Tau phosphorylation as adaptive response to metabolic dysfunction in the brain
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Citation for published version (APA):
van der Harg, J. M. (2017). Tau phosphorylation as adaptive response to metabolic dysfunction in the brain

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Introduction
Tauopathies

Tauopathies comprise a group of neurodegenerative disorders that is characterized by depositions of the tau protein in the brain. Neurodegenerative diseases with tau inclusion bodies are Alzheimer’s disease (AD), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick’s disease (PiD), argyrophilic grain disease (AGD), as well as hereditary frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [1]. Patients diagnosed with a tauopathy develop a progressive impairment of cognitive functions. The exact manifestation of the symptoms is diverse among the different tauopathies, but is directly linked to the location of the tau inclusion bodies.

AD is the most common tauopathy and therefore most extensively studied. AD is characterized not only by tangles composed of aggregated tau but also by plaques consisting of Aβ. Two types of AD can be distinguished based on their etiology: familial or early onset AD and sporadic or late onset AD. Familial AD accounts for only 5% - 10% of the cases and is characterized by an early onset of the disease (<65 years). Familial AD is caused by mutations in the genes encoding for amyloid precursor protein, presenilin-1, and presenilin-2 [2]. These mutations lead to alterations in the Aβ production. The large majority of the AD cases is sporadic AD that is characterized by a late onset of the disease (>65 years), however, the cause for sporadic AD is still elusive. APOE is the most common genetic risk factor for sporadic AD [3]. Recently, the identification of genes associated with increased risk of sporadic AD has evolved tremendously due to genome-wide association studies (GWAS). Genes involved in the immune response, cholesterol and lipid metabolism, but also in endoplasmic reticulum (ER) function are currently identified as risk factors [4, 5]. Environmental factors also contribute to the development of AD. For instance, diabetes mellitus (DM) and aging both increase the risk for AD. The later the onset of AD, the more dominant the environmental factors are in the development of the disease.

Although the genetic basis for sporadic AD is still elusive, tau aggregation closely correlates with cognitive decline in AD and other tauopathies [6]. Interestingly, FTDP-17 is directly linked to mutations in the gene encoding for tau, MAPT [7]. The mutations in the tau gene leading to FTDP-17 confirm a causal link between tau dysfunction and cognitive deficits. Since the important role of tau in the pathogenesis of tauopathies, we next will discuss the tau protein in more detail and its role in health and disease.

Physiological tau

Tau is a microtubule-associated protein that is highly expressed in neurons where it is predominantly localized in axons (Figure 1A). Alternative splicing of human tau results in six different tau isoforms [8, 9]. All tau isoforms contain an N-terminal domain, a Proline-rich domain, a microtubule binding region and a C-terminal domain. The function of tau is regulated by post-translational modifications, including O-GlcNAcylation, ubiquitination, methylation, acetylation and phosphorylation [10, 11]. Phosphorylation is the most frequent post-translational modification of tau. Since tau inclusion bodies
consist of phosphorylated tau, phosphorylation is also the most extensively studied tau modification. The phosphorylation and dephosphorylation of tau is a normal dynamic process and is regulated by a change in activity of one or multiple kinases and phosphatases. At least 30 kinases are able to phosphorylate tau in vitro [12]. Among them a wide variety of tau kinases are reported to regulate tau phosphorylation in vivo [13-16]. There are only four protein phosphatases (PPs) known to be highly expressed in the brain that dephosphorylate tau at multiple sites [15, 17].

Tau is an important protein in healthy neurons (Figure 1A). It is involved in the stabilization of microtubules. Tau binds to the microtubules via interaction of its positively charged microtubule binding domain with the negatively charged C-termini of the tubulin molecules [18-20]. Tau binding to the microtubules leaves the N- and C-terminal regions of tau free to interact with other proteins [21-24]. Phosphorylation of tau residues within the microtubule binding domain strongly reduces the binding of tau to microtubules and consequently results in destabilization of the microtubules [25, 26]. This interaction of tau with the microtubules affects neuronal outgrowth. Inhibition of tau with antisense oligonucleotides or complete knockout of tau results in decreased outgrowth of neurites [27-29]. Moreover, a mutation in MAPT gene, which disrupts tau binding to the membrane, leads to decreased neuronal extensions [30]. This indicates that the bridging of tau from the growing microtubules and the membrane of the growth cone supports neuronal outgrowth. In addition, neuronal outgrowth may be regulated by a gradient of tau phosphorylation along the axon that is observed in developing primary hippocampal neurons. Levels of phosphorylated tau are high in the proximal axon and decrease towards the distal end, thereby forming a gradient along the axon. This phospho-tau gradient also contributes to the regulation of axonal transport [31]. Binding of multiple tau proteins to microtubules results in detachment of the motor protein kinesin from the microtubules and thereby inhibit kinesin-dependent transport. Tau also directly interacts with motor proteins. For instance, overexpression of tau inhibits kinesin-dependent fast axonal transport and tau reverses the direction of the motor protein dynein [32-34]. Importantly, the phosphorylation of tau modulates its affinity for microtubules and thereby regulates neuronal outgrowth and axonal transport.

Interestingly, the different familial tau mutations uncovered several new functions of the tau protein, unrelated to its canonical functions in neuronal trafficking as described above (for review see [34]). The P301L mutation in tau showed that tau is involved in genome stabilization [35]. In addition, proteomic and functional analyses of the P301L tau transgenic mouse revealed mainly changes in metabolism-related proteins including mitochondrial proteins [36]. This is supported by studies showing that tau has an extensive effect on mitochondria that cannot be attributed to changes in mitochondrial transport. For instance, tau can bind to VDAC1, a mitochondrial pore protein, indicating that tau is involved in mitochondrial function [36]. Moreover, human tau overexpression leads to increased mitochondrial elongation, which is associated with mitochondrial dysfunction in neurons of both Drosophila and mice [37]. Restoration of the mitochondrial fission and fusion balance in these models rescued normal mitochondrial function. Together, this has altered the classical view of tau as a
microtubule binding protein to tau as a multifunctional protein. In line with this, tau has multiple binding partners from which the function in neurons is mainly unknown [38]. Therefore, additional tau functions will probably still be discovered when tau research continues. Since tau is involved in many physiological functions, it is not surprising that dysfunction of the tau protein leads to disease. Indeed, most of above described tau functions are disturbed in transgenic tau animal models and in tauopathy patients.

**Figure 1. Physiological and pathological tau**

A) In the normal situation tau is localized in the axon and is involved in several functions. Tau is involved in stabilization of microtubules, in axonal transport, in neuronal outgrowth and mitochondrial function. Since more binding partners of tau are still being discovered, tau may have additional physiological functions. B) Phosphorylation and dephosphorylation of tau by kinases and phosphatases is a normal dynamic process that regulates tau function. Pathological tau is highly phosphorylated, which eventually results in tau inclusion bodies. C) This pathological tau leads to destabilization of the microtubules and localizes to synapses where it influences synaptic function. Pathological tau may have additional effects caused by a loss or gain of function with not yet identified binding partners of tau.

**Pathological tau**

Although tau phosphorylation is a normal physiological process, phosphorylated tau is a component of tau inclusion bodies [39]. The formation of tau inclusion bodies is part of a pathological process and begins with accumulation of the tau protein (Figure 1B). Detachment of tau from the microtubules and possible other tau binding partners makes tau prone to aggregation [40]. Since phosphorylation results in dissociation of tau from its binding partners, it is likely that phosphorylation is involved in the aggregation process. Abnormally high levels of phosphorylated tau are observed in disease. At the
molecular level, the first step to increase tau phosphorylation is a disbalance in kinase and phosphatase activity. This increased tau phosphorylation can lead to tau oligomerization and tau aggregation. However, it is still elusive when tau phosphorylation is physiological and when tau phosphorylation is pathological.

Tau has approximately 85 phosphorylation sites and therefore almost 20% of the tau protein has the potential to be phosphorylated [1]. In tauopathies, tau is phosphorylated at multiple residues and therefore tau is defined as hyperphosphorylated. To investigate whether phosphorylation of specific tau residues results in tau aggregation, pathological tau phosphorylation in AD was compared to physiological tau phosphorylation during development, where tau phosphorylation is increased in the absence of tau inclusion bodies [41]. Tau phosphorylation at Ser202, Thr212, Thr217, Thr231, Ser396, Ser404, and Ser422 is significantly higher in AD than during development. Therefore, these tau residues might be involved in the switch from normal tau phosphorylation into pathological tau phosphorylation. Interestingly, some of these residues are located at the C-terminus of tau (Ser396, Ser404, and Ser422) and not in the microtubule binding domain. Mimicking tau phosphorylation at the C-terminus promotes self-aggregation of tau whereas mimicking tau phosphorylation at the N-terminus suppresses tau aggregation [42]. Although it is difficult and maybe not even possible to exactly distinguish which tau residues contribute to tau aggregation when phosphorylated, phosphorylation of residues in the C-terminus makes tau more prone to aggregate. Moreover, aggregated tau is phosphorylated at multiple residues. In vitro phosphorylation of tau on 7 or 8 residues, which are also phosphorylated in the AD brain, strongly enhanced the potential of tau to aggregate. This supports that tau phosphorylation at several residues is necessary for tau aggregation [43-45]. In addition, mutations in tau that are causative of FTDP-17 promote the self-aggregation of tau [46]. A subset of the mutations is found in the microtubule binding domain causing reduced binding of tau to the microtubules. This indicates that increased concentration of free tau is an important factor for increased tau aggregation [47].

Notably, the end-stage tau aggregations like the tangles in AD are not the most toxic form of tau. In vivo data show that tangle-bearing neurons are still functional indicating that tangles are not sufficient to fully disrupt neuronal function [48]. In addition, AD animal models have behavioral deficits without tangles and conditional transgenic tau models show that cognition is improved without change in tangle formation. Interestingly, in a mouse model oligomeric tau consistently correlates with memory loss at various disease stages [49]. Increasing evidence from in vitro and in vivo experiments shows that accumulation of early-stage aggregated tau species, before the formation of tau inclusion bodies, are the most toxic species that result in cognitive impairment [50]. Indeed, also in humans, pre-fibrillar tau correlates better with cognitive deficits than tangle formation [51, 52].

How early tau aggregates disrupt neuronal function and consequently lead to cognitive deficits is not completely understood (Figure 1C). In many tau knock-out models almost no symptoms of neurodegeneration are observed [53]. In contrast, ablation of tau in Aβ mouse model is beneficial [39]. Tau deletion protects against
excitotoxicity, it improves memory deficits and reduces Aβ toxicity without a change of Aβ expression levels. This indicates that a disruption of the normal physiological function of tau is not sufficient to lead to cognitive impairments. Nevertheless, there can be a compensatory effect for the loss of tau by other MAPs. Indeed, models with a double knock-out of tau as well as MAP1B gene show a neurodegenerative phenotype [22, 54]. This demonstrates the biological importance of proper tau function and shows that other MAP genes are able to take over tau functions. Therefore, a loss of tau function could still contribute to the pathological defects observed in tauopathies.

Tau is mostly localized in the axon, although it should be mentioned that low levels of tau are observed in pre- and postsynapses in healthy human brains [55]. Interestingly, increased tau phosphorylation enables tau to relocalize to somatodendritic compartments like synapses. This relocalization of highly phosphorylated tau might result in a toxic gain of tau function. Tau located in synapses can interact with several synaptic protein complexes [56]. For instance, tau targets the kinase Fyn to the postsynaptic NMDA receptor and thereby has an impact on synaptic function [57]. This redistribution of phosphorylated tau from the axon to the dendrites appears to cause synaptic loss [58]. Indeed, a mouse model with inducible truncated tau expression that promotes tau aggregation shows swollen synapses full with phosphorylated tau. After 13 months, accumulated oligomeric tau results in synaptic dysfunction [59]. In line with this, the level of soluble phosphorylated tau in synapses correlates better with dementia than end-stage tau aggregates [60]. This indicates that increased phosphorylated tau contributes to irreversible pathological effects by the relocalization of phosphorylated tau to the synapses which eventually can result in synaptic dysfunction. Interestingly, boosting the proteasome and autophagy in mouse models of tauopathy leads to decrease phosphorylated tau levels and improvement of cognition [61]. Moreover, these studies show that increasing the proteasome and consequently decreasing phosphorylated tau levels is only beneficial before the onset of cognitive impairments. This supports the fact that increased levels of phosphorylated tau are not pathological per se, but that additional secondary events might cause irreversible toxic effects. Prolonged relocalization of phosphorylated tau to synapses might eventually lead to the irreversible toxic effects observed in tauopathies.

In conclusion, increased phosphorylated tau is the beginning of the tau aggregation process. Increased phosphorylated tau can cause neuronal dysfunction via both loss of function and gain of function. Increased tau phosphorylation results in a loss of function by decreased interaction with its binding partners. For instance, hyperphosphorylated tau leads to destabilization of the microtubules. On the other hand, the relocalization of phosphorylated tau to synapses results in a toxic gain of function by disrupting synaptic function. Interestingly, in middle-aged tau knock-out mice, MAPs are increased, probably as a compensatory response. In contrast, in old tau knock-out mice, there was no upregulation of MAPs that may compensate for the loss of tau function. These old tau knock-out mice performed worse on the water maze task [62, 68]. This indicates that the loss of tau function could be age-related due to less efficient compensatory mechanisms. The toxic gain of tau in combination with age-related loss of tau function helps to explain the late onset of tauopathies.
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The early underlying mechanism for increased tau phosphorylation and eventually irreversible tau pathology is unknown. Interestingly, activation of the unfolded protein response (UPR) is closely associated with increased tau phosphorylation. Moreover, the UPR is only activated in neurons containing diffusely distributed phosphorylated tau, but not in neurons with densely aggregated tau. This indicates that UPR activation is an early event in tauopathies.

The unfolded protein response

The ER is a major site of synthesis, folding and post-translational modification of proteins. A disturbance in the folding and modifying capacity of the ER results in ER stress and subsequently in activation of a protective stress response called the UPR. The first discovered proteins of the UPR were called glucose-regulated proteins (GRPs) since an increase of UPR-related proteins was observed upon glucose deprivation [64]. The UPR is now perceived as a stress response activated upon multiple insults such as glucose deprivation, calcium imbalance and hypoxia [65]. The UPR consists of an intricate signaling network with various downstream targets that are all aimed to restore ER homeostasis. In higher eukaryotes, three ER transmembrane proteins act as ER stress sensors that monitor protein-folding in the ER: inositol requiring enzyme 1 (IRE1), double-stranded RNA-activated protein kinase (PKR)-like ER kinase (PERK) and activating transcription factor 6 (ATF6). All three UPR sensors can bind to glucose-regulated protein 78/immunoglobulin heavy chain-binding protein (GRP78/BiP). In the classical view of initiation of the UPR, ER stress leads to dissociation of BiP from the three sensor proteins activating the three branches of the UPR. However, the regulation of the UPR appears to be more complex [66]. For instance, deletion of the BiP-binding site in IRE1 did not alter the inducibility of this UPR pathway [67].

The three branches of the UPR are activated upon ER stress to restore ER homeostasis (Figure 2). The PERK pathway is activated by oligomerization of PERK and subsequent trans-autophosphorylation. PERK directly phosphorylates eIF2α (p-eIF2α) at Ser51. This results in overall inhibition of protein synthesis and reduces the protein load in the ER. However, the synthesis of specific proteins involved in ER homeostasis is enhanced during eIF2α phosphorylation. These proteins, for example ATF4, are able to circumvent the translational block due to a special 5'UTR [68]. The oldest branch of the UPR in an evolutionary sense is IRE1. In fact, in yeast it is the only effector of the UPR [69]. Activation of the IRE1 arm of the UPR is similar to PERK activation; IRE1 also oligomerizes and subsequently trans-autophosphorylates. IRE1 catalyzes the removal of a small part of the mRNA encoding X-box binding factor protein 1 (XBP1) resulting in the generation of an ORF encoding an active transcription factor. XBP1 results in transcription of factors that are predominantly involved in degradation of misfolded proteins. Finally, activation of ATF6 leads to transport of ATF6 to the Golgi complex where it is cleaved, to release an active transcription factor. This pathway is most important for the upregulation of ER chaperones that increase the protein-folding capacity. Overall, the activation of the UPR leads to a translational block and specific activation of ER stress responsive genes that increase the protein-folding capacity and
decrease the protein load in the ER. Although UPR activation initially protects the cell against the toxic build-up of misfolded proteins, prolonged ER stress can eventually lead to apoptosis [70]. Because of the importance of the UPR to cope with cellular stress, it is not surprising that the UPR is activated during neurodegenerative diseases, in which proteostasis is disturbed.

**UPR activation in tauopathies**

Several proteins that are part of the UPR response are highly expressed in neurons of tauopathy patients [71]. UPR activation is also found in other neurodegenerative diseases like repeat expansion diseases, amyotrophic lateral sclerosis and synucleinopathies [65, 72-76]. It is unclear in these neurodegenerative diseases whether the UPR is an early protective mechanism or a late consequence of a pathogenic process. In some neurodegenerative diseases, UPR activation occurs as a secondary event to disease progression, for example, the UPR is activated upon the disruption of ER to Golgi trafficking caused by α-synuclein accumulation in a model for Parkinson’s disease [77]. In tauopathies, UPR activation is an early event [78]. Phosphorylated PERK levels are already upregulated early in AD (Braak stage 1 and 2) and are even further increased with disease severity. Moreover, the UPR is only activated in neurons containing diffusely distributed phosphorylated tau, but not in neurons with densely aggregated tau. These data indicate that UPR activation is an early event in tauopathies that occurs before the formation of end-stage tau aggregates.

![Figure 2. Schematic overview of the Unfolded Protein Response (UPR)](image)

The unfolded protein response (UPR) consist of three signaling pathways that all aim to restore ER homeostasis. The UPR is initiated upon disturbance of the homeostasis in the ER via activation of three sensor proteins in the ER membrane: RNA-activated protein kinase R (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol requiring enzyme 1 (IRE1). Activation of these sensors transfers stress signals to the cytoplasm and the nucleus, resulting in overall inhibition of protein translation and increased transcription and translation of genes involved in the restoration of ER homeostasis. Adapted from Schepers and Hoozemans, 2015.
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Genetic data support an important role for the UPR in tauopathies. A polymorphism in the gene EIF2AK3, which encodes PERK, is associated with increased risk to develop progressive supranuclear palsy and AD [73, 74, 79]. The EIF2AK3 risk allele increases the signaling activity of the PERK pathway [80, 81]. In addition, another branch of the UPR is also associated with increased risk to develop AD [82]. A polymorphism in the promoter region of the XBP1 gene affects the expression of XBP1 and thereby its downstream targets [83].

The recent development of small-molecule inhibitors of the UPR helps to unravel the close interaction between the UPR and tau pathology. The entire UPR can be inhibited, for example with a chemical chaperone like tauroursodeoxycholic acid (TUDCA) [84, 85], but also specific branches of the UPR can be blocked. A small-molecule inhibitor of PERK signaling, GSK2606414, and 4μ8C, an inhibitor of IRE1α signaling, are widely used compounds to inhibit specific arms of the UPR [86, 87]. Interestingly, GSK2606414 has been tested in disease models. This inhibitor prevents neurodegeneration in prion-infected mice [88]. Recently, use of the same PERK inhibitor, in a mouse model of FTDP-17 showed restoration of protein synthesis, lower phosphorylated tau levels and reduced clinical signs [89]. This indicates that the UPR has a direct effect on tau phosphorylation and disease outcome. In line with this, the UPR increases the activity of glycogen synthase kinase 3, one of the major tau kinases [90]. Therefore, the UPR might be directly involved in increased tau phosphorylation and pathology.

**UPR as adaptive response to metabolic stress in tauopathies**

The UPR is initially activated to restore homeostasis. Under physiological conditions, the UPR facilitates adjustment of cells to stressful circumstances. For instance, in B-cells that are differentiated to antibody-producing plasma cells, the UPR is activated to expand the ER [91, 92]. The UPR is also activated in cells that have high secretory demands like pancreatic β-cells [93]. Also in tauopathies, the UPR might be activated as part of an adaptive response to cope with neuronal stress. However, the trigger that activates the UPR in tauopathies is unknown.

Neurons are highly dependent on glucose metabolism for normal maintenance and firing. The brain uses 25% of the total energy consumption while the brain is only 2% of the total body mass [94]. Glucose is the main energy source for the brain. Specific glucose transporters (Gluts) transfer glucose from the circulation across the blood brain barrier into neurons (Figure 3). These gluts can be either dependent or independent of insulin (Figure 4). The most important Gluts in the brain - Glut 1 expressed at the blood brain barrier and the major neuronal Glut 3 - are insulin independent [95]. Glycolysis is the process that converts glucose into pyruvate. The first step is performed by the rate limiting enzyme hexokinase. During mitochondrial respiration, pyruvate is further processed into Acetyl-CoA that enters the citric acid cycle. The net energetic balance of glycolysis is relatively low (5%) compared to mitochondrial respiration (95%) [96, 97]. Besides the importance of glucose as energy source, many intermediates that are formed in the reaction from glucose to CO2 and H2O are essential for cellular maintenance. Disturbance in glucose metabolism can result in neuronal stress.
Figure 3. Schematic overview of glucose brain metabolism
Glucose enters the neurons via glucose transporters (Gluts). 2 - 5 % of total glucose ends up in the hexosamine biosynthesis pathway that results in O-GlcNAcylation of proteins. Most of glucose is used for ATP production via glycolysis and mitochondrial respiration.

Figure 4. Schematic overview of insulin signaling
T1DM results from insulin deficiency caused by dysfunction of the β cells in the pancreas. In T2DM, the insulin receptors are less sensitive for insulin which results in overproduction of insulin and over time leads to dysfunction of β cells and reduced insulin production like in T1DM. Dysfunctional insulin signaling has an effect on Akt and its downstream signaling targets. Insulin dependent Gluts (Glut 4) will not translocate to the plasma membrane and consequently reduce glucose uptake. However, glucose uptake by insulin independent Gluts (Glut 3) will not be altered. This decreased glucose uptake can result in metabolic stress which increases AMP levels and increases phosphorylation and activation of AMPK. Modified from Gonzalez-Franquesa et al., 2012.

Abbreviations: IRS, insulin receptor substrate; P, phosphorylation; S, serine; T, tyrosine; PH, pleckstrin homology domain of the IRS-1; SHC, Src Homology 2 domain; GRB2, growth factor receptor-bound protein 2; ERK, extracellular-signal-regulated kinase or classical MAP kinases; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PDK1, phosphoinositide-dependent protein kinase 1; PKB/Akt, protein kinase B; mTOR, mammalian target of rapamycin; GSK3, glycogen synthase kinase 3; GS, glycogen synthase; AS160, 160 kDa Akt substrate; AMP, adenosine triphosphate; aPKC, atypical protein kinase C.
Interestingly, disturbed cerebral glucose metabolism that follows disease pathology is reported in several tauopathies [98, 99]. A reduced glucose metabolic rate is already observed in individuals that are genetically predisposed to develop AD before any pathological signs of the disease manifest [100, 101]. Moreover, glucose metabolism is also decreased in brains of patients with mild cognitive impairment (MCI), which is a prodromal stage to dementia [102-104]. Cognitively normal subjects with early signs of disturbed peripheral glucose metabolism have impaired glucose utilization in brain areas affected in AD [105-108]. Interestingly, cognitively normal individuals with a maternal history of sporadic AD have reduced glucose metabolic rates with a stronger decline of cerebral glucose metabolism over time while individuals with a paternal history of sporadic AD do not display a reduction in cerebral glucose metabolism [109]. This could indicate that mitochondrial dysfunction might be involved in tau pathology. In line with this, a GWAS study for cerebrospinal fluid phospho-tau levels showed that biologically related genes for metabolism are associated with increased risk for tau aggregation [110]. Moreover, a variant of GLIS3, a major risk factor for the onset of DM, was associated with increased phosphorylated tau levels in the cerebrospinal fluid. These data indicate that altered glucose metabolism is an early event in tauopathies that is not necessarily a consequence of pathology. Since metabolic dysfunction is observed in tauopathies, metabolic stress might be the trigger that activates the UPR in tauopathies.

Metabolic stress is an established physiological trigger of the UPR. 2-deoxy glucose (2DG), which blocks glucose metabolism at the first step of glycolysis, can activate the UPR [111]. The UPR is also strategically positioned to sense and integrate metabolic signals. It is an important site for crucial steps in protein, lipid and glucose metabolism. Several studies support a crucial role for the UPR in controlling glucose homeostasis [94]. For instance, PERK-deficient mice showed exocrine pancreatic insufficiency and progressive hypoglycemia [112]. Moreover, Wolcott-Rallison syndrome is a rare hereditary disease caused by impaired PERK signaling [69, 113]. These patients also show exocrine pancreatic insufficiency and develop DM. This demonstrates the importance of the UPR in normal glucose homeostasis. Although the exact role of the UPR in neuronal glucose homeostasis is still elusive, some studies indicate that the UPR has the capacity to directly restore energy homeostasis. For instance, ATF4 can induce genes involved in glucose metabolism like hexokinase and phosphoenolpyruvate carboxykinase [114]. Moreover, XBP1 is significantly enriched during ER-stress at promoters of genes regulating glucose and lipid metabolism [115]. A proteome-wide study of UPR activation in neuronal cells not only induced ER chaperones, but also increased the mitochondrial proteome indicating a direct effect of the UPR on mitochondrial bioenergetics [116]. Indeed, the ER and mitochondria can form physical connections independent of apoptosis where signaling and luminal contents are exchanged, also called the mitochondria-associated membranes (MAMs) [117]. These physical connections are crucial for mitochondrial dynamics [118] and are enhanced during UPR activation [119-121]. Overall, this indicates that the UPR is not only activated upon metabolic stress but that the UPR might also be an adaptive response to restore glucose homeostasis. In tauopathies, the activated UPR might also be an adaptive response to cope with metabolic stress, which initially is neuroprotective, but may result in tau pathology upon prolonged activation.
**Tau phosphorylation as adaptive response**

Highly phosphorylated tau is not only reported in tauopathies, but also under physiological situations (Table 1). As discussed above, increased tau phosphorylation is observed during development. Tau phosphorylation levels are higher in the fetal brain than in the adult brain [122]. Remarkably, this increased phosphorylated fetal tau does not form aggregates. Moreover, the increased levels of phosphorylated tau correlate with the exponential neurite outgrowth during development [123]. Increased tau phosphorylation in the fetal brain is possibly necessary for increased dynamics in microtubule assembly and disassembly to meet the extreme neurite outgrowth demand. Aging is the most important risk factor for neurodegenerative diseases. Strikingly, tau is also increased phosphorylated during normal aging in the monkey brain [42]. Endogenous insoluble tau is observed in the brain of aged monkeys, but in the absence of tau inclusion bodies and cognitive impairment. These findings are consistent with increased tau phosphorylation in a senescence-accelerated mouse model, SAMP8 [124]. Selective inbreeding of mice with a short lifespan created a mouse with an accelerated aging phenotype. This mouse has a lifespan of approximately 10 months and develops increased phosphorylated tau levels at 5 months. It is not known what the function of increased tau phosphorylation is during normal aging. It could be a compensatory mechanism to cope with the progressive accumulation of unrepaired changes during aging. Overall, the high levels of tau phosphorylation during development and aging suggests that increased tau phosphorylation is not pathological *per se*, but could be part of a physiological process.

Interestingly, increased endogenous tau phosphorylation is also described in starvation and torpor, which are both naturally occurring metabolic stress situations. Starvation is a condition of suffering caused by food deprivation, which occurs commonly in animals in the wild. Starvation can be used as a model to directly study the effects of energetic stress. During starvation reduced glucose levels are measured in the brain [125, 126]. Interestingly, already after 1 day of starvation endogenous tau is moderately phosphorylated. After 3 days of starvation tau phosphorylation is progressively increased in accordance with the regional selectivity observed in AD [127]. Strikingly, this increased tau phosphorylation is completely reversed upon 1 day of refeeding. Torpor is another physiological hypometabolic state whereby animals can save up to 90% of their energy expenditure [128, 129]. Torpor is a special ability of some mammalian species to save energy under limiting conditions that may be shortage of food or water [130]. In torpor, the metabolic rate is strongly reduced by an active process. In many cases this is facilitated by a reduction in body temperature, that may vary from a temperature drop of only a few degrees (e.g. in the black bear) to temperatures just above 0°C in the Arctic ground squirrel [131]. The nature and duration of torpor differs between species. Some animals have interruptions of torpor where they return to their normal metabolic rate where other species have a continuous torpor episode. Interestingly, increased endogenous tau phosphorylation is a general feature in brains of various animal species with diverse types of torpor. The residues of tau that are phosphorylated during torpor are largely corresponding to the residues phosphorylated in the AD brain. Moreover, torpor-induced
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Table 1. Overview of situations with increased levels of phosphorylated tau

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<th>Normal physiological processes</th>
<th>Tau</th>
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<td>Development</td>
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<td>E15 till P15</td>
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<td>(Watanabe et al., 1998; Yu et al., 2000)</td>
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<td>Aging</td>
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<td>Aged monkey (&gt; 25 years)</td>
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<td>(Carlyle et al., 2014)</td>
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<td>SAMP8 mice</td>
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<td>(Canudas et al., 2005; Pallas et al., 2008)</td>
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<td>Physiological metabolic stress</td>
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<td>Natural torpor in different species</td>
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<td>1 - 3 days starvation</td>
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<td>Metabolic diseases</td>
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<td>STZ model</td>
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<td>(Clodore-Miller et al., 2006; Jolivalt et al., 2008; Kim et al., 2008; Planel et al., 2007; Qu et al., 2011; Qu et al., 2014; Ramos-Rodriguez et al., 2013; Schmitz et al., 2011; Yoon et al., 2016; Zhang et al., 2010b)</td>
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<td>High-caloric diet model</td>
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<td>High-caloric diet + STZ model</td>
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Abbreviations: ↑: increase, ↓: decrease, ↔: no change.

Phospho-tau is diffuse as observed in the early stages of tau pathology and does not form dense aggregates. Strikingly, tau phosphorylation disappears completely during arousal when metabolism returns to normal [132]. The high levels of phosphorylated tau after starvation and torpor indicate that metabolic stress could be a trigger for increased tau phosphorylation. The reversibility of this tau hyperphosphorylation after removal of the metabolic stressor suggests that increased tau phosphorylation is not pathological, but part of a physiological response. Moreover, dramatically reduced neuronal activity is observed during torpor together with a decrease in synaptic complexity, but after arousal from torpor (already within 2h) the structure of dendrites and synapses is completely restored demonstrating the extreme adaptive qualities of the brain [133-136].

Interestingly, increased endogenous tau phosphorylation is also observed in animal models of DM, a severe metabolic disease (Table 1). DM is characterized by marked high levels of blood glucose and occurs in two forms: type 1 DM (T1DM) and type 2 DM (T2DM) (Figure 4). T1DM results from insulin deficiency caused by dysfunction of the B cells in the pancreas, which are the insulin producing cells. T2DM starts with overproduction of insulin due to insulin resistance and over time results like T1DM in dysfunction of B cells and extreme hyperglycemia. Dysfunctional insulin signaling in periphery and consequently disturbance in glucose uptake also affects the brain. Epidemiological studies show that both T1DM and T2DM are risk factors for AD and that the incidence of AD is higher in people with DM [137-139]. Moreover, DM is associated with higher risk for MCI [140-143]. Recently, these epidemiological observations are supported by a GWAS study that shows genetic concordance between DM and AD [144]. This suggests a link between tau pathology and DM. DM was shown to exacerbate tau aggregation in transgenic tau mice supporting a direct effect of DM on tau pathology [145]. Interestingly, various studies show also induction of endogenous
tau phosphorylation in the brains of T1DM animals (reviewed by [146, 147]). Increased levels of endogenous tau phosphorylation are also observed in rodents on a high-caloric diet, which causes insulin resistance (Table 1). However, this effect is not consistently observed in T2DM animal models.

The underlying mechanism for diabetes-induced tau phosphorylation is unknown. Metabolic dysfunction is a main characteristic of DM and therefore could be involved in increased levels of tau phosphorylation. However, other factors might also explain the diabetes-induced tau phosphorylation; for example, T1DM and T2DM are both characterized by low-grade systemic inflammation [147, 148]. This peripheral inflammation can be accompanied by neuroinflammation in specific regions of the brain. Reactive glial cells and activation of different cytokines are reported in the hypothalamus of insulin deficient [149-151] as well as insulin resistant animals and in obese humans [152]. Neuroinflammation is associated with tau pathology [153]. However, it is unknown whether neuroinflammation in DM is also observed in brain areas affected by tau pathology. Increased tau phosphorylation in metabolic stress situations like starvation and torpor supports the involvement of metabolic dysfunction in diabetes-induced tau phosphorylation. Moreover, treatment with a mitochondrial uncoupler triggers cerebral tauopathy [154-156]. However, none of these studies directly show that metabolic stress is causing increased tau phosphorylation. Therefore, further research is necessary to investigate whether metabolic stress directly induces tau phosphorylation.

**UPR activation and tau phosphorylation, adaptive responses to metabolic stress**

Overall, increased tau phosphorylation is observed in tauopathies, but also during physiological processes like development, aging, starvation and torpor. In starvation and torpor, highly phosphorylated tau is reversed upon return to a normal metabolic state. This indicates that metabolic dysfunction could induce hyperphosphorylation of tau. Since metabolic dysfunction is also observed in tauopathies, metabolic stress might be the neuronal stressor to induce abnormal tau phosphorylation in tauopathies. It has been hypothesized that initially increased tau phosphorylation is a protective mechanism which may turn pathological if it is present for a prolonged period [157]. Therefore, metabolic stress conditions might lead to increased tau phosphorylation as part of an adaptive response. For instance, increased tau phosphorylation inhibits axonal transport, which could be a mechanism to temporarily inhibit energy costly processes. If increased tau phosphorylation is part of an adaptive response, this highly phosphorylated tau will disappear when homeostasis is restored. Interestingly, the UPR is an adaptive response that can be activated upon metabolic stress. Since the UPR is strongly associated with increased tau phosphorylation in tauopathies, increased tau phosphorylation might be regulated via the UPR. UPR activation is also reported in DM [158]. UPR activation in DM is observed in the periphery, but is not yet studied in neurons. Since DM is a severe metabolic syndrome where increased tau phosphorylation is reported in the brain, DM provides an ideal model to study metabolic stress in relation to hyperphosphorylated tau. Moreover, it would be interesting to study whether the UPR is activated in diabetes-induced tau-bearing neurons.
In conclusion, we hypothesize that increased tau phosphorylation is an adaptive response to metabolic stress that initially is neuroprotective, but eventually results in irreversible pathological tau aggregates when metabolic homeostasis is not restored (Figure 5). Therefore, the pathological state with irreversible tau aggregates as observed in tauopathies is a consequence of a prolonged physiological stress situation in which restoration of neuronal homeostasis failed. In this thesis, we aim to answer the following research questions:

1. Is metabolic dysfunction a trigger for increased tau phosphorylation in tauopathies?
2. Is tau phosphorylation an adaptive response?
3. a) Is this adaptive tau response regulated via the UPR?
   b) Does the UPR directly restore metabolic homeostasis?

To investigate these research questions we used models without transgenic tau to ensure that the physiological response of tau is studied. In chapter 2, we investigate whether reversible increased tau phosphorylation in torpor is regulated via the UPR. Our findings show that UPR is activated in torpor and switched off upon removal of the metabolic stressor. In addition, we demonstrate that UPR activation upon metabolic stress is directly linked to increased tau phosphorylation by using an inhibitor of the PERK pathway. In chapter 3, we investigated in more depth how the UPR restores metabolic homeostasis. We investigated whether the UPR has a direct effect on glucose metabolism. Our findings show that the UPR reduces glucose metabolism via the IRE1 pathway. In chapter 4 and 5 we investigate the underlying mechanism of tau phosphorylation in DM. Our data in chapter 4 show that neuroinflammation is not a prerequisite for diabetes-induced tau phosphorylation. In chapter 5, we demonstrate that diabetes-induced tau phosphorylation is not regulated via the UPR upon metabolic stress, but that tau phosphorylation is a consequence of insulin deficiency that is reversed upon insulin treatment. Chapter 6 summarizes these findings and discuss the implications for tauopathies.

**Figure 5. Schematic overview of the research questions in this thesis**

We hypothesize that increased tau phosphorylation is a physiological response to cope with neuronal stress that consequently disappears when homeostasis is restored. However, if homeostasis is not restored, the neuron turns to an irreversible pathological state with tau inclusions as observed in tauopathies. In this thesis, we aim to answer the following research questions: 1) is metabolic dysfunction a trigger for increased tau phosphorylation in tauopathies? 2) is tau an adaptive response? 3) is this adaptive tau response regulated via the UPR and does the UPR directly restore metabolic homeostasis?