Trends in Endocrinology & Metabolism

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REVIEWS

Review

Timing Matters: Circadian Effects on Energy Homeostasis and Alzheimer's Disease

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Metabolic syndrome and Alzheimer's disease (AD) are two major health issues in modern society causing an extraordinary financial burden for the global health-care systems. A tight link between the pathologies of obesity and type 2 diabetes (T2D), and more recently between T2D and AD, has been discovered. Furthermore, in recent years it has become apparent that the circadian clock has an important function in controlling metabolism. This review integrates the role of the circadian clock in the development of these metabolic derangements and vice versa. Common features such as central insulin resistance, altered glycogen synthase kinase 3β (GSK3β) signalling, and central inflammation are discussed, and therapeutic interventions targeting those mechanisms are mentioned briefly.

Highlights

At the molecular level obesity, T2D and AD share common features such as central insulin resistance, chronic low-grade inflammation and altered GSK3β signalling.

Several antidiabetic drugs showed positive effects in AD patients, underpinning the molecular similarities of AD and T2D.

Recent studies have emphasised the circadian clock as an important player in whole-body energy metabolism. Circadian rhythms and clock gene expression are altered in states of metabolic derailment, and disruptions in circadian rhythms are associated with a number of different diseases including T2D and AD.

Considering the close relationship between circadian clocks and energy homeostasis, behavioural interventions, such as fasting regimens, to combat the consequences of disrupted rhythms and metabolism appear as a favourable tool.

Metabolic Disorders and the Circadian Clock

Since 1975, the worldwide prevalence of obesity has nearly tripled, accompanied by a dramatic increase of T2D, which is predicted to affect 629 million people by 2045 [1,2]. A large body of evidence links diet-induced obesity (DIO) to the development of other diseases such as T2D and AD (Box 1). Over the past few decades, these disorders have become more prevalent, causing a high financial burden for health systems and impacting quality of life. While the essential role of the brain in the pathogenesis of AD is unquestionable, its involvement in the pathogenesis of T2D has only recently started to arouse interest. Common hallmarks of DIO, T2D, and AD are central insulin resistance, altered **glycogen synthase kinase 3β (GSK3β)** (see Glossary) signalling as well as central inflammation. In this review, we discuss the shared molecular derangements that underlie these pathologies and focus on the connection between energy metabolism and **circadian rhythms**. In our modern society, these internal timekeeping systems become disrupted due to lifestyle changes and technological advances, including **social jetlag**, transmeridian travel, night shift work and constant exposure to bright artificial light sources. Here, we describe how circadian disruptions are associated with the development of metabolic derangements in both rodents and humans, as well as the efficacy of fasting regimens, such as **time-restricted feeding (TRF)**, as a therapeutic intervention to reinstate metabolic health. Finally, we highlight future challenges and open questions in this field of research.

Neuroendocrine Pathways in the Regulation of Energy Homeostasis

The importance of the brain in the regulation of energy homeostasis was proposed more than 150 years ago by Claude Bernard [3]. With the discovery of insulin in 1921 and leptin in 1994, two major players in the regulation of energy homeostasis were identified. Long considered to exert only peripheral effects, the fact that insulin exerts metabolic effects through acting in the brain was pioneered by Woods and Brüning [4,5]. The neuron-specific insulin receptor knockout (NIRKO) mouse [5] and subsequent studies by Obici *et al*. and Pocai *et al*. elucidated that insulin action in the brain is important for the regulation of whole-body glucose homeostasis [6,7]. Molecularly, binding of insulin to its receptor leads to the activation of the

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 https://doi.org/10.1016/j.tem.2018.12.001
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Box 1. Pathogenesis of AD

Clinically, AD is characterised by an initially mild impairment in memory. With its progression, individuals undergo a severe decline in cognitive abilities accompanied by neurological symptoms and behavioural changes. These range from difficulties to complete familiar tasks to pronounced disorientation and difficulties to speak, swallow, or walk [90].

Brains of AD patients display manifold histopathological changes such as Aβ plaques and NFTs, which have been the gold standard for diagnosis for decades [48]. Aβ peptides originate from the alternative processing of APP by β- and γ-secretases, resulting in hydrophobic peptides 38–43 amino acids long, which are prone to aggregation in soluble Aβ oligomers or extracellular Aβ plaques [48]. The Aβ40 and Aβ42 isoforms are the most common in AD but the Aβ42 isoform is considered to have the highest hydrophobicity and toxicity. Since Aβ42 production is enhanced at the expense of Aβ40 synthesis, the plasma Aβ42/Aβ40 ratio serves as a reliable biomarker to predict brain depositions [91]. Aβ brain load is further increased by reduced levels of Aβ degrading enzymes, decreased clearance rate, and increased brain uptake of soluble Aβ via receptors for advanced glycation end products (RAGE). Additionally, Aβ/RAGE interactions cause activation of proinflammatory signalling in endothelial cells of the blood–brain barrier, leading to endothelial apoptosis and a decrease in cerebral blood flow rate [92]. This effect of Aβ might contribute to the neurovascular changes observed in AD. Another prominent pathological hallmark are intracellular NFTs, consisting of abnormally phosphorylated τ protein, which impairs the normal ability of τ protein to stabilise microtubules. τ Protein phosphorylation is mediated by a variety of kinases such as GSK3β [48]. Hyperphosphorylation of τ protein causes its translocation into the somatodendritic area and its aggregation, thereby causing the breakdown of cytoskeletal structures and disrupted intracellular transport and signal transduction in neurons [48]. Further symptoms include oxidative stress, disturbed calcium homeostasis, and altered neurotransmitter signalling. These molecular alterations lead to a loss of synapses and finally to neuronal death [93]. Histopathological changes in the brain precede cognitive symptoms by decades, emphasising the importance of new screening methods to detect early molecular changes. PET imaging and the measurement of Aβ in CSF can estimate the Aβ burden of a patient; however, these techniques are expensive and laborious [94]. Recently, structural and functional MRI and PET studies measuring cerebral metabolism have provided promising results in presymptomatic stages of AD [57]. Novel blood tests measuring APP/Aβ42 and Aβ40/Aβ42 ratios, although still in their preliminary stages, may provide a feasible and less expensive alternative to neuroimaging [95].

phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway. The disruption of insulin signalling in the brain, which is essential for the regulation of glucose homeostasis, leads to central insulin resistance and ultimately results in the development of T2D [8]. T2D is characterised by chronically elevated blood glucose concentrations, attenuated insulin sensitivity, and hyperinsulinemia in the early stages, leading to the death of pancreatic β cells during the manifestation of the disease [9].

A major risk factor for the development of T2D is DIO [10], a state characterised by elevated circulating levels of insulin and leptin. However, both hormones are unable to mediate their catabolic effects. These phenomena are termed insulin and leptin resistance. Notably, functional leptin signalling has been shown to be crucial for insulin action in the hypothalamus [11–13]. Molecularly, leptin administration leads to elevated activation of insulin receptor substrate (IRS)1 and we could show that it alters phosphorylation of IRS1 at Ser^{612} and Ser^{307} [13], resulting in activation of the main downstream effector, the PI3 K/AKT pathway. Pharmacological inhibition of hypothalamic GSK3β was shown to have similar effects on the activation of intracellular PI3 K/AKT signalling [14]. GSK3β is a potent inhibitor of insulin signal transduction and is a key enzyme of the WNT pathway, which was recently shown to play an important role in the neuroendocrine control of energy metabolism [15–17]. Canonical WNT signal transduction leads to inactivation of GSK3β [18]. Leptin injections in rodents have been shown to activate the WNT pathway and inhibit GSK3β in leptin-deficient obese mice [16,19]. Therefore, the inhibition of GSK3β by leptin, possibly via the WNT pathway, might explain the leptin-induced sensitisation of insulin signalling (Figure 1). Furthermore, low-grade systemic and central inflammation is associated with obesity as well as T2D [20], and increased activity of proinflammatory pathways in the hypothalamus has been linked to the development of DIO and glucose intolerance [21,22]. By central inhibition of the inhibitor of nuclear factor-κB kinase β (IKKβ)/nuclear factor-κ-light-chain-enhancer of B-cells (NF-κB) pathway, the involvement of this proinflammatory

Glossary

Circadian rhythms: endogenously generated rhythms with a free-running period of approximately 24 h, meaning they persist under constant conditions. Circadian rhythms are entrainable by external cues (Zeitgebers). In mammals, light is the primary Zeitgeber, resulting in a 24-h oscillation of physiological and behavioural rhythms. Time of food intake is another prominent Zeitgeber.

Glycogen synthase kinase 3β (GSK3β): proline-directed serine/threonine protein kinase that is ubiquitously expressed and constitutively active. GSK3β activity is controlled by phosphorylation at different sites. The major inhibitory phosphorylation site is Ser^{9}. This inhibitory phosphorylation is mediated by different kinases and pathways, among which, the insulin pathway-related kinase AKT is most prominent. GSK3β is also involved in the regulation of oxidative stress, apoptosis, and gene transcription. The latter is mediated by the GSK3β regulation of diverse co-/transcription factors such as NF-κB, signal transducer and activator of transcription 3, or β-catenin. Overactivation of GSK3β is implicated in several diseases, including T2D, AD, and other neurodegenerative disorders, inflammation, cancer, and cardiovascular diseases.

Social jet lag: misalignment of biological and social time. A large proportion of the working population is exposed to this phenomenon, which is defined as the difference in mid-sleep time between work and work-free days. Social jet lag can result in metabolic consequences such as metabolic syndrome.

Suprachiasmatic nucleus (SCN): located in the anterior hypothalamus, the SCN is the central master pacemaker of mammalian circadian rhythms. The main role of the SCN is the synchronisation of peripheral circadian clocks, which are present in virtually all tissues, to ensure a tranquil coordination between physiological and behavioural processes.

Time-restricted feeding (TRF): feeding regimen in which food intake is restricted to a specified period

pathway in the development of hypothalamic leptin resistance during DIO was demonstrated in mice [21]. A possible link between both pathways is suppressor of cytokine signalling (SOCS)3, which is a potent inhibitor of leptin signalling as well as a prominent target of the NF-κB signalling cascade [23]. In line with this, we showed that genetic inhibition of IKKβ/NF-κB signalling in the ARC led to reduced *socs3* gene expression [21]. Although this finding suggests that central inflammation might be a causative factor in the development of leptin resistance further research is required to identify the origin of leptin resistance.

Common complications in many patients suffering from T2D involve neuropathy due to pathologically elevated blood glucose as well as neurodegeneration. In this regard, midlife obesity as well as pathological changes in the brain, such as central insulin resistance and central inflammation, moved into the focus of AD research as risk factors for the development of sporadic AD (≥90% of cases of AD), which is discussed later in this review.

each day, followed by an extended period of food deprivation. TRF does not necessarily imply a reduction in caloric intake, but beneficial outcomes on metabolic health are dependent on the circadian timing of caloric intake.

The Circadian Clock in Health and Disease

Reciprocal Regulation of the Circadian Clock and Energy Metabolism

The importance of the circadian clock in the control of whole-body energy metabolism has been demonstrated in numerous studies over the past decade [24]. Considerable progress in this field has derived from lesions of the **suprachiasmatic nucleus (SCN)** and studies

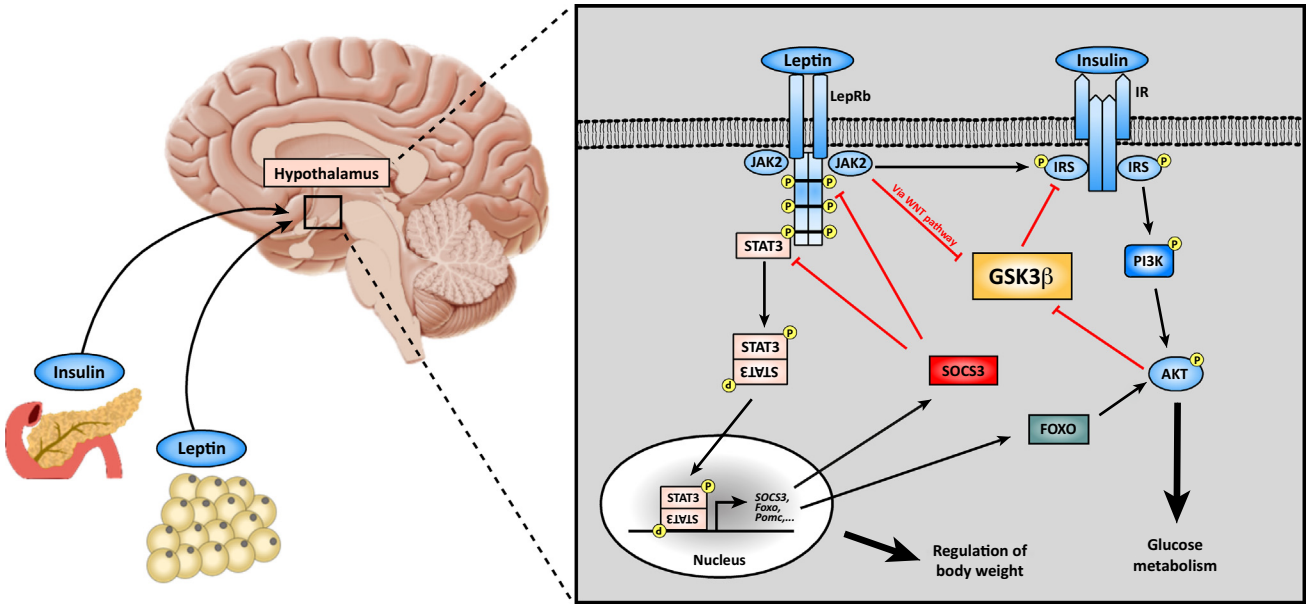

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Figure 1. Insulin and Leptin Signalling in the Brain. Insulin, secreted by pancreatic β cells, crosses the blood–brain barrier and binds to IR expressed by neurons. Upon binding of insulin to IR its intrinsic tyrosine kinase domain phosphorylates IRS1. Activated IRS1 phosphorylates PI3K leading to downstream activation of AKT. Activated AKT exhibits pleiotropic effects, most importantly controlling FOXO and GSK3β, thereby regulating key processes such as cell survival, proliferation, and metabolic adaptions. The adipokine leptin controls energy homeostasis and body weight primarily by targeting orexigenic as well as anorexigenic neurons in the hypothalamus. Binding of leptin to LepRb leads to an activation of JAK2, resulting in phosphorylation of the three intracellular receptor tyrosine residues Tyr985, Tyr1077, and Tyr1138. The phosphorylation of Tyr1138 causes recruitment of STAT3. STAT3 is subsequently phosphorylated by JAK2 at the phosphorylation site Tyr705, which leads to homodimerisation and nuclear translocation of STAT3. In the nucleus, STAT3 acts as a transcription factor and induces target gene expression, including SOCS3. SOCS3 initiates a negative feedback loop inhibiting JAK2 activation, thereby decreasing leptin action. In addition to its fundamental effects on body weight via the JAK2/STAT3 pathway, leptin also inactivates GSK3β, linking leptin signalling to various metabolic processes. Abbreviations: AKT, protein kinase B; FOXO, Forkhead box O; GSK3β, glycogen synthase kinase 3β; IR, insulin receptor; IRS1, insulin receptor substrate 1; JAK2, Janus kinase 2; PI3K, phosphoinositide 3-kinase; SOCS3, suppressor of cytokine signalling 3; STAT3, signal transducer and activator of transcription 3.

conducted in transgenic rodent models. For example, SCN lesioning in mice leads to the absence of a circadian rhythmicity in oxygen consumption, food intake, and activity, together with an increase in body weight and fat mass, as well as hepatic insulin resistance [25]. Furthermore, a missense mutation in the murine *clock* gene evokes loss of rhythmic expression of key metabolic genes in liver, skeletal muscle, and pancreas, resulting in disruption of glucose and lipid homeostasis, as well as obesity [26,27]. Deletion of the *bmal1* gene in pancreatic β cells results in a diabetic phenotype with insulin deficiency, impaired glucose tolerance, and hyperglycaemia [26], and colocalisation of the pancreatic transcription factor PDX1 with circadian locomotor output cycles kaput/brain and muscle ARNT-like protein 1 (CLOCK/BMAL-1) has been demonstrated to drive rhythmic expression of genes regulating insulin secretion [28]. These results highlight the close link between circadian rhythms and metabolic output and elucidate how circadian disruptions may contribute to the development of metabolic dysregulation and T2D.

In humans, chronic circadian misalignment has been shown to evoke symptoms of metabolic syndrome. Continuous shift-work and social jet lag lead to decreased insulin sensitivity and impaired glucose tolerance, both indicators of T2D, as well as increased body mass index and symptoms of cardiovascular disease [29–31]. Mice exposed to chronic circadian disruptions in the form of repeated jet lag display reduced hypothalamic leptin sensitivity and increased body weight [32], illustrating a potential explanation for the correlation between circadian misalignment and development of obesity, as described in humans and rodents.

Circadian rhythms and *clock* gene expression are altered simultaneously in states of metabolic derailment. DIO leads to arrhythmic patterns of locomotor activity, food intake, and *clock* gene expression [33,34]. One possible explanation for the correlation of circadian misalignments and obesity derives from the close interplay between circadian and inflammatory processes. For instance, cytokines and other immune mediators are under direct control of circadian rhythms in a broad variety of tissues [35,36]. Cryptochrome (CRY) ablation leads to constitutive activation of the proinflammatory transcription factor NF-κB [37], whereas the heterodimerisation of CLOCK and BMAL-1 results in rhythmic repression of proinflammatory gene expression [38]. Thus, altered inflammatory activity as a consequence of disrupted circadian clock rhythms might be a crucial factor for obesity and related comorbidity.

A unique involvement of the ARC in the generation of feeding rhythms has been demonstrated by ARC-targeted ablation of leptin-sensitive neurons in rats, resulting in obesity as well as impaired feeding rhythms, whereas this was not the case in other hypothalamic areas [39]. Recently, hypothalamic AgRP/NPY neurons were identified to be a crucial component of an SCN-independent, food-entrained oscillator [40].

The interaction between circadian rhythmicity and energy metabolism is further highlighted by the finding that rhythmic genomic binding and thus transcriptional activity of the CLOCK/BMAL1 complex is directly regulated by the cellular energy status. The reduced forms of NAD redox cofactors, NAD(H) and NADP(H), enhance the DNA binding of the heterodimeric complex, whereas the oxidised forms, NAD(+) and NADP(+), inhibit its binding [41]. Furthermore, rhythmic levels of NAD(+) activate the NAD(+)-dependent protein deacetylase sirtuin (SIRT)1, which oscillates and binds CLOCK/BMAL1 heterodimers in a circadian manner, thereby facilitating the degradation of PER2 and directly regulating circadian output [42]. These results demonstrate how cellular energy metabolism directly regulates the circadian clockwork machinery. The tight interplay of the clock and metabolism is illustrated in Figure 2.

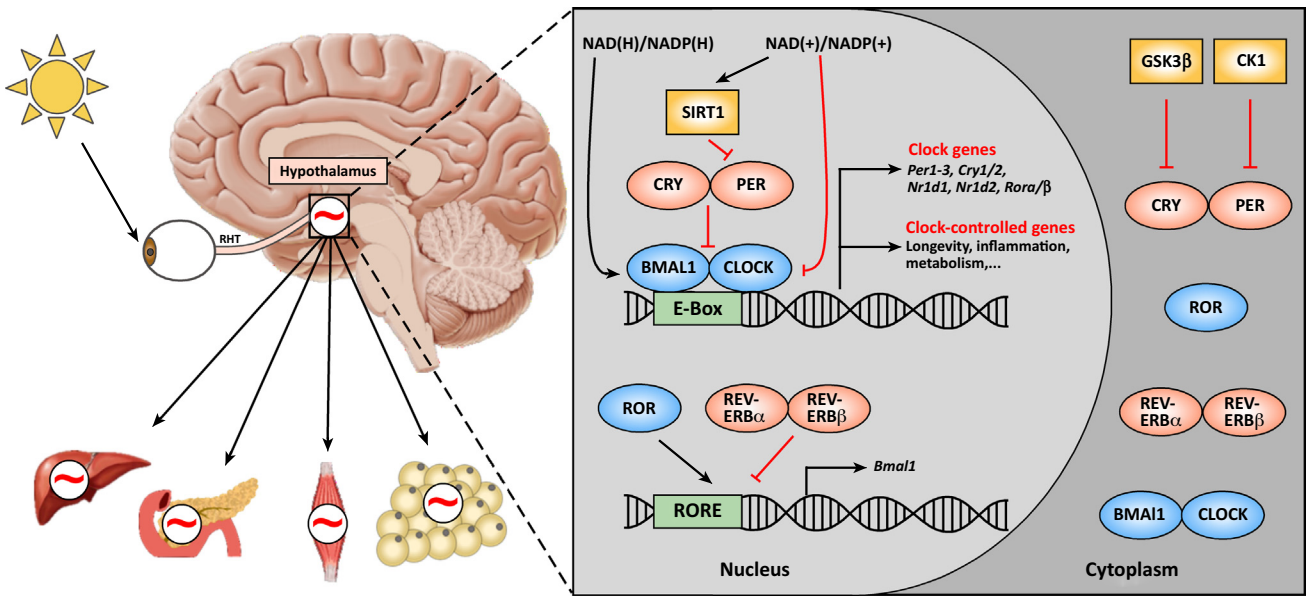

Figure 2. Regulation of Cell Autonomous Circadian Rhythms. The master pacemaker of the circadian rhythm is the central circadian clock that resides in the SCN of the hypothalamus and synchronises peripheral clocks in the body. At the molecular level, circadian rhythms are driven by a cell autonomous transcriptional translational feedback loop that occurs in virtually all cells. The positive arm of the mammalian circadian clock consists of the clock proteins CLOCK and BMAL1, which heterodimerise and bind to E-box regions in promoters of the clock genes *cry1/2* and *per1–3*, initiating their transcription. Additionally, CLOCK/BMAL1 induces *rev-erbα* and *rorα/β* gene expression. The negative arm is represented by CRY, PER, and REV-ERBα. CRY/PER heterodimers translocate into the nucleus and repress the binding of the CLOCK/BMAL1 complex to target promoter regions, thereby inhibiting their own transcription. Furthermore, REV-ERBα negatively regulates *bmal1* expression, while RORα/β induces its expression. CLOCK/BMAL1 are directly regulated by NAD(H) and NADP(H), which enhance the transcriptional activity of the complex. Their oxidised forms, NAD(+) and NADP(+), inhibit CLOCK/BMAL1 binding to DNA. Furthermore, SIRT1 oscillates and binds CLOCK/BMAL1 heterodimers in a circadian manner, facilitating the degradation of PER2 and directly regulating circadian rhythms. Another regulatory mechanism for this rhythmic machinery is represented by the cytosolic enzymes CK1 and GSK3β, which label CRY and PER for degradation. This transcriptional translational feedback loop is repeated approximately once every 24 h. Abbreviations: BMAL1, brain and muscle ARNT-like protein 1; CK1, casein kinase 1; CLOCK, circadian locomotor output cycles kaput; CRY, cryptochrome; GSK3β, glycogen synthase kinase 3β; SCN, suprachiasmatic nucleus; SIRT1, sirtuin 1.

TRF as a Therapeutic Intervention to Combat Circadian Disruptions and Metabolic Diseases

Considering the close relationship between circadian clocks and energy homeostasis, behavioural interventions, such as fasting regimens, to combat the consequences of disrupted rhythms and metabolism appear to be a favourable tool. Intriguingly, fasting and caloric restriction (CR) protocols increase SIRT1 activity in the hypothalamus as well as the periphery [43]. SIRT1 possesses potent anti-inflammatory capacities and improves metabolic health by its ability to interact with a variety of transcription factors that control energy homeostasis [43]. These findings offer an explanation for the beneficial effects of intermittent fasting (IF) and TRF on the detrimental consequences of DIO and related metabolic disorders. TRF is a form of IF in which food intake is restricted to specified hours each day. In a broad variety of species from fruit flies to mice and humans, CR and TRF lead to an extension of lifespan, promote weight loss, prevent the development of metabolic diseases, and reduce levels of proinflammatory cytokines [44–46]. Recent studies suggest that the beneficial effects of TRF strongly depend on the timing of food intake. Time-restricted access to high-fat diet (HFD) during the active (dark) phase of mice prevents body weight gain, hyperinsulinemia, glucose intolerance, and systemic inflammation, whereas mice with access to HFD both *ad libitum* and exclusively during their inactive (light) phase, develop metabolic disorders, even when caloric intake is identical [34,45].

Timing of food intake is such a strong circadian entrainment cue that TRF of HFD can restore the expression phase of the hepatic clock genes *Clock* and *Cry1* and phase-advance the expression of *Bmal1*, *Per1/2*, *Cry2*, among others, compared with mice fed HFD *ad libitum* [47]. These findings suggest that TRF can be used as a behavioural intervention strategy to counteract the detrimental effects of obesity and/or circadian disruptions.

Studies to determine the therapeutic effects of food as a time cue to resynchronise peripheral clocks are underway. Restricting meal times to certain hours of the day may be a promising long-term strategy to combat metabolic disease.

Neuroendocrine Disruptions in the Development of AD

AD is the most common cause of dementia. Progressive loss of synapses and neuronal death underlie the pathology of AD, resulting in cognitive decline and memory loss. Extracellular amyloid plaques (Aβ), caused by alternative processing of the amyloid precursor protein (APP) by β-secretase (BACE)1 and γ-secretase, as well as intraneuronal neurofibrillary tangles (NFTs) consisting of hyperphosphorylated and aggregated τ protein, are the two traditional hallmarks of this disease [48] (Box 1).

A common feature of AD and metabolic diseases such as obesity is central inflammation, contributing to both pathologies in multiple ways [49]. In this regard, TLR-4-mediated PTEN/PI3K/AKT/NF-κB signalling was proposed as a link between DIO, T2D, and AD [50,51]. Basal NF-κB signalling can promote neuronal survival and regeneration, whereas sustained inflammation is associated with increased Aβ deposition and pathological tissue damage [50,51].

The term type 3 diabetes (Box 2) was proposed by Steen and colleagues in 2005 to highlight the profound role of impaired insulin signalling in the development and progression of AD [52]. Insulin receptors (IRs) are expressed in many brain areas by neurons and glial cells with high abundance in the hypothalamus and hippocampus, where they regulate neuronal survival and differentiation, cytoskeletal rearrangements, neuronal plasticity, neurotransmitter release, gene expression, protein synthesis, as well as central and whole-body energy homeostasis [53]. *Ex vivo* studies in postmortem brains from nondiabetic aged patients with and without AD have provided strong evidence for central insulin resistance as a prominent feature of the neurodegenerative disease. Talbot and colleagues stimulated postmortem hippocampal tissue samples with physiological doses of insulin and revealed reduced phosphorylation of IR, IRS1, AKT, and GSK3β in AD patients in comparison to age-matched healthy controls, indicating a decreased action of the hormone [54]. Furthermore, these aberrations correlated positively with Aβ load and the extent of τ protein phosphorylation, as well as negatively with cognition and memory scores of patients [54]. Additionally, decreases in gene expression and protein levels of insulin pathway molecules such as insulin, IR, and IRS1 were observed [52]. Furthermore, insulin signal transduction was shown to be negatively correlated with ageing and the pathogenesis of AD due to declining hormone levels in the cerebrospinal fluid (CSF) and

Box 2. Type 3 Diabetes

Central insulin resistance can have a high impact on the histopathological changes seen in AD brains due to impaired glucose uptake and utilisation and altered signal transduction, as described in the main text. Accordingly, patients suffering from T2D have a 50–65% higher risk of developing AD and other forms of dementia [96]. Emphasising the similarities between both diseases, the term type 3 diabetes was coined for patients suffering from both medical conditions, T2D and AD/dementia. Common molecular features of both diseases, beside central insulin resistance, are the occurrence of amyloidogenic protein aggregates in the brain and/or pancreas, increased proinflammatory signalling, severe oxidative stress, neurodegeneration, microvascular changes, and an advanced production of glycation end-products.

decreased binding capacity of insulin to its receptor [55]. In line with those results, insulin-mediated gene expression declines with AD progression.

Since glucose is the main energy source of the brain, the disturbances in glucose homeostasis caused by deteriorated central insulin signalling lead to a starvation state of the brain and thus to oxidative stress and cell death [56]. Even though the uptake of glucose into the brain and neurons is mediated by insulin-independent GLUT-3 and GLUT-1 glucose transporters, respectively, a direct association between insulin resistance and a reduction in brain glucose metabolism was demonstrated by fluorodeoxyglucose (FDG) positron emission tomography (PET) in AD patients [57]. Accordingly, reduced glucose uptake in the brain measured by PET is an early and accurate indicator of neurodegeneration in humans and goes along with the severity of cognitive decline and memory impairments [58]. Age-dependent impairments in central glucose homeostasis can also be observed in mouse models of AD [59]. Here, decreased brain glucose levels are associated with accelerated disease progression, and GLUT-1 deficiency in endothelial cells was shown to enhance neuronal atrophy, Aβ plaque formation, and cognitive impairments. In turn, overexpression of GLUT-1 improved neurodegeneration and behavioural alterations in a fruit fly model of AD [60].

However, the negative effects of diminished insulin pathway activation are not only due to direct effects on energy metabolism, but also to changes in the regulation of important downstream pathways and molecules. A direct link between central insulin action and cognitive function was demonstrated by studies that depleted insulin receptors in the brain or inhibited central insulin signalling in rodents, causing profound cognitive impairments and AD-like molecular and biochemical changes [61,62]. Insulin exhibits antiapoptotic and anti-inflammatory properties via inhibition of Forkhead box (FOX)O, Bcl-2-associated death promoter (BAD), GSK3β, and NF-κB pathways. In the case of insulin deficiency, these detrimental pathways remain activated, causing central inflammation and apoptosis. Moreover, the formation of cytotoxic Aβ plaques and of hyperphosphorylated τ protein aggregates is promoted at different levels. On a transcriptional level, insulin is involved in the regulation of τ protein and APP expression, causing elevated levels of APP and decreased levels of *tau* mRNA in postmortem AD brains [52]. Since functional insulin signalling is also essential for the trafficking of Aβ peptides from their production site in the trans-Golgi network to the plasma membrane, for secretion and degradation via insulin-degrading enzymes (IDEs), disturbed insulin signalling leads to reduced Aβ clearance [63]. IDE expression and membrane localisation are induced by insulin stimulation in cultured astrocytes, where insulin facilitates the degradation of exogenous Aβ [64]. In turn, Aβ oligomers inhibit IRS1 through activation of the JNK pathway, decrease the binding affinity of insulin, reduce IR surface expression, and interfere with PI3K-mediated activation of AKT, thereby exacerbating insulin signal transduction [65].

GSK3β, which is inhibited by the insulin pathway, can phosphorylate τ protein at multiple sites, promoting the aggregation of hyperphosphorylated τ protein and causing the formation of NFTs [66]. Eventually, this leads to a disruption of intraneuronal transport mechanisms, collapse of the cytoskeletal structures, neurite retraction, and loss of synaptic connections.

Antidiabetic Drugs in the Treatment of AD

Based on the shared features between T2D and AD, it is not surprising that antidiabetic drugs not only improve symptoms of T2D but also exhibit positive effects in AD patients. Recent studies have shown promising results in rodent models, and several drugs have been tested in clinical trials in AD patients. The most prominent treatment linking T2D to AD is intranasally administered insulin. In patients with mild cognitive impairment and AD, both acute and chronic treatment improved memory and plasma Aβ 40/42 ratio [67,68].

Various other diabetes medications have been tested in animal models of AD. Promising results have been demonstrated for glucagon-like peptide-1 receptor agonists (GLP-1RAs), dipeptidyl peptidase-4 inhibitors, metformin, and thiazolidinediones. Systemic GLP-1RA administration in rodents and non-human primates, for instance, greatly improved numerous markers of memory function, neuronal survival, insulin signalling, and inflammation. Additionally, hippocampal Aβ load and τ protein pathology were reduced [69–71]. So far, only a limited number of controlled studies with yet inconclusive outcomes has been conducted in humans. Taken together, these data demonstrate the close link between disrupted insulin action and AD. However, whether this effect is direct or indirect is still controversial and is addressed in the final chapter.

Circadian Disruptions in AD

Beside metabolic derangements, disruptions in circadian rhythms are associated with a number of different diseases such as cancer, mood disorders, and AD. In fruit fly and rodent models, mutations or knockouts of the clock genes *bmal1* and *period* cause an accelerated aging phenotype characterised by earlier decline in cognitive functions, a high rate of oxidative tissue damage, and an overall shorter lifespan [72,73].

The influence of the pathology of AD on circadian rhythms was observed several decades ago. More recently, it has become clear that circadian disturbances are one of the earliest symptoms of AD and often they precede the onset of cognitive and motor symptoms by several years [74]. AD patients exhibit abnormal behavioural rhythms such as disturbed sleep/wake cycles and emotional imbalances. While nocturnal sleep becomes increasingly fragmented, night-time activity and daytime sleepiness intensify in these patients [75,76]. This is accompanied by the sundown syndrome that describes a state of increased distress, emotional volatility, and aggression in the evening. Physiological and biochemical processes such as hormone release patterns or antioxidative defence rhythms are also changed [77]. The hormone melatonin, which is produced by the pineal gland, is involved in the entrainment of circadian rhythms in the SCN and acts as an effective free-radical scavenger. In healthy individuals its release peaks during night-time and is suppressed by daylight. However, in AD patients and individuals with preclinical cognitive symptoms of dementia, melatonin levels are reduced and its rhythmic oscillation is flattened [77,78]. Further circadian abnormalities were detected in the core body temperature rhythm, which is delayed and its amplitude decreased [79]. In line with these results, postmortem AD brains display a severe loss of hypothalamic tissue, including SCN cells and lowered melatonin receptor (MT1) levels [80].

Recent studies in animal models of AD indicate changes in the rhythmic expression patterns of the clock genes *bmal1*, *per1*, and *cry1* in the brain. Although *bmal1* expression remains rhythmic in several brain regions and peripheral tissues, the temporal phase relationship between different areas seems to vary in comparison to healthy controls [76]. Additionally, the *bmal1*, *per1*, and *cry1* mRNA rhythmicity is lost in cells of the pineal gland [81]. Regarding the pathology of AD, *presilin-2* expression is regulated by CLOCK/BMAL1 heterodimers at a transcriptional and post-transcriptional level in peripheral tissue [82]. Mutations in *presilin-2* are associated with the development and Aβ pathology of familial, early-onset AD.

A direct link between desynchronised body clocks and Aβ pathology was recently shown in different cell culture and mouse models. The treatment of human skin fibroblasts, human A172 glioma cells, as well as primary cortical and hippocampal mouse neurons with physiological concentrations of $A\beta_{1-42}$ peptides caused a dampening in metabolic ATP level oscillations and mitochondrial respiration rhythms, leading to increased oxidative stress [83]. In turn, the disruption of circadian rhythmicity either globally or locally in the brain, achieved by deletion

of the clock gene *bmal1* in a β-amyloidosis mouse model, exposed a direct effect of central circadian disruptions on daily Aβ fluctuations in the hippocampal interstitial fluid, promoting Aβ plaques deposition [84]. Furthermore, a global *bmal1* deletion resulted in elevated *apoe* expression in the brain parenchyma and the formation of fibrillary plaques [84].

Key Figure

Obesity, Type 2 Diabetes and Alzheimer's Disease Are Tightly Linked Disorders with Obesity Being a Major Risk Factor for the Development of T2D and AD

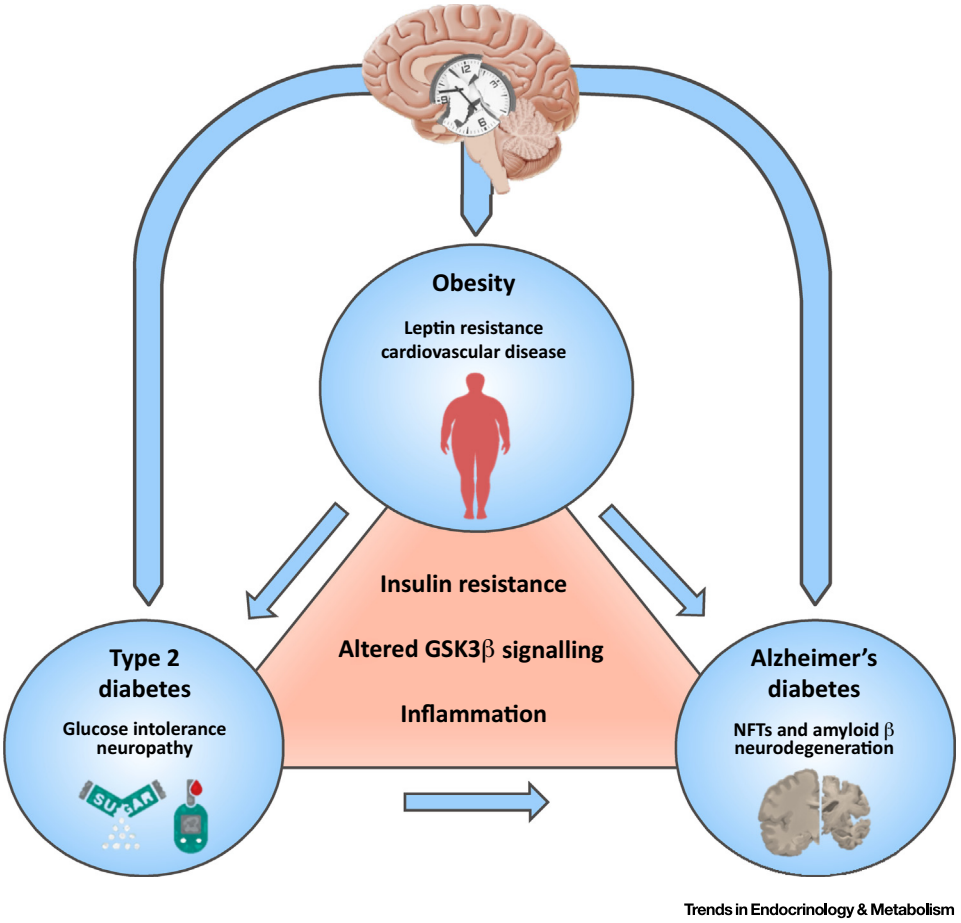

Figure 3. Impaired glucose homeostasis in T2D also increases the risk for the development of sporadic AD. Pathological changes such as insulin resistance, altered GSK3β signalling, and central inflammation are common features of all three diseases. Recent research also provides evidence that disruption of the circadian clock can further promote the progression of all three disorders. Abbreviations: AD, Alzheimer's disease; GSK3β, glycogen synthase kinase 3β; NFTs, neurofibrillary tangles; T2D, type 2 diabetes.

The number of circadian abnormalities in AD and metabolic syndrome leads inevitably to the question of whether circadian disruptions are the cause or consequence of the pathogenesis of AD and other neurodegenerative diseases. So far, this question cannot be answered easily even though there is some evidence that changes in the master clock deteriorate rather than initiate pathological processes in AD [85].

Further evidence for the close link between circadian disruptions and AD is provided by circadian-focussed therapies that are suggested to improve the symptoms of AD. These interventions, including bright light therapy and timed administration of melatonin resulted in inconsistent outcomes, thus their benefit is still controversial [86–89].

Concluding Remarks

The brain plays a crucial role in the development of obesity, T2D, and AD, and disruptions of neuroendocrine signalling cascades deteriorate metabolic health. Central insulin resistance, altered GSK3β signalling, and an increase in inflammatory processes are shared hallmarks of these metabolic diseases. The development of one of these disorders can therefore facilitate further metabolic derangement. Furthermore, the close regulatory relationship between energy metabolism as well as cognitive health and the circadian clock identifies circadian disruptions as a major risk factor in the development of obesity, T2D, and AD (Figure 3, Key Figure). It is still unclear as to whether the detrimental effects of central insulin resistance directly contribute to the pathological changes that lead to AD, or whether the secondary consequences of central insulin resistance on energy metabolism result in neurodegeneration. However, the occurrence of central insulin resistance in nondiabetic patients with AD [54] points towards a direct link between insulin resistance and AD. Likewise, the question arises whether the disruption of the circadian clock directly triggers neurodegeneration or rather is a consequence of AD. Further research is urgently needed to unravel the fascinating underlying molecular mechanisms linking circadian disruption, T2D, and AD to find novel treatment modalities that target the root cause of these debilitating conditions (see Outstanding Questions).

Outstanding Questions

How important is the timing of meals for the synchronisation of the master clock, and do abnormal eating patterns lead to a disruption of circadian rhythms?

Are neuroendocrine pathways, which control energy metabolism, regulated in a circadian manner in the brain?

Are central insulin resistance, altered GSK3β signalling and chronic low-grade inflammation accompanying symptoms of a disrupted clock?

Are circadian disruptions the cause or consequence of the pathogenesis of AD and other neurodegenerative diseases? Recent evidence suggests that circadian disruptions deteriorate rather than initiate pathological processes in AD but little is known about the underlying mechanisms.

Can IF/TRF restore a misalignment of the circadian clock and thereby alleviate/reverse metabolic derangements and what are the molecular mechanisms?

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