression of mutant huntingtin (69Q) in 832/13 INS-1 β-cells resulted in the formation of aggregates, both cytoplasmic and nuclear, while no aggregates were found in cells expressing normal huntingtin (17Q). By static incubation experiments we revealed that 69Q cells were unable to elevate insulin secretion to the same degree as 17Q cells when exposed to a high glucose concentration (figure 10A). Defective metabolism could explain this finding and an initial methyl-tetrazolium (MTS)-assay indicated that metabolic activity and redox state were altered by expression of mutant huntingtin. As reduced NADPH levels

could contribute to both the MTS-assay finding and the reduced insulin secretion[19], we measured the NADPH/$NADP^+$ ratio. We observed no differences in 69Q compared to 17Q cells. Glucose oxidation measured by release of radiolabeled CO_2 from ^{14}C-glucose was also normal (figure 10B). Gene expression analysis by low density array revealed a few significant changes of metabolic enzymes. However, these alterations were not of a magnitude that is likely to explain the secretory defect. Finally, we did not detect any changes in mitochondrial respiration (figure 10C).

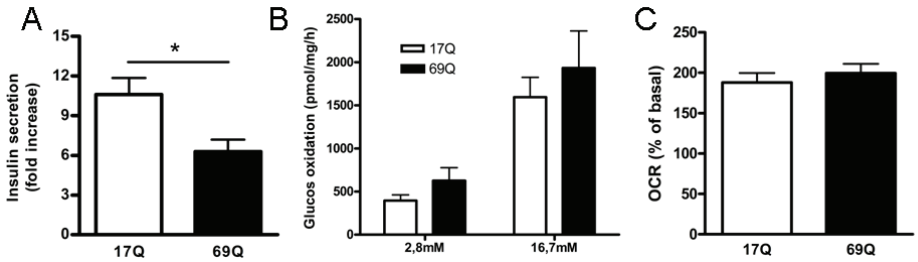

Figure 10. Expression of mutant huntingtin in 832/13 INS-1 cells cause an impairment of GSIS (A). This is not due to altered glucose oxidation (B) or impaired mitochondrial respiration (C).

Discussion

We show that metabolic defects do not contribute to the dysfunction in our β-cell model. However, this does not refute metabolic aberrations completely. Due to toxicity, we could not investigate the effects of mutant huntingtin after more than 48 hours and it is possible that metabolic defects require more time to develop. Indeed, results from HD patients and animal models show that ETC perturbations are not a primary event in HD pathogenesis[158]. The strong toxicity likely depends on the high levels of huntingtin expression due to the use of a cytomegalovirus promoter in our vectors. A weaker promoter would most likely allow us to investigate the model after prolonged expression. We are therefore working on lentiviral vectors that will allow us to regulate the permanent expression of our transgene by changing the tetracycline concentrations in the culture medium. Such a vector may allow us to examine changes over several passages.

Paper IV

As glucose metabolism was not altered in our cell model (paper III), the secretory defect must instead be caused by other factors. In paper IV we investigated the effects of mutant huntingtin on trafficking of insulin granules along the microtubule network.

Results

As in paper III, we found that expression of 69Q huntingtin blunted insulin secretion in 832/13 INS-1 cells. This was neither due to reduced insulin content nor cell death. The latter was increased, but not to levels explaining the defective secretion. We next used a VSVG-YFP construct to investigate intracellular trafficking. Post-Golgi trafficking was perturbed in the 69Q cells while ER to Golgi transport was normal. In FRAP and iFRAP experiments we obtained results that support these claims. To investigate how this defect affected exocytosis of insulin granules, we used a CD4-phogrin construct. This fusion protein localizes to the insulin granules and allowed us to get an indirect measurement of granule exocytosis (see Models and Methods section above) over time. Exocytosis at the early time points, corresponding to first phase of insulin secretion, was normal in 69Q cells (figure 11). However, sustained release was severely blunted. Transport of insulin granules along microtubules is a prerequisite for sustained insulin release at a high level. We therefore tracked insulin granules visualized by an eGFP-IAPP fusion protein and found that fast moving insulin granules were fewer in 69Q cells compared to 17Q cells. In an effort to identify the cause of this aberration, we performed co-immunoprecipitation followed by MALDI-TOF MS to identify proteins interacting with mutant huntingtin. In this screen we identified β-tubulin as preferentially interacting with mutant huntingtin. We verified this by co-immunoprecipitation followed by western blot (figure 12). To test if this interaction affected microtubule dynamics we depolymerized the microtubular network by incubating cells in medium containing nocodazol. We then allowed the microtubule to repolymerize for up to 30 minutes. Polymerized

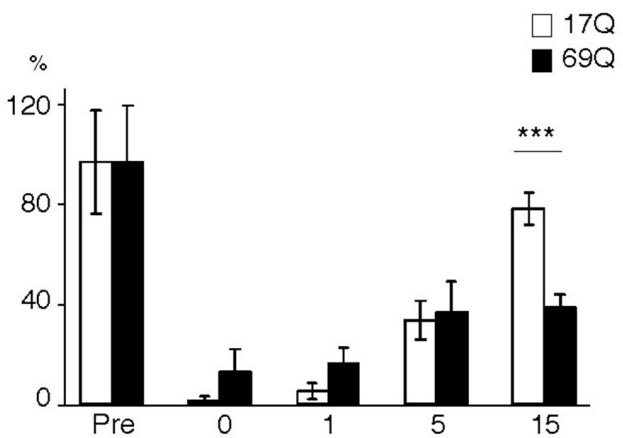

Figure 11. Cells expressing mutant huntingtin exhibit normal first phase of insulin secretion. However, reduced numbers of CD4-phogrin-positive granules at the later time point indicates that the second phase of insulin release is blunted.

tubulin was isolated by centrifugation and the ratio of polymerized and soluble tubulin was determined by western blot. We detected no changes in the efficiency of microtubule assembly or in the amount of polymerized tubulin at steady state in cells expressing mutant compared to normal huntingtin.

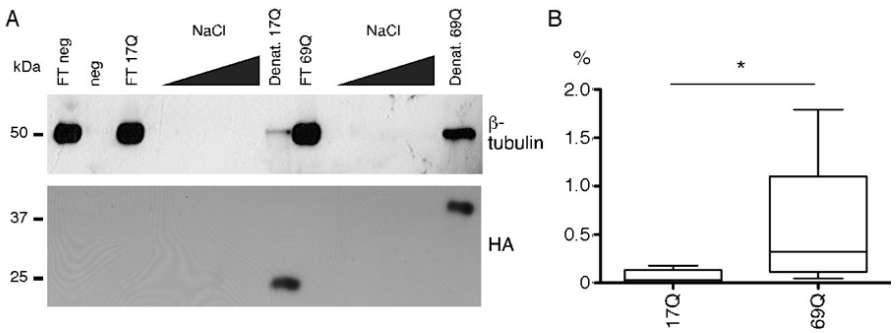

Figure 12. The β-tubulin/huntingtin interaction is enhanced by an expanded polyQ domain. The left panel shows a western blot of HA-tag immunoprecipitates. The right panel shows quantification of the fraction of β-tubulin co-immunoprecipitated by the two huntingtin proteins.

Discussion

We propose that mutant huntingtin causes an insulin secretion defect due to an aberration of the insulin granule transport, a process known to be dependent on microtubule filaments[41]. Previously, phosphorylation and inactivation of kinesin due to polyQ induced JNK activation has been shown to stall fast axonal transport[219]. However, in our model, inhibiting JNK does not improve the secretory defect. Also, we could not identify any direct interaction of our huntingtin transgenes with kinesin. Instead, we found that the polyQ expansion enhanced the strength of the huntingtin/β-tubulin interaction. This interaction had no effect on the dynamics of microtubular polymerization. We therefore propose that mutant huntingtin acts a physical block of transport, much like a speed bump, thereby reducing the efficiency with which insulin vesicles can travel in the cell. Such a defect is unlikely to be specific to insulin granule transport and could therefore also contribute to the trafficking defects seen in neuronal cells. Therefore, breaking up the huntingtin/β-tubulin interaction might be a target for therapeutic intervention in HD.

Major conclusions

- R6/2 mice develop glucose intolerance due to a reduced β-cell mass and decreased insulin secretion capacity
- Pancreatic islet morphology is normal in non-diabetic HD patients
- Short term expression of mutant huntingtin does not cause metabolic perturbations
- Mutant huntingtin aberrantly interacts with β-tubulin and disrupts insulin granule translocation

Sammanfattning på Svenska (Summary in Swedish)

Vid Huntingtons sjukdom förstörs regioner i hjärnan som är involverade i bland annat kontroll av rörelser, minne och humör. Det gör att patienterna lider av demens, personlighetsförändringar, depression och mycket karakteristiska dansrörelser (därav det ursprungliga namnet Huntingtons chorea, chorea = dans på grekiska). Idag finns inget botemedel mot denna sjukdom, och den leder därför till döden ungefär 15-20 år efter att de första motoriska symptomen bryter ut. Sjukdomen orsakas av en mutation i den gen som kodar för proteinet huntingtin. När detta protein är förändrat stör det den normala cellulära funktionen och klumpar ihop sig i så kallade aggregat inne i cellen. Exakt vilka funktionella förändringar i cellen som bidrar till sjukdomsutvecklingen är fortfarande oklart och detsamma gäller proteinets normala funktion Det har dock föreslagits att förändringar i ämnesomsättning och transport inne i cellen är viktiga.

Som flera andra hjärnsjukdomar är Huntingtons sjukdom förknippad med en ökad risk för diabetes. Vad detta beror på är oklart men med tanke på de funktionella likheterna mellan nervceller och de insulinproducerande β-cellerna är det inte osannolikt att samma sjukdomsmekanismer föreligger i båda celltyperna. I det arbete som ligger till grund för denna avhandling har jag försökt klargöra dessa mekanismer, i förhoppningen om att hitta nya angreppspunkter för framtida behandling.

I det första arbetet undersökte vi en musmodell för Huntingtons sjukdom. Det är sedan tidigare känt att denna musmodell utvecklar ett diabetesliknande tillstånd, och detta har föreslagits bero på ett sänkt insulininnehåll i β-cellerna. Vår studie bekräftade detta fynd, men vi upptäckte även att flera andra förändringar föreligger. Vi såg att det bildas huntingtinaggregat i en stor majoritet av β-cellerna. Parallellt med detta sker en minskning av den totala β-cellmassan, och det visade sig bero på att cellerna inte fortplantar sig normalt. I insulinfrisättningsförsök upptäcktes dessutom att β-cellerna från de sjuka djuren släpper ut mycket mindre insulin än celler från de friska djuren när de stimuleras med glukos (socker). För att ta reda på orsaken till detta använde vi oss av elektronmikroskopi, vilket ger bilder med mycket stor förstoring. Då såg vi att de små insulininnehållande blåsor som normalt finns inne i cellen var nästan helt försvunna. Därför drog vi slutsatserna att dessa möss utvecklar diabetes på grund av att de har ett reducerat antal β-celler. Dessutom har de kvarvarande cellerna en förminskad kapacitet att frisätta insulin.

För att undersöka om liknande förändringar föreligger i patienter med Huntingtons sjukdom analyserade vi obduktionsmaterial från nio patienter (arbete II). Detta material undersökte vi med hjälp av antikroppar mot de hormoner som bildas i pankreas, nämligen insulin, glukagon, somatostatin, pankreatisk polypeptid och ghrelin. Vi kunde dock inte hitta några förändringar vare sig i mängden hormon eller totala mängden β-celler. Denna metod kan dock vara väldigt okänslig, så vi mätte insulinmängden även med en metod som mäter insulin-mRNA, ett förstadum till proteinet. Resultatet förblev dock det samma. Slutligen undersökte vi om det fanns några huntingtinaggregat i materialet, men även detta försök gav ett negativt resultat. De förändringar vi kunde se i musen finns alltså inte i patienter med Huntingtons sjukdom. Detta stämmer med tidigare studier som visar att just den musmodellen lider av en väldigt allvarlig sjukdom, till och med värre än den genomsnittlige patienten. Baserat på resultaten drog vi slutsatsen att inga synliga förändringar finns i de insulinproducerande cellerna från patienter med Huntingtons sjukdom. De defekter som då finns måste i stället bero på funktionella störningar som vi inte kunde upptäcka i detta patientmaterial.

För att klarlägga dessa störningar har vi tagit fram en cellmodell för Huntingtons sjukdom. I denna modell använder vi oss av speciella virus som vi tillverkat. Med dessa kan vi föra in genen för muterat huntingtin i en cellinje vi odlar i petriskålar. Resultat från försök med celler uttryckande det muterade proteinet visade att de frisätter mindre insulin när de stimuleras med glukos. Denna förändring kan bero på många saker. I arbete III undersökte vi hur muterat huntingtin påverkar ämnesomsättningen inne i cellen. Eftersom dessa processer är mycket viktiga för regleringen av hur insulinet frisätts är det möjligt att rubbningen av dem kan orsaka den defekta insulinfrisättningen. Det visade sig dock att alla undersökningar vi gjorde gav normala resultat, och därmed drog vi slutsatsen att metabola förändringar inte bidrar till den funktionella defekten.

En annan process som krävs för en väl fungerande insulinfrisättning är transport av insulinblåsorna från cellens inre till dess yttervägg. Hur detta fungerar undersökte vi i arbete IV. Till skillnad från musmodellen fanns det normala mängder insulinblåsor i de celler vi introducerat muterat huntingtin i. Detta belyser återigen hur allvarlig mössens sjukdom är. Insulinfrisättning kan delas upp i en första och en andra fas varav den andra är beroende av transport av blåsorna. Våra försök visade att just denna fas påverkades av muterat huntingtin. I nästföljande försök använde vi oss därför av ett protein som finns i samma blåsor som insulinet. Eftersom det dessutom lyser när det utsätt för laserljus av en viss våglängd kan man med mikroskop följa hur de individuella blåsorna förflyttar sig inne i cellen. Detta försök visade att i celler med muterat huntingtin var det färre blåsor som rörde sig och fler som var stilla. I

ett försök att ta reda på orsaken till detta använde vi oss av en metod som kan identifiera de proteiner som huntingtin binder till. Då fann vi att tubulin, en byggsten i de vägar som blåsorna transporteras längsmed inne i cellen binder till huntingtin. Vi kunde dessutom se att muterat huntingtin band starkare till tubulin än vad det friska gjorde. Med dessa resultat som bakgrund har vi formulerat följande hypotes: muterat huntingtin binder till insulinblåsornas transportvägar och fungerar ungefär som vägbulor. Därmed saktas transporten av blåsorna och mindre insulin frisätts. Vi tror dessutom att detta fenomen även kan påverka nervcellerna. Om detta visar sig vara sant skulle substanser som kan bryta upp dessa vägbulor kunna fungera som framtida läkemedel mot Huntingtons sjukdom.

Acknowledgements

Only one name is written on the cover of this thesis. However, without the help of many people, not one experiment would have succeeded and not a single word would have been written. And most of all, these past years would have been a LOT less fun. My sincere apologies to those I inevitably forget to mention.

Hindrik, during these years I have never felt supervised. Instead, I have felt supported. You have allowed and encouraged me to run my projects (at least somewhat) as I like. That freedom has been invaluable! And when things went wrong, I hate to admit it but sometimes they did, you always stayed positive and kept me on track.

My co-supervisor, Patrik Brundin, although it's been a long time since we had regular HD/endo meetings, your drive and enthusiasm towards science that shines through when we meet for discussions are really inspiring.

Thanks to the past and present members of the molecular metabolism unit. You are all so positive and helpful I could not have asked for a better environment to work in. Maria, my first roomie, thanks for letting me in on your projects and for introducing me to the Cheese cake factory in Boston (that peanut butter and fudge cheese cake was fantastic!). Thanks to Malin for laughing at my stupid jokes although they were not about your favourite topic, yes I'm talking about poo. Thanks also for taking time out of your vacation to isolate islets for me. Ulrika, you actually deserve the biggest thank you. If I had not run into you at the BMC gym that afternoon in the winter of 03/04 I would never have known there was an open position in the lab and would have done my PhD somewhere else. Marloes, I don't think I have met anyone else who smiles as much as you do. Being met by such a happy face every day in our shared office made many mornings easier. Cecilia, thanks for taking such a BIG responsibility for keeping things running in the lab, but most of all for being great company in Amsterdam, Rome and otherwise. Olga, thanks for doing some rather acute NADPH measurement on you spare time and for always seeing the bright side of things. Peter "Vad fan är en sniff" Spégel, thank you for aiding me in the fight against the female dominance in the lab and great gym company. Vladimir, thank you for never saying no when I need help and for the guided tour of Stockholm. Siri, thanks for improving my fighting skills and for making sure that we fika. Anders, my fellow gourmand, what ever happened to that neighbour of

yours? ☺. Karin, your office space might be hijacked, but I think you should come and spend some more time with us! Thomas and Jelena, although I have not been around so much since you started in the lab a few weeks ago I'm sure you'll make great additions to the group. Thanks also to the students that were bold enough to let me supervise them and Anna, Hedvig and Ashkan (who even returned!) for being particularly nice "guests" in the lab.

The Neuronal survival unit:
Jia-Yi Li, although not officially my supervisor, you have sort of co-co-supervised me. Thanks for your commitment to the trafficking project. It ended up as a pretty good story!
Ruben, thanks for great collaboration and many dinners! When the paper is published we should celebrate properly! We certainly have deserved it. Jorien, I never thought spending hours rummaging for food crumbs could be so much fun. Thanks to Valentina, Denis and Pontus for spending hours analyzing data. Thanks also to Åsa for fruitful collaborations.

Thanks to Nisse Wierup for coauthoring my paper and helping me find invisible islets.
The people at B and C11. You are too many to mention but thanks for all the help when we shared space and, not to forget, all the fredagskakor! A special thanks to the CRC fugitives Ola and Peter for happily (well, Ola at least) helping me with big molecular biology issues the last year. Bitte, thank you for islet help and always replying with a smile even though we have harassed you with questions ever since we moved to the CRC. We all wish that we had a Bitte in the lab.

Thanks also to my friends for making me forget the lab from time to time, be it through floorball, poker nights (your financial contributions have been highly appreciated), sports and hamburgers at Glorias, lagfest (lagfest IV anyone?), the occasional badminton game, travels, fika or whatever. You are awesome!

My families, the Bensow-Hejnæs, Bacos and Nilsson-Landbergs (were those the best summers a young boy could ever ask for or what?!), thank you for always believing in me and for accepting that I have been a bit to enclosed in my "PhD bubble" for some time now.
Finally, mamma, you may be a bit of a nag at times, but without your love and support the last 30 years I would be absolutely nothing.

References

1. Bonner-Weir, S. The anatomy of the islet of Langerhans, in *The endocrine pancreas.* (ed. E. Samols) 15-27 (Raven Press, New York; 1991).

2. Wierup, N., Svensson, H., Mulder, H. & Sundler, F. The ghrelin cell: a novel developmentally regulated islet cell in the human pancreas. *Regulatory peptides* **107**, 63-69 (2002).

3. Henquin, J.C. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* **49**, 1751-1760 (2000).

4. Johnson, J.H., Newgard, C.B., Milburn, J.L., Lodish, H.F. & Thorens, B. The high Km glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has an identical primary sequence. *The Journal of biological chemistry* **265**, 6548-6551 (1990).

5. Iynedjian, P.B., Mobius, G., Seitz, H.J., Wollheim, C.B. & Renold, A.E. Tissue-specific expression of glucokinase: identification of the gene product in liver and pancreatic islets. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 1998-2001 (1986).

6. Jensen, M.V. *et al.* Metabolic cycling in control of glucose-stimulated insulin secretion. *American journal of physiology* **295**, E1287-1297 (2008).

7. Dudkina, N.V., Sunderhaus, S., Boekema, E.J. & Braun, H.P. The higher level of organization of the oxidative phosphorylation system: mitochondrial supercomplexes. *Journal of bioenergetics and biomembranes* **40**, 419-424 (2008).

8. Ashcroft, F.M., Harrison, D.E. & Ashcroft, S.J. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature* **312**, 446-448 (1984).

9. Ashcroft, F.M. & Rorsman, P. Electrophysiology of the pancreatic beta-cell. *Progress in biophysics and molecular biology* **54**, 87-143 (1989).

10. Gilon, P. & Henquin, J.C. Influence of membrane potential changes on cytoplasmic Ca2+ concentration in an electrically excitable cell, the insulin-secreting pancreatic B-cell. *The Journal of biological chemistry* **267**, 20713-20720 (1992).

11. Gembal, M., Gilon, P. & Henquin, J.C. Evidence that glucose can control insulin release independently from its action on ATP-sensitive K+ channels in mouse B cells. *The Journal of clinical investigation* **89**, 1288-1295 (1992).

12. Wollheim, C.B. & Maechler, P. Beta-cell mitochondria and insulin secretion: messenger role of nucleotides and metabolites. *Diabetes* **51 Suppl 1**, S37-42 (2002).

13. Eliasson, L., Renstrom, E., Ding, W.G., Proks, P. & Rorsman, P. Rapid ATP-dependent priming of secretory granules precedes Ca(2+)-induced exocytosis in mouse pancreatic B-cells. *The Journal of physiology* **503 (Pt 2)**, 399-412 (1997).

14. Detimary, P., Gilon, P., Nenquin, M. & Henquin, J.C. Two sites of glucose control of insulin release with distinct dependence on the energy state in pancreatic B-cells. *The Biochemical journal* **297 (Pt 3)**, 455-461 (1994).

15. Detimary, P., Van den Berghe, G. & Henquin, J.C. Concentration dependence and time course of the effects of glucose on adenine and guanine nucleotides in mouse pancreatic islets. *The Journal of biological chemistry* **271**, 20559-20565 (1996).

16. Kibbey, R.G. *et al.* Mitochondrial GTP regulates glucose-stimulated insulin secretion. *Cell metabolism* **5**, 253-264 (2007).

17. Watkins, D.T. & Moore, M. Uptake of NADPH by islet secretion granule membranes. *Endocrinology* **100**, 1461-1467 (1977).

18. Ronnebaum, S.M. *et al.* A pyruvate cycling pathway involving cytosolic NADP-dependent isocitrate dehydrogenase regulates glucose-stimulated insulin secretion. *The Journal of biological chemistry* **281**, 30593-30602 (2006).

19. Ivarsson, R. *et al.* Redox control of exocytosis: regulatory role of NADPH, thioredoxin, and glutaredoxin. *Diabetes* **54**, 2132-2142 (2005).

20. Maechler, P. & Wollheim, C.B. Mitochondrial glutamate acts as a messenger in glucose-induced insulin exocytosis. *Nature* **402**, 685-689 (1999).

21. Hoy, M. *et al.* Increase in cellular glutamate levels stimulates exocytosis in pancreatic beta-cells. *FEBS letters* **531**, 199-203 (2002).

22. Bertrand, G., Ishiyama, N., Nenquin, M., Ravier, M.A. & Henquin, J.C. The elevation of glutamate content and the amplification of insulin secretion in glucose-stimulated pancreatic islets are not causally related. *The Journal of biological chemistry* **277**, 32883-32891 (2002).

23. MacDonald, M.J. & Fahien, L.A. Glutamate is not a messenger in insulin secretion. *The Journal of biological chemistry* **275**, 34025-34027 (2000).

24. Prentki, M. *et al.* Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *The Journal of biological chemistry* **267**, 5802-5810 (1992).

25. Mulder, H. *et al.* Overexpression of a modified human malonyl-CoA decarboxylase blocks the glucose-induced increase in malonyl-CoA level but has no impact on insu-

lin secretion in INS-1-derived (832/13) beta-cells. *The Journal of biological chemistry* **276**, 6479-6484 (2001).

26. Dachicourt, N., Serradas, P., Giroix, M.H., Gangnerau, M.N. & Portha, B. Decreased glucose-induced cAMP and insulin release in islets of diabetic rats: reversal by IBMX, glucagon, GIP. *The American journal of physiology* **271**, E725-732 (1996).

27. Schuit, F.C. & Pipeleers, D.G. Regulation of adenosine 3',5'-monophosphate levels in the pancreatic B cell. *Endocrinology* **117**, 834-840 (1985).

28. Yang, S. *et al.* Enhanced cAMP protein kinase A signaling determines improved insulin secretion in a clonal insulin-producing beta-cell line (INS-1 832/13). *Molecular endocrinology (Baltimore, Md* **18**, 2312-2320 (2004).

29. Ammala, C., Ashcroft, F.M. & Rorsman, P. Calcium-independent potentiation of insulin release by cyclic AMP in single [beta]-cells. *Nature* **363**, 356-358 (1993).

30. Renstrom, E., Eliasson, L. & Rorsman, P. Protein kinase A-dependent and -independent stimulation of exocytosis by cAMP in mouse pancreatic B-cells. *The Journal of physiology* **502 (Pt 1)**, 105-118 (1997).

31. Ozaki, N. *et al.* cAMP-GEFII is a direct target of cAMP in regulated exocytosis. *Nature cell biology* **2**, 805-811 (2000).

32. Eliasson, L. *et al.* SUR1 regulates PKA-independent cAMP-induced granule priming in mouse pancreatic B-cells. *The Journal of general physiology* **121**, 181-197 (2003).

33. Olofsson, C.S. *et al.* Fast insulin secretion reflects exocytosis of docked granules in mouse pancreatic B-cells. *Pflugers Arch* **444**, 43-51 (2002).

34. Rorsman, P. & Renstrom, E. Insulin granule dynamics in pancreatic beta cells. *Diabetologia* **46**, 1029-1045 (2003).

35. Hirokawa, N. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science (New York, N.Y* **279**, 519-526 (1998).

36. Hirokawa, N. & Noda, Y. Intracellular transport and kinesin superfamily proteins, KIFs: structure, function, and dynamics. *Physiological reviews* **88**, 1089-1118 (2008).

37. Meng, Y.X., Wilson, G.W., Avery, M.C., Varden, C.H. & Balczon, R. Suppression of the expression of a pancreatic beta-cell form of the kinesin heavy chain by antisense oligonucleotides inhibits insulin secretion from primary cultures of mouse beta-cells. *Endocrinology* **138**, 1979-1987 (1997).

38. Varadi, A., Tsuboi, T., Johnson-Cadwell, L.I., Allan, V.J. & Rutter, G.A. Kinesin I and cytoplasmic dynein orchestrate glucose-stimulated insulin-containing vesicle movements in clonal MIN6 [beta]-cells. *Biochemical and Biophysical Research Communications* **311**, 272-282 (2003).

39. Varadi, A., Ainscow, E.K., Allan, V.J. & Rutter, G.A. Involvement of conventional kinesin in glucose-stimulated secretory granule movements and exocytosis in clonal pancreatic beta-cells. *J Cell Sci* **115**, 4177-4189 (2002).

40. Donelan, M.J. *et al.* Ca2+-dependent dephosphorylation of kinesin heavy chain on beta-granules in pancreatic beta-cells. Implications for regulated beta-granule transport and insulin exocytosis. *The Journal of biological chemistry* **277**, 24232-24242 (2002).

41. Ivarsson, R., Obermuller, S., Rutter, G.A., Galvanovskis, J. & Renstrom, E. Temperature-sensitive random insulin granule diffusion is a prerequisite for recruiting granules for release. *Traffic* **5**, 750-762 (2004).

42. Ivarsson, R., Jing, X., Waselle, L., Regazzi, R. & Renstrom, E. Myosin 5a controls insulin granule recruitment during late-phase secretion. *Traffic* **6**, 1027-1035 (2005).

43. Varadi, A., Tsuboi, T. & Rutter, G.A. Myosin Va transports dense core secretory vesicles in pancreatic MIN6 beta-cells. *Molecular biology of the cell* **16**, 2670-2680 (2005).

44. Barg, S. *et al.* Priming of insulin granules for exocytosis by granular Cl(-) uptake and acidification. *J Cell Sci* **114**, 2145-2154 (2001).

45. Jahn, R. & Scheller, R.H. SNAREs [mdash] engines for membrane fusion. *Nat Rev Mol Cell Biol* **7**, 631-643 (2006).

46. Lang, J. Molecular mechanisms and regulation of insulin exocytosis as a paradigm of endocrine secretion. *European journal of biochemistry / FEBS* **259**, 3-17 (1999).

47. Martin, F., Moya, F., Gutierrez, L.M., Reig, J.A. & Soria, B. Role of syntaxin in mouse pancreatic beta cells. *Diabetologia* **38**, 860-863 (1995).

48. Regazzi, R. *et al.* VAMP-2 and cellubrevin are expressed in pancreatic beta-cells and are essential for Ca(2+)-but not for GTP gamma S-induced insulin secretion. *The EMBO journal* **14**, 2723-2730 (1995).

49. Sadoul, K. *et al.* SNAP-25 is expressed in islets of Langerhans and is involved in insulin release. *The Journal of cell biology* **128**, 1019-1028 (1995).

50. Kwan, E.P. & Gaisano, H.Y. New insights into the molecular mechanisms of priming of insulin exocytosis. *Diabetes, obesity & metabolism* **9 Suppl 2**, 99-108 (2007).

51. Tomas, A., Meda, P., Regazzi, R., Pessin, J.E. & Halban, P.A. Munc 18-1 and granuphilin collaborate during insulin granule exocytosis. *Traffic* **9**, 813-832 (2008).

52. Gauthier, B.R. *et al.* Synaptotagmin VII splice variants alpha, beta, and delta are expressed in pancreatic beta-cells and regulate insulin exocytosis. *Faseb J* **22**, 194-206 (2008).

53. Gustavsson, N. *et al.* Impaired insulin secretion and glucose intolerance in synaptotagmin-7 null mutant mice. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 3992-3997 (2008).

54. Iezzi, M., Eliasson, L., Fukuda, M. & Wollheim, C.B. Adenovirus-mediated silencing of synaptotagmin 9 inhibits Ca2+-dependent insulin secretion in islets. *FEBS letters* **579**, 5241-5246 (2005).

55. Barnard, R.J., Morgan, A. & Burgoyne, R.D. Stimulation of NSF ATPase activity by alpha-SNAP is required for SNARE complex disassembly and exocytosis. *The Journal of cell biology* **139**, 875-883 (1997).

56. Vikman, J., Ma, X., Tagaya, M. & Eliasson, L. Requirement for N-ethylmaleimide-sensitive factor for exocytosis of insulin-containing secretory granules in pancreatic beta-cells. *Biochemical Society transactions* **31**, 842-847 (2003).

57. Ostenson, C.G., Gaisano, H., Sheu, L., Tibell, A. & Bartfai, T. Impaired gene and protein expression of exocytotic soluble N-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients. *Diabetes* **55**, 435-440 (2006).

58. Muoio, D.M. & Newgard, C.B. Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. *Nat Rev Mol Cell Biol* **9**, 193-205 (2008).

59. Butler, A.E. *et al.* Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102-110 (2003).

60. Anello, M. *et al.* Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. *Diabetologia* **48**, 282-289 (2005).

61. Deng, S. *et al.* Structural and functional abnormalities in the islets isolated from type 2 diabetic subjects. *Diabetes* **53**, 624-632 (2004).

62. Lenzen, S., Drinkgern, J. & Tiedge, M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues. *Free radical biology & medicine* **20**, 463-466 (1996).

63. Kubisch, H.M. *et al.* Transgenic copper/zinc superoxide dismutase modulates susceptibility to type I diabetes. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 9956-9959 (1994).

64. Cnop, M., Igoillo-Esteve, M., Cunha, D.A., Ladriere, L. & Eizirik, D.L. An update on lipotoxic endoplasmic reticulum stress in pancreatic beta-cells. *Biochemical Society transactions* **36**, 909-915 (2008).

65. Laybutt, D.R. *et al.* Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. *Diabetologia* **50**, 752-763 (2007).

66. Cunha, D.A. *et al.* Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. *J Cell Sci* **121**, 2308-2318 (2008).

67. Elouil, H. *et al.* Acute nutrient regulation of the unfolded protein response and integrated stress response in cultured rat pancreatic islets. *Diabetologia* **50**, 1442-1452 (2007).

68. Huntington, G. On Chorea. *The Medical and Surgical Reporter: A Weekly Journal* **26**, 317-321 (1872).

69. Harper, P.S. The epidemiology of Huntington's disease. *Human genetics* **89**, 365-376 (1992).

70. Group, H.s.D.C.R. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* **72**, 971-983 (1993).

71. McNeil, S.M. *et al.* Reduced penetrance of the Huntington's disease mutation. *Human molecular genetics* **6**, 775-779 (1997).

72. Myers, R.H. Huntington's disease genetics. *NeuroRx* **1**, 255-262 (2004).

73. Duyao, M. *et al.* Trinucleotide repeat length instability and age of onset in Huntington's disease. *Nature genetics* **4**, 387-392 (1993).

74. Nance, M.A. & Myers, R.H. Juvenile onset Huntington's disease--clinical and research perspectives. *Mental retardation and developmental disabilities research reviews* **7**, 153-157 (2001).

75. Wexler, N.S. *et al.* Venezuelan kindreds reveal that genetic and environmental factors modulate Huntington's disease age of onset. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 3498-3503 (2004).

76. Squitieri, F. *et al.* Homozygosity for CAG mutation in Huntington disease is associated with a more severe clinical course. *Brain* **126**, 946-955 (2003).

77. Bates, G., Harper, P.S. & Jones, L. *Huntington's disease*, Edn. 3rd. (Oxford University Press, Oxford ; New York; 2002).

78. Walker, F.O. Huntington's Disease. *Seminars in neurology* **27**, 143-150 (2007).

79. Vonsattel, J.P. *et al.* Neuropathological classification of Huntington's disease. *Journal of neuropathology and experimental neurology* **44**, 559-577 (1985).

80. Halliday, G.M. *et al.* Regional specificity of brain atrophy in Huntington's disease. *Exp Neurol* **154**, 663-672 (1998).

81. Kassubek, J., Juengling, F.D., Ecker, D. & Landwehrmeyer, G.B. Thalamic atrophy in Huntington's disease co-varies with cognitive performance: a morphometric MRI analysis. *Cereb Cortex* **15**, 846-853 (2005).

82. Kremer, H.P., Roos, R.A., Dingjan, G., Marani, E. & Bots, G.T. Atrophy of the hypothalamic lateral tuberal nucleus in Huntington's disease. *Journal of neuropathology and experimental neurology* **49**, 371-382 (1990).

83. Fennema-Notestine, C. *et al.* In vivo evidence of cerebellar atrophy and cerebral white matter loss in Huntington disease. *Neurology* **63**, 989-995 (2004).

84. Spargo, E., Everall, I.P. & Lantos, P.L. Neuronal loss in the hippocampus in Huntington's disease: a comparison with HIV infection. *Journal of neurology, neurosurgery, and psychiatry* **56**, 487-491 (1993).

85. Rosas, H.D. *et al.* Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. *Neurology* **60**, 1615-1620 (2003).

86. Sorensen, S.A. & Fenger, K. Causes of death in patients with Huntington's disease and in unaffected first degree relatives. *Journal of medical genetics* **29**, 911-914 (1992).

87. Ristow, M. Neurodegenerative disorders associated with diabetes mellitus. *Journal of molecular medicine (Berlin, Germany)* **82**, 510-529 (2004).

88. Podolsky, S. Hormone studies in patients with Huntington's disease. *AGE* **2**, 17-22 (1979).

89. Podolsky, S. & Leopold, N.A. Abnormal glucose tolerance and arginine tolerance tests in Huntington's disease. *Gerontology* **23**, 55-63 (1977).

90. Podolsky, S., Leopold, N.A. & Sax, D.S. Increased frequency of diabetes mellitus in patients with Huntington's chorea. *Lancet* **1**, 1356-1358 (1972).

91. Schubotz, R., Hausmann, L., Kaffarnik, H., Zehner, J. & Oepen, H. [Fatty acid patterns and glucose tolerance in Huntington's chorea (author's transl)]. *Research in experimental medicine* **167**, 203-215 (1976).

92. Kremer, H.P. *et al.* Endocrine functions in Huntington's disease. A two-and-a-half years follow-up study. *Journal of the neurological sciences* **90**, 335-344 (1989).

93. Davidson, M.B., Green, S. & Menkes, J.H. Normal glucose, insulin, and growth hormone responses to oral glucose in Huntington's disease. *Journal of Laboratory and Clinical Medicine* **84**, 807-812 (1974).

94. Phillipson, O.T. & Bird, E.D. Plasma Glucose, Non-Esterified Fatty-Acids and Amino-Acids in Huntingtons-Chorea. *Clinical Science and Molecular Medicine* **52**, 311-318 (1977).

95. Keogh, H.J., Johnson, R.H., Nanda, R.N. & Sulaiman, W.R. Altered growth hormone release in Huntington's chorea. *Journal of neurology, neurosurgery, and psychiatry* **39**, 244-248 (1976).

96. Lavin, P.J., Bone, I. & Sheridan, P. Studies of hypothalamic function in Huntington's chorea. *Journal of neurology, neurosurgery, and psychiatry* **44**, 414-418 (1981).

97. Lalic, N.M. *et al.* Glucose homeostasis in Huntington disease: abnormalities in insulin sensitivity and early-phase insulin secretion. *Archives of neurology* **65**, 476-480 (2008).

98. Farrer, L.A. Diabetes mellitus in Huntington disease. *Clinical genetics* **27**, 62-67 (1985).

99. Andreassen, O.A. *et al.* Dichloroacetate exerts therapeutic effects in transgenic mouse models of Huntington's disease. *Annals of neurology* **50**, 112-117 (2001).

100. Bjorkqvist, M. *et al.* Progressive alterations in the hypothalamic-pituitary-adrenal axis in the R6/2 transgenic mouse model of Huntington's disease. *Human molecular genetics* **15**, 1713-1721 (2006).

101. Chou, S.Y. *et al.* CGS21680 attenuates symptoms of Huntington's disease in a transgenic mouse model. *Journal of neurochemistry* **93**, 310-320 (2005).

102. Hunt, M.J. & Morton, A.J. Atypical diabetes associated with inclusion formation in the R6/2 mouse model of Huntington's disease is not improved by treatment with hypoglycaemic agents. *Experimental brain research. Experimentelle Hirnforschung* **166**, 220-229 (2005).

103. Hurlbert, M.S. *et al.* Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. *Diabetes* **48**, 649-651 (1999).

104. Luesse, H.G. *et al.* Evaluation of R6/2 HD transgenic mice for therapeutic studies in Huntington's disease: behavioral testing and impact of diabetes mellitus. *Behavioural brain research* **126**, 185-195 (2001).

105. Ma, T.C. *et al.* Metformin therapy in a transgenic mouse model of Huntington's disease. *Neuroscience letters* **411**, 98-103 (2007).

106. Miller, T.W., Shirley, T.L., Wolfgang, W.J., Kang, X. & Messer, A. DNA vaccination against mutant huntingtin ameliorates the HDR6/2 diabetic phenotype. *Mol Ther* **7**, 572-579 (2003).

107. Nguyen, T., Hamby, A. & Massa, S.M. Clioquinol down-regulates mutant huntingtin expression in vitro and mitigates pathology in a Huntington's disease mouse model. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 11840-11845 (2005).

108. Tanaka, M. *et al.* Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nature medicine* **10**, 148-154 (2004).

109. Andreassen, O.A. *et al.* Huntington's disease of the endocrine pancreas: insulin deficiency and diabetes mellitus due to impaired insulin gene expression. *Neurobiol Dis* **11**, 410-424 (2002).

110. Rocic, B., Lovrencic, M.V., Poje, M. & Ashcroft, S.J. Effect of creatine on the pancreatic beta-cell. *Exp Clin Endocrinol Diabetes* **115**, 29-32 (2007).

111. Ferrante, R.J. *et al.* Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* **20**, 4389-4397 (2000).

112. Hansson, O. *et al.* Transgenic mice expressing a Huntington's disease mutation are resistant to quinolinic acid-induced striatal excitotoxicity. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 8727-8732 (1999).

113. van Dellen, A., Blakemore, C., Deacon, R., York, D. & Hannan, A.J. Delaying the onset of Huntington's in mice. *Nature* **404**, 721-722 (2000).

114. Josefsen, K. *et al.* Impaired glucose tolerance in the R6/1 transgenic mouse model of Huntington's disease. *Journal of neuroendocrinology* **20**, 165-172 (2008).

115. Andreassen, O.A. *et al.* Creatine increase survival and delays motor symptoms in a transgenic animal model of Huntington's disease. *Neurobiol Dis* **8**, 479-491 (2001).

116. Duan, W. *et al.* Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 2911-2916 (2003).

117. Martin, B. *et al.* Exendin-4 improves glycemic control, ameliorates brain and pancreatic pathologies, and extends survival in a mouse model of Huntington's disease. *Diabetes* **58**, 318-328 (2009).

118. Craft, S. & Watson, G.S. Insulin and neurodegenerative disease: shared and specific mechanisms. *Lancet neurology* **3**, 169-178 (2004).

119. Yamamoto, A., Cremona, M.L. & Rothman, J.E. Autophagy-mediated clearance of huntingtin aggregates triggered by the insulin-signaling pathway. *The Journal of cell biology* **172**, 719-731 (2006).

120. Li, S.H. *et al.* Huntington's disease gene (IT15) is widely expressed in human and rat tissues. *Neuron* **11**, 985-993 (1993).

121. Strong, T.V. *et al.* Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nature genetics* **5**, 259-265 (1993).

122. Hoogeveen, A.T. *et al.* Characterization and localization of the Huntington disease gene product. *Human molecular genetics* **2**, 2069-2073 (1993).

123. Sharp, A.H. *et al.* Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron* **14**, 1065-1074 (1995).

124. Trottier, Y. *et al.* Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nature genetics* **10**, 104-110 (1995).

125. DiFiglia, M. *et al.* Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* **14**, 1075-1081 (1995).

126. Gutekunst, C.A. *et al.* Identification and localization of huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 8710-8714 (1995).

127. Hoffner, G., Kahlem, P. & Djian, P. Perinuclear localization of huntingtin as a consequence of its binding to microtubules through an interaction with beta-tubulin: relevance to Huntington's disease. *J Cell Sci* **115**, 941-948 (2002).

128. Choo, Y.S., Johnson, G.V., MacDonald, M., Detloff, P.J. & Lesort, M. Mutant huntingtin directly increases susceptibility of mitochondria to the calcium-induced permeability transition and cytochrome c release. *Human molecular genetics* **13**, 1407-1420 (2004).

129. Gutekunst, C.A. *et al.* The cellular and subcellular localization of huntingtin-associated protein 1 (HAP1): comparison with huntingtin in rat and human. *J Neurosci* , 7674-7686 (1998).

130. Duyao, M.P. *et al.* Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science (New York, N.Y* **269**, 407-410 (1995).

131. Nasir, J. *et al.* Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell* **81**, 811-823 (1995).

132. Zeitlin, S., Liu, J.P., Chapman, D.L., Papaioannou, V.E. & Efstratiadis, A. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nature genetics* **11**, 155-163 (1995).

133. Qin, Z.H. & Gu, Z.L. Huntingtin processing in pathogenesis of Huntington disease. *Acta pharmacologica Sinica* **25**, 1243-1249 (2004).

134. Graham, R.K. *et al.* Cleavage at the caspase-6 site is required for neuronal dysfunction and degeneration due to mutant huntingtin. *Cell* **125**, 1179-1191 (2006).

135. Davies, S.W. *et al.* Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* **90**, 537-548 (1997).

136. DiFiglia, M. *et al.* Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science (New York, N.Y* **277**, 1990-1993 (1997).

137. Lunkes, A. *et al.* Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. *Mol Cell* **10**, 259-269 (2002).

138. Saft, C. *et al.* Mitochondrial impairment in patients and asymptomatic mutation carriers of Huntington's disease. *Mov Disord* **20**, 674-679 (2005).

139. Sathasivam, K. *et al.* Formation of polyglutamine inclusions in non-CNS tissue. *Human molecular genetics* **8**, 813-822 (1999).

140. Saudou, F., Finkbeiner, S., Devys, D. & Greenberg, M.E. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* **95**, 55-66 (1998).

141. Slow, E.J. *et al.* Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin inclusions. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 11402-11407 (2005).

142. Arrasate, M., Mitra, S., Schweitzer, E.S., Segal, M.R. & Finkbeiner, S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* **431**, 805-810 (2004).

143. Browne, S.E. Mitochondria and Huntington's disease pathogenesis: insight from genetic and chemical models. *Annals of the New York Academy of Sciences* **1147**, 358-382 (2008).

144. Mochel, F. *et al.* Early energy deficit in Huntington disease: identification of a plasma biomarker traceable during disease progression. *PLoS ONE* **2**, e647 (2007).

145. Trejo, A. *et al.* Assessment of the nutrition status of patients with Huntington's disease. *Nutrition (Burbank, Los Angeles County, Calif* **20**, 192-196 (2004).

146. Myers, R.H. *et al.* Factors associated with slow progression in Huntington's disease. *Archives of neurology* **48**, 800-804 (1991).

147. Mazziotta, J.C. *et al.* Reduced cerebral glucose metabolism in asymptomatic subjects at risk for Huntington's disease. *The New England journal of medicine* **316**, 357-362 (1987).

148. Reynolds, N.C., Jr., Prost, R.W. & Mark, L.P. Heterogeneity in 1H-MRS profiles of presymptomatic and early manifest Huntington's disease. *Brain research* **1031**, 82-89 (2005).

149. Beal, M.F. *et al.* Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid. *J Neurosci* **13**, 4181-4192 (1993).

150. Arenas, J. *et al.* Complex I defect in muscle from patients with Huntington's disease. *Annals of neurology* **43**, 397-400 (1998).

151. Goebel, H.H., Heipertz, R., Scholz, W., Iqbal, K. & Tellez-Nagel, I. Juvenile Huntington chorea: clinical, ultrastructural, and biochemical studies. *Neurology* **28**, 23-31 (1978).

152. Mihm, M.J. *et al.* Cardiac dysfunction in the R6/2 mouse model of Huntington's disease. *Neurobiol Dis* **25**, 297-308 (2007).

153. Squitieri, F. *et al.* Severe ultrastructural mitochondrial changes in lymphoblasts homozygous for Huntington disease mutation. *Mechanisms of ageing and development* **127**, 217-220 (2006).

154. Wang, H., Lim, P.J., Karbowski, M. & Monteiro, M.J. Effects of overexpression of huntingtin proteins on mitochondrial integrity. *Human molecular genetics* **18**, 737-752 (2009).

155. Banoei, M.M. *et al.* Huntington's disease and mitochondrial DNA deletions: event or regular mechanism for mutant huntingtin protein and CAG repeats expansion?! *Cellular and molecular neurobiology* **27**, 867-875 (2007).

156. Horton, T.M. *et al.* Marked increase in mitochondrial DNA deletion levels in the cerebral cortex of Huntington's disease patients. *Neurology* **45**, 1879-1883 (1995).

157. Chen, X., Bonilla, E., Sciacco, M. & Schon, E.A. Paucity of deleted mitochondrial DNAs in brain regions of Huntington's disease patients. *Biochimica et biophysica acta* **1271**, 229-233 (1995).

158. Guidetti, P. *et al.* Early degenerative changes in transgenic mice expressing mutant huntingtin involve dendritic abnormalities but no impairment of mitochondrial energy production. *Exp Neurol* **169**, 340-350 (2001).

159. Parker, W.D., Jr., Boyson, S.J., Luder, A.S. & Parks, J.K. Evidence for a defect in NADH: ubiquinone oxidoreductase (complex I) in Huntington's disease. *Neurology* **40**, 1231-1234 (1990).

160. Benchoua, A. *et al.* Involvement of mitochondrial complex II defects in neuronal death produced by N-terminus fragment of mutated huntingtin. *Molecular biology of the cell* **17**, 1652-1663 (2006).

161. Browne, S.E. *et al.* Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Annals of neurology* **41**, 646-653 (1997).

162. Butterworth, J., Yates, C.M. & Reynolds, G.P. Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase and gamma-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. *Journal of the neurological sciences* **67**, 161-171 (1985).

163. Gu, M. *et al.* Mitochondrial defect in Huntington's disease caudate nucleus. *Annals of neurology* **39**, 385-389 (1996).

164. Mann, V.M. *et al.* Mitochondrial function and parental sex effect in Huntington's disease. *Lancet* **336**, 749 (1990).

165. Stahl, W.L. & Swanson, P.D. Biochemical abnormalities in Huntington's chorea brains. *Neurology* **24**, 813-819 (1974).

166. Tabrizi, S.J. *et al.* Biochemical abnormalities and excitotoxicity in Huntington's disease brain. *Annals of neurology* **45**, 25-32 (1999).

167. Brennan, W.A., Jr., Bird, E.D. & Aprille, J.R. Regional mitochondrial respiratory activity in Huntington's disease brain. *Journal of neurochemistry* **44**, 1948-1950 (1985).

168. Bae, B.I. *et al.* p53 mediates cellular dysfunction and behavioral abnormalities in Huntington's disease. *Neuron* **47**, 29-41 (2005).

169. Fukui, H. & Moraes, C.T. Extended polyglutamine repeats trigger a feedback loop involving the mitochondrial complex III, the proteasome and huntingtin aggregates. *Human molecular genetics* **16**, 783-797 (2007).

170. Gizatullina, Z.Z. *et al.* Low stability of Huntington muscle mitochondria against Ca2+ in R6/2 mice. *Annals of neurology* **59**, 407-411 (2006).

171. Majumder, P., Raychaudhuri, S., Chattopadhyay, B. & Bhattacharyya, N.P. Increased caspase-2, calpain activations and decreased mitochondrial complex II activity in cells expressing exogenous huntingtin exon 1 containing CAG repeat in the pathogenic range. *Cellular and molecular neurobiology* **27**, 1127-1145 (2007).

172. Tabrizi, S.J. *et al.* Mitochondrial dysfunction and free radical damage in the Huntington R6/2 transgenic mouse. *Annals of neurology* **47**, 80-86 (2000).

173. Milakovic, T. & Johnson, G.V. Mitochondrial respiration and ATP production are significantly impaired in striatal cells expressing mutant huntingtin. *The Journal of biological chemistry* **280**, 30773-30782 (2005).

174. Klivenyi, P. *et al.* Mice deficient in dihydrolipoamide dehydrogenase show increased vulnerability to MPTP, malonate and 3-nitropropionic acid neurotoxicity. *Journal of neurochemistry* **88**, 1352-1360 (2004).

175. Sorolla, M.A. *et al.* Proteomic and oxidative stress analysis in human brain samples of Huntington disease. *Free radical biology & medicine* **45**, 667-678 (2008).

176. Higgins, D.S., Hoyt, K.R., Baic, C., Vensel, J. & Sulka, M. Metabolic and glutamatergic disturbances in the Huntington's disease transgenic mouse. *Annals of the New York Academy of Sciences* **893**, 298-300 (1999).

177. Perluigi, M. *et al.* Proteomic analysis of protein expression and oxidative modification in r6/2 transgenic mice: a model of Huntington disease. *Mol Cell Proteomics* **4**, 1849-1861 (2005).

178. Sorbi, S., Bird, E.D. & Blass, J.P. Decreased pyruvate dehydrogenase complex activity in Huntington and Alzheimer brain. *Annals of neurology* **13**, 72-78 (1983).

179. Cui, L. *et al.* Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* **127**, 59-69 (2006).

180. Weydt, P. *et al.* Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. *Cell metabolism* **4**, 349-362 (2006).

181. Taherzadeh-Fard, E., Saft, C., Andrich, J., Wieczorek, S. & Arning, L. PGC-1alpha as modifier of onset age in Huntington disease. *Molecular neurodegeneration* **4**, 10 (2009).

182. Weydt, P. *et al.* The gene coding for PGC-1alpha modifies age at onset in Huntington's Disease. *Molecular neurodegeneration* **4**, 3 (2009).

183. Stack, E.C., Matson, W.R. & Ferrante, R.J. Evidence of oxidant damage in Huntington's disease: translational strategies using antioxidants. *Annals of the New York Academy of Sciences* **1147**, 79-92 (2008).

184. Gines, S. *et al.* Specific progressive cAMP reduction implicates energy deficit in presymptomatic Huntington's disease knock-in mice. *Human molecular genetics* **12**, 497-508 (2003).

185. Lodi, R. *et al.* Abnormal in vivo skeletal muscle energy metabolism in Huntington's disease and dentatorubropallidoluysian atrophy. *Annals of neurology* **48**, 72-76 (2000).

186. Seong, I.S. *et al.* HD CAG repeat implicates a dominant property of huntingtin in mitochondrial energy metabolism. *Human molecular genetics* **14**, 2871-2880 (2005).

187. Fernandes, H.B., Baimbridge, K.G., Church, J., Hayden, M.R. & Raymond, L.A. Mitochondrial sensitivity and altered calcium handling underlie enhanced NMDA-induced apoptosis in YAC128 model of Huntington's disease. *J Neurosci* **27**, 13614-13623 (2007).

188. Milakovic, T., Quintanilla, R.A. & Johnson, G.V. Mutant huntingtin expression induces mitochondrial calcium handling defects in clonal striatal cells: functional consequences. *The Journal of biological chemistry* **281**, 34785-34795 (2006).

189. Oliveira, J.M. *et al.* Mitochondrial-dependent Ca2+ handling in Huntington's disease striatal cells: effect of histone deacetylase inhibitors. *J Neurosci* **26**, 11174-11186 (2006).

190. Oliveira, J.M. *et al.* Mitochondrial dysfunction in Huntington's disease: the bioenergetics of isolated and in situ mitochondria from transgenic mice. *Journal of neurochemistry* **101**, 241-249 (2007).

191. Panov, A.V., Burke, J.R., Strittmatter, W.J. & Greenamyre, J.T. In vitro effects of polyglutamine tracts on Ca2+-dependent depolarization of rat and human mitochondria: relevance to Huntington's disease. *Archives of biochemistry and biophysics* **410**, 1-6 (2003).

192. Panov, A.V. *et al.* Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nature neuroscience* **5**, 731-736 (2002).

193. Sawa, A. *et al.* Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. *Nature medicine* **5**, 1194-1198 (1999).

194. Fan, M.M., Fernandes, H.B., Zhang, L.Y., Hayden, M.R. & Raymond, L.A. Altered NMDA receptor trafficking in a yeast artificial chromosome transgenic mouse model of Huntington's disease. *J Neurosci* **27**, 3768-3779 (2007).

195. Lee, J.M. *et al.* Unbiased gene expression analysis implicates the huntingtin polyglutamine tract in extra-mitochondrial energy metabolism. *PLoS genetics* **3**, e135 (2007).

196. Bird, E.D., Gale, J.S. & Spokes, E.G. Huntington's chorea: post mortem activity of enzymes involved in cerebral glucose metabolism. *Journal of neurochemistry* **29**, 539-545 (1977).

197. Kish, S.J. *et al.* Brain glyceraldehyde-3-phosphate dehydrogenase activity in human trinucleotide repeat disorders. *Archives of neurology* **55**, 1299-1304 (1998).

198. Powers, W.J. *et al.* Selective defect of in vivo glycolysis in early Huntington's disease striatum. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 2945-2949 (2007).

199. Block-Galarza, J. *et al.* Fast transport and retrograde movement of huntingtin and HAP 1 in axons. *Neuroreport* **8**, 2247-2251 (1997).

200. Caviston, J.P., Ross, J.L., Antony, S.M., Tokito, M. & Holzbaur, E.L. Huntingtin facilitates dynein/dynactin-mediated vesicle transport. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 10045-10050 (2007).

201. Li, X.J. *et al.* A huntingtin-associated protein enriched in brain with implications for pathology. *Nature* **378**, 398-402 (1995).

202. Engelender, S. *et al.* Huntingtin-associated protein 1 (HAP1) interacts with the p150Glued subunit of dynactin. *Human molecular genetics* **6**, 2205-2212 (1997).

203. Li, S.H., Gutekunst, C.A., Hersch, S.M. & Li, X.J. Interaction of huntingtin-associated protein with dynactin P150Glued. *J Neurosci* **18**, 1261-1269 (1998).

204. McGuire, J.R., Rong, J., Li, S.H. & Li, X.J. Interaction of Huntingtin-associated protein-1 with kinesin light chain: implications in intracellular trafficking in neurons. *The Journal of biological chemistry* **281**, 3552-3559 (2006).

205. Colin, E. *et al.* Huntingtin phosphorylation acts as a molecular switch for anterograde/retrograde transport in neurons. *The EMBO journal* (2008).

206. Gauthier, L.R. *et al.* Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell* **118**, 127-138 (2004).

207. Her, L.S. & Goldstein, L.S. Enhanced sensitivity of striatal neurons to axonal transport defects induced by mutant huntingtin. *J Neurosci* **28**, 13662-13672 (2008).

208. Rong, J. *et al.* 14-3-3 protein interacts with Huntingtin-associated protein 1 and regulates its trafficking. *The Journal of biological chemistry* **282**, 4748-4756 (2007).

209. del Toro, D. *et al.* Mutant huntingtin impairs the post-Golgi trafficking of brain-derived neurotrophic factor but not its Val66Met polymorphism. *J Neurosci* **26**, 12748-12757 (2006).

210. Sinadinos, C. *et al.* Live axonal transport disruption by mutant huntingtin fragments in Drosophila motor neuron axons. *Neurobiol Dis* (2009).

211. Szebenyi, G. *et al.* Neuropathogenic forms of huntingtin and androgen receptor inhibit fast axonal transport. *Neuron* **40**, 41-52 (2003).

212. Chang, D.T., Rintoul, G.L., Pandipati, S. & Reynolds, I.J. Mutant huntingtin aggregates impair mitochondrial movement and trafficking in cortical neurons. *Neurobiol Dis* **22**, 388-400 (2006).

213. Lee, W.C., Yoshihara, M. & Littleton, J.T. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 3224-3229 (2004).

214. Gunawardena, S. *et al.* Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. *Neuron* **40**, 25-40 (2003).

215. Trushina, E. *et al.* Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. *Mol Cell Biol* **24**, 8195-8209 (2004).

216. Dompierre, J.P. *et al.* Histone deacetylase 6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation. *J Neurosci* **27**, 3571-3583 (2007).

217. Orr, A.L. *et al.* N-terminal mutant huntingtin associates with mitochondria and impairs mitochondrial trafficking. *J Neurosci* **28**, 2783-2792 (2008).

218. del Toro, D. *et al.* Mutant huntingtin impairs post-Golgi trafficking to lysosomes by delocalizing optineurin/Rab8 complex from the Golgi apparatus. *Molecular biology of the cell* **20**, 1478-1492 (2009).

219. Morfini, G. *et al.* JNK mediates pathogenic effects of polyglutamine-expanded androgen receptor on fast axonal transport. *Nature neuroscience* **9**, 907-916 (2006).

220. Smith, R., Brundin, P. & Li, J.Y. Synaptic dysfunction in Huntington's disease: a new perspective. *Cell Mol Life Sci* **62**, 1901-1912 (2005).

221. Mangiarini, L. *et al.* Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87**, 493-506 (1996).

222. Stack, E.C. *et al.* Chronology of behavioral symptoms and neuropathological sequela in R6/2 Huntington's disease transgenic mice. *The Journal of comparative neurology* **490**, 354-370 (2005).

223. Asfari, M. *et al.* Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* **130**, 167-178 (1992).

224. Hohmeier, H.E. *et al.* Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes* **49**, 424-430 (2000).

225. Becker, T.C. *et al.* Use of recombinant adenovirus for metabolic engineering of mammalian cells. *Methods in cell biology* **43 Pt A**, 161-189 (1994).

226. Mittereder, N., March, K.L. & Trapnell, B.C. Evaluation of the concentration and bioactivity of adenovirus vectors for gene therapy. *Journal of virology* **70**, 7498-7509 (1996).

227. Toomre, D., Keller, P., White, J., Olivo, J.C. & Simons, K. Dual-color visualization of trans-Golgi network to plasma membrane traffic along microtubules in living cells. *J Cell Sci* **112 (Pt 1)**, 21-33 (1999).

228. Wasmeier, C. & Hutton, J.C. Molecular cloning of phogrin, a protein-tyrosine phosphatase homologue localized to insulin secretory granule membranes. *The Journal of biological chemistry* **271**, 18161-18170 (1996).

229. Torii, S. *et al.* Cytoplasmic transport signal is involved in phogrin targeting and localization to secretory granules. *Traffic* **6**, 1213-1224 (2005).

230. Lukinius, A., Korsgren, O., Grimelius, L. & Wilander, E. Expression of islet amyloid polypeptide in fetal and adult porcine and human pancreatic islet cells. *Endocrinology* **138**, 5319-5325 (1997).

231. Phair, R.D. & Misteli, T. High mobility of proteins in the mammalian cell nucleus. *Nature* **404**, 604-609 (2000).

232. Dundr, M. *et al.* A kinetic framework for a mammalian RNA polymerase in vivo. *Science (New York, N.Y* **298**, 1623-1626 (2002).

233. Soderberg, O. *et al.* Direct observation of individual endogenous protein complexes in situ by proximity ligation. *Nat Methods* **3**, 995-1000 (2006).

234. Hortin, G.L. The MALDI-TOF mass spectrometric view of the plasma proteome and peptidome. *Clinical chemistry* **52**, 1223-1237 (2006).

235. McNiven, M.A. & Thompson, H.M. Vesicle formation at the plasma membrane and trans-Golgi network: the same but different. *Science (New York, N.Y* **313**, 1591-1594 (2006).

236. Burnett, B.G., Andrews, J., Ranganathan, S., Fischbeck, K.H. & Di Prospero, N.A. Expression of expanded polyglutamine targets profilin for degradation and alters actin dynamics. *Neurobiol Dis* **30**, 365-374 (2008).

237. Holbert, S. *et al.* Cdc42-interacting protein 4 binds to huntingtin: neuropathologic and biological evidence for a role in Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 2712-2717 (2003).

238. Modregger, J., DiProspero, N.A., Charles, V., Tagle, D.A. & Plomann, M. PACSIN 1 interacts with huntingtin and is absent from synaptic varicosities in presymptomatic Huntington's disease brains. *Human molecular genetics* **11**, 2547-2558 (2002).

239. Modregger, J., Schmidt, A.A., Ritter, B., Huttner, W.B. & Plomann, M. Characterization of Endophilin B1b, a brain-specific membrane-associated lysophosphatidic acid acyl transferase with properties distinct from endophilin A1. *The Journal of biological chemistry* **278**, 4160-4167 (2003).

240. Qin, Z.H. *et al.* Huntingtin bodies sequester vesicle-associated proteins by a polyproline-dependent interaction. *J Neurosci* **24**, 269-281 (2004).

241. Rangone, H. *et al.* Phosphorylation of arfaptin 2 at Ser260 by Akt Inhibits PolyQ-huntingtin-induced toxicity by rescuing proteasome impairment. *The Journal of biological chemistry* **280**, 22021-22028 (2005).

242. Singaraja, R.R. *et al.* HIP14, a novel ankyrin domain-containing protein, links huntingtin to intracellular trafficking and endocytosis. *Human molecular genetics* **11**, 2815-2828 (2002).

243. Waelter, S. *et al.* The huntingtin interacting protein HIP1 is a clathrin and alpha-adaptin-binding protein involved in receptor-mediated endocytosis. *Human molecular genetics* **10**, 1807-1817 (2001).

244. Aziz, N.A. *et al.* Increased hypothalamic-pituitary-adrenal axis activity in Huntington's disease. *The Journal of clinical endocrinology and metabolism* (2009).

245. Leblhuber, F. *et al.* Serum dehydroepiandrosterone and cortisol measurements in Huntington's chorea. *Journal of the neurological sciences* **132**, 76-79 (1995).

246. Andrews, R.C. & Walker, B.R. Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci (Lond)* **96**, 513-523 (1999).

247. Gamberino, W.C. & Brennan, W.A., Jr. Glucose transporter isoform expression in Huntington's disease brain. *Journal of neurochemistry* **63**, 1392-1397 (1994).

248. Colin, E. *et al.* Akt is altered in an animal model of Huntington's disease and in patients. *The European journal of neuroscience* **21**, 1478-1488 (2005).