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REVIEW

Effects of ketone bodies in Alzheimer's disease in relation to neural hypometabolism, β-amyloid toxicity, and astrocyte function

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Abstract

Diet supplementation with ketone bodies (acetoacetate and β-hydroxybuturate) or medium-length fatty acids generating ketone bodies has consistently been found to cause modest improvement of mental function in Alzheimer's patients. It was suggested that the therapeutic effect might be more pronounced if treatment was begun at a pre-clinical stage of the disease instead of well after its manifestation. The pre-clinical stage is characterized by decade-long glucose hypometabolism in brain, but ketone body metabolism is intact even initially after disease manifestation. One reason for the impaired glucose metabolism may be early destruction of the noradrenergic brain stem nucleus, locus coeruleus, which stimulates glucose metabolism, at least in astrocytes. These glial cells are essential in Alzheimer pathogenesis. The β-amyloid peptide Aβ interferes with their cholinergic innervation, which impairs synaptic function because of diminished astrocytic glutamate release. Aβ also reduces glucose metabolism and causes hyperexcitability. Ketone bodies are similarly used against seizures, but the effectively used concentrations are so high that they must interfere with glucose metabolism and *de novo* synthesis of neurotransmitter glutamate, reducing neuronal glutamatergic signaling. The lower ketone body concentrations used in Alzheimer's disease may owe their effect to support of energy metabolism, but might also inhibit release of gliotransmitter glutamate.

Keywords: Alzheimer's disease, astrocytes, Aβ, gliotransmitter, hypometabolism, subcortical nuclei.

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A moderate improvement in Alzheimer's disease symptomatology has been seen after supplementation of the diet with relatively small amounts of ketone bodies. These amounts are much smaller than those that can be effective against seizures, and the mechanism(s) of action are probably very different. The effect of ketone body supplementation is interesting because Alzheimer's disease is preceded by decades of glucose hypometabolism, and earlier dietary intervention might have greater therapeutic effect. Moreover, the potent toxicity of the soluble oligomeric Aβ peptide is being realized and this compound is used experimentally. A previously suggested hypothesis that initial damage of subcortical nuclei is important for development of the disease is getting renewed support. While it is well known that microglia play a major role in Alzheimer's disease, it is becoming obvious that impairment of astrocytic functions is also important. These cells are essential for the pathogenesis of Alzheimer's disease, owing to their large contribution to brain energy metabolism and inflammatory and anti-inflammatory events. Recent research has also shown the importance of their release of glutamate and other compounds as gliotransmitters.

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Abbreviations used: APP, amyloid precursor protein; NBM, nucleus basalis of Meynert; PARP-1, poly(ADP-ribose) polymerase 1; ROS, reactive oxygen species; TCA, tricarboxylic acid.

Brain hypometabolism precedes clinical signs of Alzheimer's disease

Frackowiak *et al.* (1981) found a decline in cerebral blood flow and mean cerebral oxygen utilization, i.e., hypometabolism, which is correlated with the severity of dementia. Global oxygen extraction ratio was not increased, providing evidence against chronic ischemia. Shortly afterward de Leon *et al.* (1983) found that aged patients with senile dementia showed consistent diminutions in regional glucose use compared to elderly normal persons, also with significant correlation between hypometabolism and decreased cognitive functioning. Thirteen additional studies (till 2009) all showed glucose hypometabolism (Cunnane *et al.* 2011), and since then glucose hypometabolism has repeatedly been confirmed in Alzheimer's patients. In contrast, metabolism of ketone bodies is unaltered, at least in early stages of the disease (Castellano *et al.* 2015).

Alzheimer's patients expressing the apolipoprotein E4 (ApoE4), a risk factor for the disease, show a more severe medial temporal hypometabolism than matched ApoE4-negative patients in spite of lower global amyloid burden (Lehmann *et al.* 2014). Brain glucose metabolism is also reduced in late-middle-aged healthy ApoE4 carriers (Reiman *et al.* 2005). A confounding factor is that glucose metabolism decreases in healthy aging, but the decrease is more pronounced in ApoE4 carriers than in age-matched non-carriers (Reiman *et al.* 2005). When this decline is taken into account, the most severe hypometabolism seems to occur in younger Alzheimer's patients. Thus, glucose hypometabolism in brain may indicate a risk for future development or worsening of dementia (Dukart *et al.* 2013). Consistent with this, reduced glucose metabolism can be demonstrated not only in ApoE4 carriers but also in other persons at risk for developing Alzheimer's disease decades before the onset of the disease (Mosconi *et al.* 2008). As mitochondrial DNA is maternally inherited in humans, it is interesting that brain glucose metabolism is lower in elderly with a maternal family history of Alzheimer's disease than in those with a paternal family history and in controls with a negative family history of Alzheimer's disease (Mosconi *et al.* 2007). Furthermore, in cognitively normal persons between 32 and 72 years of age with a family history of Alzheimer's disease, brain glucose metabolism is lowest in those where both parents had suffered from the disease, intermediate in those with a maternal history of the disorder and highest in those with a paternal family history (Mosconi *et al.* 2014). The importance of brain energy metabolism is also indicated by the observation that reduction in blood pressure in hypertonic patients is associated with memory decline (Glodzik *et al.* 2014).

More detailed information about brain metabolism can be obtained by studies of the metabolic fate of ^{13}C-labeled glucose or the astrocyte-specific substrate acetate by nuclear magnetic resonance (^{13}C-NMR). Nilsen *et al.* (2014) performed a ^{13}C-NMR study in the transgenic McGill-R-Thy1-APP rat model of Alzheimer's disease. In frontal cortex the tricarboxylic acid (TCA) cycle turnover was reduced in both neurons (glutamatergic and GABAergic) and astrocytes. Pyruvate carboxylation, an astrocytic metabolic process, which is essential for *de novo* synthesis of transmitter glutamate and GABA via the glutamine–glutamate-GABA cycle (see legend of Fig. 1), was decreased, and the level of glutamate, glutamine, GABA, and aspartate was reduced in some regions. This observation is in agreement with a pronounced decrease in pyruvate carboxylase activity in brains of patients having suffered from Alzheimer's disease (Hertz *et al.* 2000). Nilsen *et al.* (2014) found the contents of glucose and of [1-^{13}C]glucose in brain to be unchanged (indicating that uptake across the blood–brain barrier was not reduced). In another rat model of Alzheimer's disease, the metabolic response to forepaw stimulation as well as evoked neural activity were reduced by ~ 50% (Sanganahalli *et al.* 2013). This may reflect synaptic degeneration and/or glutamate deficiency. A small ^{13}C-MRS study in Alzheimer's patients using ^{13}C-labeled glucose and with no attempt to study astrocytic metabolism showed also a clear-cut reduction in neuronal TCA cycle turnover rate, which was combined with reduced formation of glutamate from glutamine (Lin *et al.* 2003). Another study from the same group using ^{13}C-labeled acetate in Alzheimer's patients and measuring bicarbonate (CO_2) formation correlated an increase in astrocytic TCA cycle metabolism with an increase in the presumed astrocytic marker myo-inositol and glial activation as an indication of inflammation (Sailasuta *et al.* 2011). This is consistent with elevated activity of beta-glucuronidase, a lysosomal enzyme, which occurs in reactive astrocytes (McGeer *et al.* 1989), a hallmark of Alzheimer's disease. Increased physiological activity by 'activated' astrocytes (as indicated by up-regulation of glial fibrillary acidic protein) has also been shown by Seidel *et al.* (2015). However, the 'activated' astrocytes may well be metabolically and functionally different from astrocytes in normal brain.

Glucose hypometabolism is dangerous not only because it acutely limits the metabolic capabilities of the cells, but also because it leads to oxidative stress. Oxidative stress owing to glucose deprivation is well known in human tumor cells (Lee *et al.* 1998; Blackburn *et al.*, 1999) and in primary cultures of rat thymocytes (Aulwurm and Brand 2000). The first step of most glucose degradation is conversion to pyruvate by cytosolic glycolysis, initiated by hexokinase-catalyzed glucose phosphorylation. The hexokinase isoforms I and II are also bound to the mitochondrial membrane (Polakis and Wilson 1985; Henderson *et al.* 2006; Sun *et al.* 2008). The binding occurs to the voltage-dependent anion channel, also known as mitochondrial porin, located at the outer membrane (Pastorino and Hoek 2008). This channel is associated with

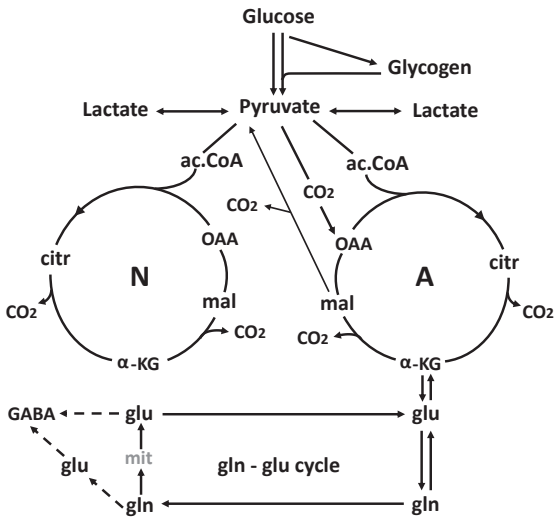

Fig. 1 Cartoon of glucose metabolism via pyruvate in neurons (left – N) and astrocytes (right – A) and of glutamine–glutamate (GABA) cycling. One molecule glucose is metabolized by glycolysis in the cytosol to two molecules of pyruvate in a complex and strictly regulated pathway, where one oxidative process requires transfer of reducing equivalents to the mitochondria (see, e.g., Hertz and Dienel 2002). In both neurons and astrocytes pyruvate metabolism via acetyl Coenzyme A (ac.CoA) leads to formation of citrate by condensation with pre-existing oxaloacetate (OAA) in the tricarboxylic acid (TCA) cycle, an end-result of the previous turn of the cycle. Citrate oxidation in the TCA cycle includes two decarboxylations, leading to re-formation of oxaloacetate, ready for another turn of the cycle, and to reduction of NADH, leading to large amounts of energy (ATP) via re-oxidation in the electron transport chain. Pyruvate carboxylation, which is active in astrocytes, but absent in neurons (reviewed in Hertz 2013), creates a new molecule of oxaloacetate, which after condensation with acetyl Coenzyme A, derived from a second molecule of pyruvate, forms a new molecule of citrate. α-Ketoglutarate (α-KG), one of the intermediates of the TCA cycle can leave the cycle to form glutamate (glu), catalyzed by either aspartate aminotransferase or glutamate dehydrogenase. Further metabolism by the cytosolic and astrocyte-specific enzyme glutamine synthetase leads to the formation of glutamine (gln). After release from astrocytes glutamine is accumulated in glutamatergic and GABAergic neurons [lower line of the glutamine-glutamate (GABA) cycle (glu-gln cycle], converted to glutamate (and in GABAergic cells onward to GABA) and released as transmitter. In glutamatergic neurons all glutamate formed by deamidation of glutamine enters the mitochondria (mit) and is returned to the cytosol in a complex process, which requires simultaneous glucose metabolism. In GABAergic neurons this is only the case for some of the glutamate, whereas the remainder enters the cytosol directly. This difference may make GABA production less sensitive to replacement of glucose as the substrate with β-hydroxybutyrate in patients receiving very high amounts of ketone bodies to prevent seizures. Released glutamate is almost quantitatively re-accumulated in astrocytes, together with at least part of the released GABA [upper line of the glutamine–glutamate (GABA) cycle (gln–glu cycle)] and re-accumulated in the astrocytic cytosol. Here, about 75% is converted to glutamine and re-enters the glutamine–glutamate (GABA) cycle. The remaining ~ 25% is oxidatively degraded, via one of two partly different pathways. In both α-ketoglutarate is re-converted to malate. In one malate exits to the cytosol, is decarboxylated by cytosolic malic enzyme to pyruvate, which is oxidized in the TCA cycle via acetyl Coenzyme A. In the other malate does not exit the TCA cycle but may be further metabolized to α-ketoglutarate after condensation with acetyl Coenzyme A, allowing re-synthesis of another molecule of glutamate from only one molecule pyruvate. In either case the degraded glutamate must in the long term be replaced by a quantitatively similar production of glutamate from glucose, in the first case by complete *de novo* synthesis from one molecule glucose, in the second from one half of a glucose molecule. However, temporary fluctuations in the content of glutamate occur. The initial part of GABA metabolism is different, as all GABA is metabolized via succinate and α-ketoglutarate to glutamate. Modified from Hertz (2013).

the ADP/ATP carrier (adenine nucleotide translocator), which mediates the exchange of ADP and ATP through the inner mitochondrial membrane (Vyssokikh and Brdiczka 2003; Azoulay-Zohar *et al.*, 2004). Appropriate function of voltage-dependent anion channel is essential to prevent oxidative damage and cell death as the channel also transports reactive oxygen species (ROS) (Shoshan-Barmatz *et al.* 2014). Hexokinase binding to the mitochondrial membrane reduces channel conductance and ROS transport (Shoshan-Barmatz *et al.* 2015). Thus, besides the acute effect on energy supply, continued glucose hypometabolism and hexokinase hypoactivity may be an important inducer of oxidative stress, known to play a major role in Alzheimer's disease (Subbarao *et al.* 1990; Mosconi *et al.* 2008). However, formation of mitochondrial ROS is also increased by β-amyloid, which accumulates in mitochondria before its deposit as extracellular amyloid plaques (Readnower *et al.* 2011). Mitochondrial Ca^{2+} mobilization may play a role in this process (Camello-Almaraz *et al.* 2006). An association between β-amyloid toxicity, mitochondrial dysfunction, oxidative stress and neuronal damage and death in Alzheimer's disease has been emphasized by Albrekkan and Kelly-Worden (2013). Recently, an ATP synthase has also been recognized on the surface of neural cells which is inhibited by the β-amyloid peptide, Aβ or Abeta (Schmidt *et al.* 2008; Xing *et al.* 2012).

Toxicity of A*β*1–42 and related peptides

Aβ1-42 is a soluble cleavage product of amyloid precursor protein (APP). APP is a family of transmembrane proteins that can be cleaved at several sites. Neurons are the principal source of APP in the normal cerebral cortex (Cole *et al.* 1991), but both astrocytes and microglia can rapidly synthesize APP, when stimulated by neuronal injury (Siman *et al.* 1989; Töpper *et al.* 1995). APP is most commonly cleaved by α-secretase at a site on the extracellular, N-terminal domain, close to the cell membrane, yielding a soluble, diffusible APP fragment, sAPPα, which is neuro-

protective and may play a role during development (Ho *et al.* 1996; Barger 2000). APP can also be cleaved a little further from the cell membrane by β-secretases, leading to the release of sAPPβ. Although this protein is only slightly different from sAPPα, it does not share the neuroprotective effect of sAPPα. Combined action of β-secretases with γ-secretases, which act intracellularly, generates β-amyloid peptide (Aβ) 40 or 42 together with peptides of different length (Zhang and Xu 2007). Although it has been suggested that Aβ in the brain only becomes toxic when it precipitates from solution and forms aggregates, the soluble oligomeric polypeptide itself induces a multitude of functional abnormalities in brain tissue (Zilberter *et al.* 2013). Consistent with this, the research focus is shifting from amyloid plaques toward soluble oligomeric Aβ as the toxic species, which strongly correlates with the severity of dementia (McLean *et al.* 1999).

Aβ is toxic to primary cultures of rodent hippocampal neurons (Barger *et al.* 1995; Bales *et al.* 1998) and inhibits long-term potentiation in cocultures (Lambert *et al.* 1998). Aβ1-42 is neurotoxic, although it has no effect on $[Ca^{2+}]_i$ in neurons but rapidly increases $[Ca^{2+}]_i$ in nearby astrocytes in hippocampal cocultures (Abramov *et al.* 2003). This effect is dependent on extracellular Ca^{2+}, and it may be the reason for an increased rate of generation of ROS causing mainly neuronal death and depletion of glutathione, a ROS scavenger (Morris *et al.* 2014), in both neurons and astrocytes. Malfunctioning Ca^{2+} homeostasis in astrocytes (Lim *et al.* 2014) may also affect Ca^{2+}-dependent gliotransmitter release and in line with this Aβ induces glutamate release in cultured astrocytes (Talantova *et al.* 2013). In the additional presence of neurons the released glutamate causes extrasynaptic NMDA receptor activation and synaptic loss. Similar observations have been made in normal astrocytes of hippocampal slices, where acute exposure to Aβ1-42 acts rapidly at the α7 nicotinic cholinergic receptor, where it increases $[Ca^{2+}]_i$ and releases gliotransmitter glutamate that subsequently activates neuronal NMDA receptors (Pirttimaki *et al.* 2013). The same authors found that spontaneous astrocytic $[Ca^{2+}]_i$ elevations took place at increased frequency in a 3–4-month-old mouse Alzheimer model, in which synaptic or cognitive deficits had not yet developed. This indicated enhanced gliotransmission, probably because of increase in Aβ. White matter myelin and oligodendrocytes are also damaged by Aβ (Jantaratnotai *et al.* 2003).

A study in neuronal-astrocytic cocultures by Abeti *et al.* (2011) showed a direct and rapid inhibition by Aβ of astrocytic and neuronal glucose uptake. It also demonstrated a rapid decrease in rate of oxygen consumption and mitochondrial depolarization in astrocytes. This was caused by activation of astrocytic poly(ADP-ribose) polymerase 1 (PARP-1), a nuclear DNA repair enzyme, and it was triggered by oxidative stress. These events, as well as neuronal death after 24 h, could all be prevented by addition of pyruvate (Abeti *et al.* 2011; Abeti and Duchen 2012) and might thus be secondary to inhibited metabolism of glucose. The data obtained by Abeti and Duchen (2012) suggested that PARP-1 activation initially depletes mainly cytosolic NAD^+ which is required for glycolysis, but not for oxidation of substrates that enter the TCA cycle directly, such as β-hydroxybutyrate and pyruvate. Abnormal NAD(P)H signaling together with neuronal hyperactivity and interictal-like spontaneous synchronized discharges that began after 40 min of exposure to Aβ1-42 has also been shown in hippocampal slices (Zilberter *et al.* 2013). Whether or not this is enough to explain the increased incidence of seizures in Alzheimer's patients (Spencer 2014) remains unknown. In addition, Zilberter *et al.* (2013) showed that synaptic function, long-term potentiation and GABA-ergic activity were impaired. All effects of Aβ were prevented by medium supplementation with pyruvate (5 mM) and β-hydroxybutyrate (4 mM), concentrations that are high compared to those in blood after administration of octanoic acid in usual doses. Zilberter *et al.* (2013) also found that brain slices from a transgenic Alzheimer mouse model fed a standard diet showed signs of glucose hypometabolism (abnormal NAD(P)H signaling, reduced tolerance to hypoglycemia and two-fold reduction in brain glycogen level) together with some neuronal dysfunction. Dietary supplementation with *small* amounts of pyruvate or β-hydroxybutyrate (26 mg/day) for 5 weeks decreased neuronal hyper-excitability and prevented glycogen depletion. Pyruvate also prevented the development of age-dependent cognitive deficits in a mouse model of Alzheimer's disease without reducing amyloid and tau pathology (Isopi *et al.* 2014).

Subcortical nuclei are vulnerable in Alzheimer's Disease which may affect glucose metabolism, inflammation and release of gliotransmitter

A contributing factor to the development of brain hypometabolism early in the prodromal stage of Alzheimer's disease may be impairment of adrenergic stimuli of brain metabolism. The brain receives its entire adrenergic stimulation from a small brain stem nucleus, locus coeruleus (Moore and Bloom 1979). There is profound neurodegeneration and expression of neurofibrillary tangles in locus coeruleus in Alzheimer's disease (German *et al.* 1987; Zweig *et al.* 1988; Chan-Palay 1991; Busch *et al.* 1997; Haglund *et al.* 2006; Grudzien *et al.* 2007; Takahashi *et al.* 2015). Clinical manifestations associated with this subcortical pathology unfortunately remain undetected in most pathological studies, because current NIH consensus criteria (Hyman *et al.* 2012) for diagnosis of Alzheimer's disease focus on cortical pathology. This is in spite of occasional suggestions during the last 25 years that Alzheimer's disease might be an anterograde degeneration originating in the brainstem and secondarily affecting the brain cortex (Hertz 1989; Marien

et al. 2004; Weinshenker 2008; Simic *et al.* 2009; Chalermpalanupap *et al.* 2013). The most direct support for this hypothesis is a recent paper by Braak and Del Tredici (2013) concluding that 'the most likely candidates for generating Aβ, in our view, are neurons of brainstem nuclei with diffuse projections to the cerebral cortex. In fact, the existence of Aβ plaques in the cerebellum (Braak et al. 1989) can only be explained by such a phenomenon, i.e., the release of Aβ via terminal axons of nerve cells with tau pathology, insofar as the neuronal types within the cerebellum itself do not develop tau-associated lesions'. The authors go on to explain that cerebellar neurons are well supplied with dense networks of fibers originating from brainstem nuclei, above all locus coeruleus, where abnormal tau occurs in remarkably young individuals (Braak and Del Tredici 2011; Braak *et al.* 2011; Elobeid *et al.* 2012).

Reduced noradrenergic input to the brain has important consequences. A well-established reason for a detrimental effect of deficient noradrenergic innervation of cortical brain cells is interruption of normal anti-inflammatory effects in microglia and astrocytes. This topic has recently been authoritatively reviewed by Braun *et al.* (2014), a review which should be consulted for additional information. It concludes that an increase in intracellular levels of cAMP by noradrenergic stimulation of β-adrenergic receptors suppresses the activity of inflammatory transcription factors, causes alterations in nuclear localization of proteins, and induces gene expression via cAMP-response element binding protein activation. These effects reduce inflammatory events and contribute to neuroprotective actions by increasing expression of neurotrophic substances including brain-derived neurotrophic factor (BDNF), Glial cell-derived neurotrophic factor (GDNF), and nerve growth factor. Their absence contributes to the progression of Alzheimer's disease.

A less known effect of noradrenergic innervation is that on energy metabolism. Besides stimulating glycogenolysis (Magistretti *et al.* 1981; Ververken *et al.* 1982; Subbarao and Hertz 1990; Hertz *et al.* 2015) noradrenaline has additional effects on energy metabolism. After unilateral chemical lesion of locus coeruleus in rats the resulting noradrenaline depletion was not associated with changes in resting metabolism in cerebral cortex of anaesthetized rats (LaManna *et al.* 1981). However, the noradrenaline depletion slowed the transient metabolic response of both cytochrome oxidase and NAD^+ to sudden increases in energy demand produced by direct cortical electrical stimulation.

Cell culture experiments have shown that glycolysis in isolated astrocytes is stimulated by noradrenaline and the α_1-adrenergic agonist phenylephrine, but not by the α_2-adrenergic agonist clonidine. Both subtypes of α-adrenergic agonists stimulated TCA cycle activity, measured as production of labeled CO_2 from [1-^{14}C]glutamate or [1-^{14}C] pyruvate (Subbarao and Hertz 1991; Chen and Hertz 1999; Chen *et al.* 2000). The effect of α_2 agonists in these isolated cells is exerted on post-junctional receptors (Hertz *et al.* 2010). The effect on oxidative metabolism was less potent than the very potent effect of noradrenaline on glycolysis (Subbarao and Hertz 1991), which therefore may be its primary target under normal conditions. These results are consistent with an increased release of lactate from the prefrontal cortex after local perfusion with noradrenaline (Takita *et al.* 1992) and inhibition of glucose utilization in most cortical regions by both non-selective α-adrenergic inhibitors and the α_2-adrenergic antagonist yohimbine (Savaki *et al.* 1982). The non-selective α-adrenergic effect is probably mediated via an increase in mitochondrial Ca^{2+}, causing a stimulation of metabolic rate of several TCA cycle enzymes including the pyruvate dehydrogenase complex (Denton 2009). This enzyme shows a decreased activity in Alzheimer's disease (Sheu *et al.* 1985; Bubber *et al.* 2005). The effect of α_2-adrenergic inhibition in whole brain is most likely owing to a reduction in adrenergic brain activity by stimulation of pre-junctional inhibitory autoreceptors, reducing stimulation of neurons and astrocytes, which both are all major targets for noradrenaline (Bekar *et al.* 2008; O'Donnell *et al.* 2012). However, with the exception of noradrenaline's effect on Na^+,K^+-ATPase activity (Hajek *et al.* 1996) metabolic effects of noradrenaline have to our knowledge not been demonstrated in isolated neurons, but the stimulation of Na^+,K^+-ATPase activity is likely to increase glucose utilization. Neuronal depolarization also causes increased respiration because of increased activity of Ca^{2+}-dependent intramitochondrial enzymes (Duchen 1992), but it was not studied if noradrenaline may have a similar effect. Noradrenaline effects have also not been studied on pyruvate carboxylation in brain, but it is enhanced in liver by α-adrenergic stimulation (Garrison and Borland 1979).

Other transmitters released from subcortical structures also include astrocytes in their targets. Recently, Chen *et al.* (2012) showed potentiation of neuronal responses in visual cortex to stimuli from nucleus basalis of Meynert (NBM), which was mediated by a cholinergic muscarinic increase in $[Ca^{2+}]_i$ in astrocytes. Muscarinic innervation of astrocytes and $[Ca^{2+}]_i$ response requires functional inositol trisphosphate (IP_3) receptors (Takata *et al.* 2011; Chen *et al.* 2012). Cholinergic activation is normally associated with attention and vigilance, and Takata *et al.* (2011) demonstrated astrocytic involvement in cortical plasticity. They showed that combined stimulation of mouse whiskers and NBM enhanced whisker-evoked local field potential by an effect on muscarinic and *N*-methyl-D-aspartic acid (NMDA) receptors. The elevation of astrocytic $[Ca^{2+}]_i$ was blocked by muscarinic antagonists, and whisker plasticity could not be induced in IP_3 receptor type 2 knock-out mice. In wild-type mice NBM stimulation led to an increase in the extracellular concentration of the NMDA receptor coagonist d-serine, and plasticity in the knock-out mice could be rescued by external supply of d-serine. Thus, stimulation of gliotransmitter

release represents another important mechanism by which astrocytic stimulation of subcortical nuclei can modify function in the normal brain. This effect should be added to inhibition of inflammation and stimulation of glucose metabolism.

It has long been known that NBM is involved in Alzheimer pathology at an early stage (Saper *et al.* 1985; Rasool *et al.* 1986; Mesulam *et al.* 2004). Injection of Aβ into the rat NBM triggers extracellular elevation of excitatory amino acids and increase in Ca^{2+} uptake in the injected area as well as loss of cholinergic projections to neocortex (Harkany *et al.* 2000). Thus, Aβ causes impairment of normal cholinergic innervation by dual mechanisms, as it also leads to a direct cholinomimetic activation of cortical tissue as described by Talantova *et al.* (2013) and Pirttimaki *et al.* (2013). This makes it no wonder that augmentation of cholinergic activity by cholinesterase inhibitors has only marginal therapeutic effects in Alzheimer's disease.

Effects of ketogenic diet

Over the last ~ 10 years, a ketogenic diet has been used with some effect in patients suffering from Alzheimer's disease (see below) or Parkinson's disease (Vanitallie *et al.* 2005; Hashim and VanItallie 2014). Veech *et al.* (2001) were the first to recommend a ketone-rich diet for treatment of these conditions; the suggested daily amount of β-hydroxybutyrate was relatively (but not extremely) high (100–150 g). Lower doses were used by the Henderson group, where Reger *et al.* (2004) demonstrated an acute beneficial effect of a single 40 mL oral dose of octanoate on memory in probable Alzheimer's patients with mild to moderate memory impairment (Fig. 2). The positive response was restricted to patients who tested negative for APOE4, whereas APOE4-positive patients showed no effect or even a minor deterioration (in spite of the more pronounced hypometabolism in APOE4 carriers described in section 2). Less than 15% of total calories were supplied from the medium-chain fatty acid octanoate, which is metabolized via acetoacetate/β-hydroxybutyrate. The serum β-hydroxybutyrate concentration only increased to ~ 0.5 mM, but there was a significant correlation between the increase and the improvement of memory (Fig. 2). The effects are moderate, but earlier treatment of individuals at risk for developing the disease might have greater therapeutic impact. A second clinical trial of the same compound showed identical effects (Costantini *et al.* 2008). Additional studies found memory-enhancing effects after 45 and 90 days of treatment (Henderson *et al.* 2009) in human patients. The beneficial effects persisted 14 days after termination of the administration in a study comprising 152 patients (Henderson and Poirier 2011), although they were reduced after the discontinuation of the drug. This study confirmed that the beneficial effect excluded APOE4 carriers, and it also showed that some single-nucleotide polymorphisms in the genes of interleukin 1B and of the insulin degrading enzyme played a role in determining the efficacy of the treatment. Insulin degrading enzyme degrades several short polypeptides including both insulin and Aβ (Miners *et al.* 2008). A commercial preparation with octanoate as the active ingredient (Axona) is now available on prescription.

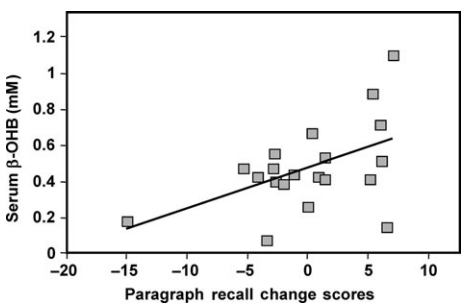

Fig. 2 Hydroxybutyrate levels at the time of cognitive testing and change in paragraph recall following acute treatment of Alzheimer's patients with octanoic acid. Each subject received 40 mL of an octanoate triglyceride preparation or placebo 90 min before the cognitive test and the determination of plasma β-hydroxybutyrate (mM). $r = 0.50$. $p = 0.02$. From Reger *et al.* (2004).

Another commercial preparation (from a different company), 'Fuel for Thought', consisting of coconut oil fortified with octanoate presently undergoes a clinical trial in Alzheimer's disease. The same company has submitted a patent application for a β-hydroxybutyrate ester, which has been found capable of producing plasma ketone body levels comparable to those achieved by rigorous ketogenic diet after oral administration (Hashim and VanItallie 2014). Following administration of a single dose of the ketone monoester plasma levels of β-hydroxybutyrate and acetoacetate were elevated, whereas the intact ester was not detected (Clarke *et al.* 2012). A case report showing improvement in a single Alzheimer's patient treated with this preparation (~ 29 g three times daily) has recently been published (Newport *et al.* 2015). The treatment was well tolerated and the patient improved markedly in daily activity performance, as evaluated by mood, affect, self-care and cognitive ability. Noticeable improvements in conversation and interaction was found at higher compared to lower plasma levels of β-hydroxybutyrate, which reached levels of ~ 5 mM 1 h after the administration but subsequently decreased rapidly (half-life 1–1.5 h).'Virgin' (cold-pressed) coconut oil is also a good source of medium-chain fatty acids but in addition contains some higher fatty acids (mainly C-12 and C-14), (Shilling *et al.* 2013) but no glucose. All the fatty acids are metabolized via the ketone body acetoacetate. Unfortunately none of the fatty acids is odd-numbered. This might have improved the effect as each odd-numbered fatty acid gives rise to one molecule of succinyl CoA, which enters the TCA cycle directly, providing a new molecule of a TCA cycle intermediate. This might compensate for the reduced

pyruvate carboxylase expression in Alzheimer brain. This concept is supported by the finding that supplementation of a ketogenic diet with triheptanoin, a triglyceride composed of three C-7 fatty acids, reduces the memory impairment in an Alzheimer mouse model and decreases astrocytic inflammatory responses (Aso *et al.* 2013).

None of these preparations 'cures' the neurodegenerative disease, but also Vanitallie (2013) pointed out that best results can be expected by early treatment before any neurodegeneration has occurred. Didic *et al.* (2011) similarly emphasized the importance of early diagnosis of Alzheimer's disease, because development of effective therapeutic agents requires reliable identification of patients when neuropathological changes in the brain are minimal. Although cognitive deficit, initially as isolated loss of ability to remember recent events (episodic memory) is generally the initial event leading to assessment of patients for Alzheimer's disease this can be a difficult task. This is because normal aging and mild cognitive impairment (MCI) also affect memory, but it may be possible to reveal differences from Alzheimer's disease in these conditions by psychological testing (Koen and Yonelinas 2014). Moreover, biomarkers for Alzheimer's disease (Aβ1-42, tau, phosphorylated tau in cerebrospinal fluid; expression of specific genes) are becoming of increased value and this field is constantly developing with the aim of enabling non-invasive and early detection of the disease (Humpel and Hochstrasser 2011).

As cognitive impairment is the catastrophic result of Alzheimer's disease, learning experiments in animals are of importance for evaluating the influence of ketone bodies on memory establishment and its mechanisms. In a mouse model of Alzheimer's disease, Kashiwaya *et al.* (2013) found that a diet where 21.5% of the calories had been switched from carbohydrate to a β-hydroxybutyrate ester improved performance in some conventional memory tests. Other learning experiments have been carried out after exposure to oligomeric Aβ1-42. It inhibits learning when injected intracerebrally in mice (Mishra *et al.* 2013) or into the avian equivalent of the cerebral cortex in day-old chickens, 45 min before training (Gibbs *et al.* 2009, 2010).

During learning in the day-old chicken neuronal glucose uptake and metabolism is essential immediately after training, whereas astrocytic glucose uptake is needed 30 min later (Gibbs *et al.* 2008). In addition, astrocytic pyruvate carboxylation also occurs immediately after training and is required for formation of glutamate, which is indispensable for learning (Gibbs and Hertz 2005). Based on this knowledge, Gibbs *et al.* (2009) investigated whether inhibition of learning caused by administration of Aβ1-42 to the avian equivalent of the mammalian cortex 45 min before training could be reversed by addition of different metabolic substrates (glucose, lactate, octanoate, β-hydroxybutyrate, and the astrocyte-specific substrate acetate). None of them had any effect when injected before the neuronal glucose demand immediately after training. However, memory loss after injection of Aβ1-42 was prevented when almost any of the metabolic substrates tested was injected 20 min post training. This is illustrated in Fig. 3(a) for acetate, a further indication that the provision of metabolic substrate rescued learning by an effect on astrocytes. This effect must be exerted on energy metabolism, not pyruvate carboxylation (see Fig. 1 and its legend), as β-hydroxybutyrate was effective and since lactate had no effect immediately after

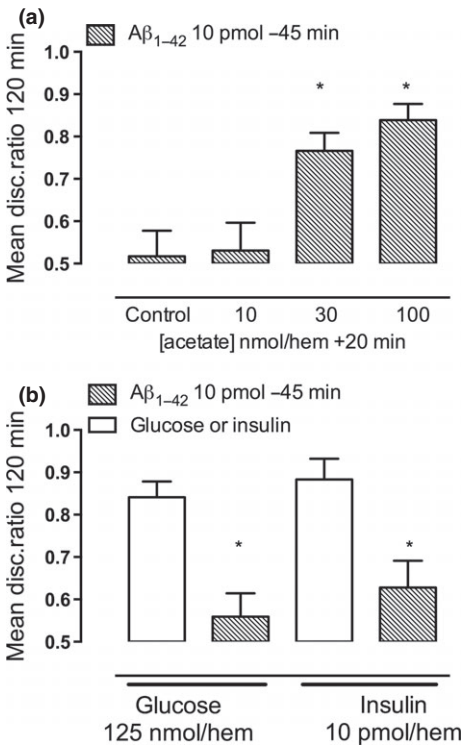

Fig. 3 Ability of acetate, but not of glucose or insulin to restore learning after its inhibition by Aβ1-42. (a) Effect of different doses of acetate injected at 20 min after training in day-old chicks treated with 10 pmol/hemisphere of freshly prepared Aβ1–42 45 min before aversive training, carried out by briefly exposing the chicks to beads of two colors, of which one but not the other was tainted with a drug of aversive taste. The ability to remember association between color and aversive or non-aversive taste of an offered bead was determined 120 min post training as the discrimination ratio between pecks on a bead of the previously aversive and the previously non-aversive color, of which neither was tainted. Perfect learning would result in a discrimination ratio of 1, and total inhibition would result in a ratio of 0.5. Normal learning generally results in a ratio of ~ 0.9, similar to that seen after administration of acetate. Results are means ± SEM for group sizes of 15–16, $*p < 0.005$ for difference from control (saline injection). Lactate, β-hydroxybutyrate and octanoic acid had similar effect as the high dose of acetate (data not shown). (b) Inability of glucose or insulin to restore learning in the Aβ-treated chickens, although both could restore memory impairment after weakly reinforced training, i.e., training on a diluted aversant (Glucose or Insulin). Group sizes of 15–16, $*p < 0.01$ for effect of Aβ. From Gibbs *et al.* (2009).

training. The only substrate that did not rescue memory after Aβ1-42 injection in the Gibbs experiments was glucose (Fig. 3b), which was interpreted as an inhibition of either glucose uptake or glycolysis by Aβ.

It has often been concluded that insulin signaling is impaired in Alzheimer brain (e.g., Hoyer 1991, 1998). However, insulin did not counteract the inhibition of learning in day-old chicken by Aβ1-42 (Fig. 3b), although it can rescue learning impaired by other means (Gibbs *et al.* 2009, 2010). This might be related to the absence of the insulin-sensitive glucose transporter GLUT-4 in chicken, although another insulin-stimulated transporter, GLUT-8 is present in chicken brain (Seki *et al.* 2003). An ability of increased glucose supply to cause some improvement of memory in Alzheimer's patients is contingent upon appropriate insulin function, but it is not known if this is because of a neuronal or astrocytic effect (Watson and Craft 2004). That the memory-enhancing effect is exerted on the brain is shown by the observation that intranasal administration of insulin in patients with mild cognitive impairment or Alzheimer's disease causes improvements in memory tasks and glucose metabolism (Hölscher 2014), although adult brain expresses little GLUT4 (Vannucci *et al.* 2000).

Potential mechanism(s) of action of ketogenic diets: supplementation of energy production or inhibition of glucose-dependent gliotransmitter function

The ketone bodies acetoacetate and β-hydroxybutyrate are of major nutritional importance in brain neonatally when their plasma concentrations are high and the blood–brain barrier expression of monocarboxylate transporters is substantial (Cremer *et al.* 1976). In adults their blood concentrations are normally ≤0.5 mM (Laffel 1999), i.e., 10 times lower than that of glucose, and the expression of their transporter into brain is greatly reduced. Nevertheless, ketone bodies at saturating concentrations can cover up to 60% of brain metabolism in the slightly anesthetized rat or normal human (but total metabolism in the deeply anesthetized animal), whereas the remaining 40% must be supplied by glucose (Owen *et al.* 1967; Chowdhury *et al.* 2014). Moreover, although cultured glutamatergic neurons metabolize both glucose and lactate avidly, only utilization of glucose, not that of lactate, is increased during NMDA-induced synaptic activity (Bak *et al.* 2006). Therefore, a very high intake of ketone bodies combined with reduced glucose intake may inhibit some aspects of brain function. On the other hand, less drastic supplementation of the diet with ketone bodies can support metabolism, including brain metabolism. The simplicity of ketone body metabolism, compared to that of glucose may be important for this function.

Fatty acids are metabolized in the liver to the ketone body acetoacetate, which is carried in blood, mainly after reduction to β-hydroxybutyrate, to the cells where it is used. Acetoacetate is metabolized intramitochondrially to two molecules acetyl coenzyme A, which enter the TCA cycle directly. β-Hydroxybutyrate is oxidized to acetoacetate, generating one molecule of NADH. This is similar to lactate oxidation to pyruvate with the exception that lactate oxidation occurs in the cytosol and competes with an oxidation occurring during glycolysis. In contrast to the simple metabolism of ketone bodies, glucose must first be converted in the cytosol to pyruvate by glycolysis (Fig. 1). This is a complicated process. One oxidation in the cytosol necessitates cytosolic-mitochondrial transport of reducing equivalents in the malate–aspartate shuttle. Oxidation of acetoacetate in the TCA cycle via acetyl CoA requires the presence of oxaloacetate, which can be deficient when glucose metabolism is severely inhibited, e.g., in diabetes. If that is the case ketone body accumulation leads to acidosis. Glucose metabolism is not compromised in Alzheimer's disease to a degree inhibiting ketone body metabolism as seen by the rapid decline in plasma β-hydroxybutyrate in the case study by Newport *et al.* (2015).

A strict 4 : 1 diet (grams of fat vs. grams of protein plus carbohydrate) has shown therapeutic effect in some epileptic children (Vining *et al.* 1998). A less restrictive more palatable diet based on medium-chain fatty acid triglycerides as well as a modified Atkins diet may also have some anti-epileptic effect (Liu and Wang 2013; Sharma and Jain 2014). However, additional seizure control can be obtained by switching from modified Atkins diet to the classical 4 : 1 ketogenic diet (Kossoff *et al.* 2010). β-Hydroxybutyrate levels in blood correlate with seizure control, and children with levels above 4 mM are significantly more likely to show decreased seizure frequency than those with lower levels (Gilbert *et al.* 2000).

Considering that fat oxidation provides twice the amount of calories per g as oxidation of glucose or amino acids, the strict ketogenic diet is extremely low in glucose, which might be a reason for its therapeutic effect. Such a profound reduction in carbohydrates may deliver insufficient glucose to maintain brain functions dependent upon a certain utilization of glucose. This includes provision of the main excitatory transmitter glutamate, which is essential for conscious brain function, including learning (Gibbs and Hertz 2005; Gibbs *et al.* 2007). Synthesis of glutamate occurs via the glutamine–glutamate cycle. The flux within this extremely active cycle amounts to 75% of total cortical glucose utilization (Sibson *et al.* 1998; Rothman *et al.* 2011). It is initiated by production of glutamate from glucose via the TCA cycle constituent α-ketoglutarate. This occurs in astrocytes but not in neurons, because neurons lack an enzyme, pyruvate carboxylase, required for net synthesis of TCA cycle constituents (Fig. 1). At least some glucose is required for the synthesis of glutamate. This is partly because the glutamate generated in glutamatergic neurons from

glutamine is released into the mitochondrial matrix and depends upon transport in a 'pseudo-malate-aspartate-cycle' to reach the cytosol (Palaiologos *et al.* 1989; Bak *et al.* 2008). The function of this cycle requires a concomitant oxidative process in the cytosol, generally pyruvate formation (reviewed by Hertz 2013; Verkhratsky *et al.* 2015). The energy derived from this glucose oxidation is not utilized in the glutamine–glutamate cycle but might be used for energizing synaptic activity, which requires glucose metabolism (Bak *et al.* 2006). This could be because the neuronal vesicular glutamate transporter requires glucose for optimum function (Ikemoto *et al.* 2003). The 4 : 1 ketogenic diet may therefore prevent or reduce seizures by compromising formation of the excitatory neurotransmitter glutamate. It is consistent with this hypothesis that replacement of glucose in the medium with β-hydroxybutyrate reduces availability of transmitter glutamate in cultured neurons (Lund *et al.* 2009). The inhibitory transmitter GABA is synthesized via astrocyte-generated glutamate which is also carried to neurons in the glutamine–glutamate (GABA) cycle. Nevertheless, GABAergic activity may be better maintained if cycle activity is impaired, because a fraction of precursor glutamate is formed directly from glutamine without entrance into mitochondria and decarboxylated to GABA (the direct pathway). Another fraction undergoes transamination to α-ketoglutarate in the same way as in glutamatergic neurons (the indirect pathway), although with some added complexity (Waagepetersen *et al.* 2001; Walls *et al.* 2010, 2011; Leke *et al.* 2011). Both pathways contribute to vesicular GABA and are involved in determining the GABAergic tone. Moreover, released GABA is to a considerable extent re-accumulated directly into GABAergic neurons (Schousboe *et al.* 2013; see, however also Patel *et al.* 2015), whereas almost all released glutamate is taken up by astrocytes (Danbolt 2001; Zhou and Danbolt 2013) and returned to neurons in the glutamine–glutamate/GABA cycle, again requiring concomitant glycolysis. Thus, inhibition of glutamate production during anti-epileptic treatment with ketone bodies would not be accompanied by a similar decrease in formation of the inhibitory transmitter GABA.

Some glutamate is also utilized in astrocytes, e.g., as a gliotransmitter released in response to receptor activation or for synthesis of glutathione. This glutamate is formed in a similar manner as astrocytic glutamate used for production of neurotransmitter glutamate and GABA, but obviously no glutamine–glutamate/GABA cycling is involved. The release of glutamate from astrocytes is orders of magnitude smaller than that from glutamatergic neurons (Vardjan *et al.* 2014), but it is important because the activated glutamate receptors are not located post-synaptically. This matters because stimulation of extrasynaptic glutamate receptors may jeopardize neuronal viability (Molokanova *et al.* 2014).

Whether astrocyte dysfunction and hypometabolism (Abeti *et al.* 2011) contribute to the neuronal malfunction in Aβ-treated hippocampal slices observed by Zilberter *et al.* (2013) remains unknown. A potentially decreased metabolism may seem in disagreement with the maintained or increased astrocytic energy metabolism found by Sailasuta *et al.* (2011) in Alzheimer's patients. However, it should be recalled that this study dealt with metabolism of acetate and therefore provides no information about glucose metabolism. Glucose metabolism would be especially sensitive to damage induced by increased PARP-1- (Abeti *et al.* 2011; Abeti and Duchen 2012). Unfortunately, no study seems to specifically show glucose metabolism in astrocytes of Alzheimer's patients or in animal models of the disease.

The astrocyte-specific rescue of Aβ1-42-learning shown in Fig. 3(a) by acetate could be consistent with the astrocyte-selective PARP-1 effect. However, preliminary experiments by D. Song, L. Hertz and L. Peng also confirmed effects on ATP synthase by Aβ (Schmidt *et al.* 2008; Xing *et al.* 2012) in cultured astrocytes which differed when the substrate was glucose and when it was pyruvate or β-hydroxybutyrate. Nevertheless, there is also the possibility that the rescue might be because of prevention of gliotransmitter release, shown to be deleterious in the presence of Aβ by Talantova *et al.* (2013) and Pirttimaki *et al.* (2013). The reason for this is that in the presence of glucose in the medium addition of lactate or glutamate (substrates that like β-hydroxybutyrate enter the TCA cycle directly) causes a rapid decrease in intracellular glucose concentration in astrocytes (Prebil *et al.* 2011). As already mentioned, glucose is essential for the vesicular glutamate transporter, which is also operating in astrocytes, and a drastic decrease in glucose concentration might reduce or abolish release of gliotransmitter glutamate and ensuing excitatory events. Aβ-induced transmitter release from microglia (Wu *et al.* 2004) might be affected in a similar manner and reduce inflammation, as transmitter release from microglia also can be vesicular (Imura *et al.* 2013).

Concluding remarks

Alzheimer's disease as well as some forms of epilepsy can be treated more or less effectively with ketogenic diet. In epilepsy, it appears advantageous to administer the highest possible fraction of the patients' calorie need in the form of ketone bodies, which may reduce glucose metabolism sufficiently to impair glutamate production in neurons. Much lower doses of ketone bodies can have therapeutic effect in Alzheimer's disease by different mechanisms. Enabling ketone bodies to supply a fraction of needed ATP may partly compensate for the deficiency in glucose metabolism in Alzheimer's patients. An alternative mechanism of action could be to prevent or reduce gliotransmitter release of glutamate. Stimulation from subcortical nuclei can induce gliotransmitter release besides decreasing inflammation and enhancing metabolism. The early destruction of these nuclei

has up till now provided little or no impetus for drug development, but the recent conclusions regarding their importance by Heiko Braak, a giant in Alzheimer research, may hopefully lead to a change.

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References

Abeti R. and Duchen M. R. (2012) Activation of PARP by oxidative stress induced by beta-amyloid: implications for Alzheimer's disease. *Neurochem. Res.* **37**, 2589–2596.

Abeti R., Abramov A. Y. and Duchen M. R. (2011) Beta-amyloid activates PARP causing astrocytic metabolic failure and neuronal death. *Brain* **134**, 1658–1672.

Abramov A. Y., Canevari L. and Duchen M. R. (2003) Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. *J. Neurosci.* **23**, 5088–5095.

Albrekkan F. M. and Kelly-Worden M. (2013) Mitochondrial dysfunction and Alzheimer's disease. *Open J. Endocr. Metab. Dis.* **3**, 14–19.

Aso E., Semakova J., Joda L., Semak V., Halbaut L., Calpena A., Escolano C., Perales J. C. and Ferrer I. (2013) Triheptanoin supplementation to ketogenic diet curbs cognitive impairment in APP/PS1 mice used as a model of familial Alzheimer's disease. *Curr. Alzheimer Res.* **10**, 290–297.

Aulwurm U. R. and Brand K. A. (2000) Increased formation of reactive oxygen species due to glucose depletion in primary cultures of rat thymocytes inhibits proliferation. *Eur. J. Biochem.* **267**, 5693–5698.

Azoulay-Zohar H., Israelson A., Abu-Hamad S. and Shoshan-Barmatz V. (2004) In self-defence: hexokinase promotes voltage-dependent anion channel closure and prevents mitochondria-mediated apoptotic cell death. *Biochem J.* **377**, 347–355.

Bak L. K., Schousboe A., Sonnewald U. and Waagepetersen H. S. (2006) Glucose is necessary to maintain neurotransmitter homeostasis during synaptic activity in cultured glutamatergic neurons. *J. Cereb. Blood Flow Metab.* **26**, 1285–1297.

Bak L. K., Zieminska E., Waagepetersen H. S., Schousboe A. and Albrecht J. (2008) Metabolism of [U-13C]glutamine and [U-13C] glutamate in isolated rat brain mitochondria suggests functional phosphate-activated glutaminase activity in matrix. *Neurochem. Res.* **33**, 273–278.

Bales K. R., Du Y., Dodel R. C., Yan G. M., Hamilton-Byrd E. and Paul S. M. (1998) The NF-kappaB/Rel family of proteins mediates Abeta-induced neurotoxicity and glial activation. *Brain Res. Mol. Brain Res.* **57**, 63–72.

Barger S. W. (2000) Production and functional assays of recombinant secreted amyloid precursor protein (APP) (sAPPalpha). *Methods Mol. Med.* **32**, 63–78.

Barger S. W., Horster D., Furukawa K., Goodman Y., Krieglstein J. and Mattson M. P. (1995) Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca^{2+} accumulation. *Proc. Natl Acad. Sci. USA* **92**, 9328–9332.

Bekar L. K., He W. and Nedergaard M. (2008) Locus coeruleus alpha-adrenergic-mediated activation of cortical astrocytes in vivo. *Cereb. Cortex* **18**, 2789–2795.

Blackburn R. V., Spitz D. R., Liu X., Galoforo S. S., Sim J. E., Ridnour L. A., Chen J. C., Davis B. H., Corry P. M. and Lee Y. J. (1999) Metabolic oxidative stress activates signal transduction and gene expression during glucose deprivation in human tumor cells. *Free Radic. Biol. Med.* **26**, 419–430.

Braak H. and Del Tredici K. (2011) The pathological process underlying Alzheimer's disease in individuals under thirty. *Acta Neuropathol.* **121**, 171–181.

Braak H. and Del Tredici K. (2013) Amyloid-beta may be released from non-junctional varicosities of axons generated from abnormal tau-containing brainstem nuclei in sporadic Alzheimer's disease: a hypothesis. *Acta Neuropathol.* **126**, 303–306.

Braak H., Braak E., Bohl J. and Lang W. (1989) Alzheimer's disease: amyloid plaques in the cerebellum. *J. Neurol. Sci.* **93**, 277–287.

Braak H., Thal D. R., Ghebremedhin E. and Del Tredici K. (2011) Stages of the pathologic process in Alzheimer disease: age categories from 1 to 100 years. *J. Neuropathol. Exp. Neurol.* **70**, 960–969.

Braun D., Madrigal J. L. M. and Feinstein D. L. (2014) Noradrenergic regulation of glial activation: molecular mechanisms and therapeutic implications. *Curr. Neuropharmacol.* **12**, 342–352.

Bubber P., Haroutunian V., Fisch G., Blass J. P. and Gibson G. E. (2005) Mitochondrial abnormalities in Alzheimer brain: mechanistic implications. *Ann. Neurol.* **57**, 695–703.

Busch C., Bohl J. and Ohm T. G. (1997) Spatial, temporal and numeric analysis of Alzheimer changes in the nucleus coeruleus. *Neurobiol. Aging* **18**, 401–406.

Camello-Almaraz C., Gomez-Pinilla P. J., Pozo M. J. and Camello P. J. (2006) Mitochondrial reactive oxygen species and Ca^{2+} signaling. *Am. J. Physiol. Cell Physiol.* **291**, C1082–C1088.

Castellano C. A., Nugent S., Paquet N., Tremblay S., Bocti C., Lacombe G., Imbeault H., Turcotte É., Fulop T. and Cunnane S. C. (2015) Lower brain 18F-fluorodeoxyglucose uptake but normal 11C-acetoacetate metabolism in mild Alzheimer's disease dementia. *J. Alzheimers Dis.* **43**, 1343–1353.

Chalermpalanupap T., Kinkead B., Hu W. T., Kummer M. P., Hammerschmidt T., Heneka M. T., Weinshenker D. and Levey A. I. (2013) Targeting norepinephrine in mild cognitive impairment and Alzheimer's disease. *Alzheimers Res. Ther.* **5**, 21.

Chan-Palay V. (1991) Alterations in the locus coeruleus in dementias of Alzheimer's and Parkinson's disease. *Prog. Brain Res.* **88**, 625–630.

Chen Y. and Hertz L. (1999) Noradrenaline effects on pyruvate decarboxylation: correlation with calcium signaling. *J. Neurosci. Res.* **58**, 599–606.

Chen Y., Zhao Z., Code W. E. and Hertz L. (2000) A correlation between dexmedetomidine-induced biphasic increases in free cytosolic calcium concentration and energy metabolism in astrocytes. *Anesth. Analg.* **91**, 353–357.

Chen N., Sugihara H., Sharma J., Perea G., Petravicz J., Le C. and Sur M. (2012) Nucleus basalis-enabled stimulus-specific plasticity in the visual cortex is mediated by astrocytes. *Proc. Natl Acad. Sci. USA* **109**, E2832–E2841.

Chowdhury G. M., Jiang L., Rothman D. L. and Behar K. L. (2014) The contribution of ketone bodies to basal and activity-dependent neuronal oxidation in vivo. *J. Cereb. Blood Flow Metab.* **34**, 1233–1242.

Clarke K., Tchabanenko K., Pawlosky R. *et al.* (2012) Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regul. Toxicol. Pharmacol.* **63**, 401–408.

Cole G. M., Masliah E., Shelton E. R., Chan H. W., Terry R. D. and Saitoh T. (1991) Accumulation of amyloid precursor fragment in Alzheimer plaques. *Neurobiol. Aging* **12**, 85–91.

Costantini L. C., Barr L. J., Vogel J. L. and Henderson S. T. (2008) Hypometabolism as a therapeutic target in Alzheimer's disease. *BMC Neurosci.* **9**(Suppl 2), S16.

Cremer J. E., Braun L. D. and Oldendorf W. H. (1976) Changes during development in transport processes of the blood-brain barrier. *Biochim. Biophys. Acta* **448**, 633–637.

Cunnane S., Nugent S., Roy M. *et al.* (2011) Brain fuel metabolism, aging, and Alzheimer's disease. *Nutrition* **27**, 3–20.

Danbolt N. C. (2001) Glutamate uptake. *Prog. Neurobiol.* **65**, 1–105.

Denton R. M. (2009) Regulation of mitochondrial dehydrogenases by calcium ions. *Biochim. Biophys. Acta* **1787**, 1309–1316.

Didic M., Barbeau E. J., Felician O., Tramoni E., Guedj E., Poncet M. and Ceccaldi M. (2011) Which memory system is impaired first in Alzheimer's disease? *J. Alzheimers Dis.* **27**, 11–22.

Duchen M. R. (1992) Ca(2+)-dependent changes in the mitochondrial energetics in single dissociated mouse sensory neurons. *Biochem. J.* **283**(Pt 1), 41–50.

Dukart J., Mueller K., Villringer A., Kherif F., Draganski B., Frackowiak R. and Schroeter M. L. (2013) Relationship between imaging biomarkers, age, progression and symptom severity in Alzheimer's disease. *Neuroimage Clin.* **3**, 84–94.

Elobeid A., Soininen H. and Alafuzoff I. (2012) Hyperphosphorylated tau in young and middle-aged subjects. *Acta Neuropathol.* **123**, 97–104.

Frackowiak R. S., Pozzilli C., Legg N. J., Du Boulay G. H., Marshall J., Lenzi G. L. and Jones T. (1981) Regional cerebral oxygen supply and utilization in dementia. A clinical and physiological study with oxygen-15 and positron tomography. *Brain* **104**, 753–778.

Garrison J. C. and Borland M. K. (1979) Regulation of mitochondrial pyruvate carboxylation and gluconeogenesis in rat hepatocytes via an alpha-adrenergic, adenosine 3':5'-monophosphate-independent mechanism. *J. Biol. Chem.* **254**, 1129–1133.

German D. C., White C. L., 3rd and Sparkman D. R. (1987) Alzheimer's disease: neurofibrillary tangles in nuclei that project to the cerebral cortex. *Neuroscience* **21**, 305–312.

Gibbs M. E. and Hertz L. (2005) Importance of glutamate-generating metabolic pathways for memory consolidation in chicks. *J. Neurosci. Res.* **81**, 293–300.

Gibbs M. E., Lloyd H. G., Santa T. and Hertz L. (2007) Glycogen is a preferred glutamate precursor during learning in 1-day-old chick: biochemical and behavioral evidence. *J. Neurosci. Res.* **85**, 3326–3333.

Gibbs M. E., Hutchinson D. S. and Summers R. J. (2008) Role of beta-adrenoceptors in memory consolidation: beta3-adrenoceptors act on glucose uptake and beta2-adrenoceptors on glycogenolysis. *Neuropsychopharmacology* **33**, 2384–2397.

Gibbs M. E., Gibbs Z. and Hertz L. (2009) Rescue of Abeta(1-42)-induced memory impairment in day-old chick by facilitation of astrocytic oxidative metabolism: implications for Alzheimer's disease. *J. Neurochem.* **109**(Suppl 1), 230–236.

Gibbs M. E., Maksel D., Gibbs Z., Hou X., Summers R. J. and Small D. H. (2010) Memory loss caused by beta-amyloid protein is rescued by a beta(3)-adrenoceptor agonist. *Neurobiol. Aging* **31**, 614–624.

Gilbert D. L., Pyzik P. L. and Freeman J. M. (2000) The ketogenic diet: seizure control correlates better with serum beta-hydroxybutyrate than with urine ketones. *J. Child Neurol.* **15**, 787–790.

Glodzik L., Rusinek H., Pirraglia E. *et al.* (2014) Blood pressure decrease correlates with tau pathology and memory decline in hypertensive elderly. *Neurobiol. Aging* **35**, 64–71.

Grudzien A., Shaw P., Weintraub S., Bigio E., Mash D. C. and Mesulam M. M. (2007) Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. *Neurobiol. Aging* **28**, 327–335.

Haglund M., Sjobeck M. and Englund E. (2006) Locus ceruleus degeneration is ubiquitous in Alzheimer's disease: possible implications for diagnosis and treatment. *Neuropathology* **26**, 528–532.

Hajek I., Subbarao K. V. and Hertz L. (1996) Acute and chronic effects of potassium and noradrenaline on Na+, K+-ATPase activity in cultured mouse neurons and astrocytes. *Neurochem. Int.* **28**, 335–342.

Harkany T., Abraham I., Timmerman W. *et al.* (2000) Beta-amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis. *Eur. J. Neurosci.* **12**, 2735–2745.

Hashim S. A. and VanItallie T. B. (2014) Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester. *J. Lipid Res.* **55**, 1818–1826.

Henderson S. T. and Poirier J. (2011) Pharmacogenetic analysis of the effects of polymorphisms in APOE, IDE and IL1B on a ketone body based therapeutic on cognition in mild to moderate Alzheimer's disease; a randomized, double-blind, placebo-controlled study. *BMC Med. Genet.* **12**, 137.

Henderson S. T., Bonafe M. and Johnson T. E. (2006) daf-16 Protects the nematode Caenorhabditis elegans during food deprivation. *J. Gerontol. A Biol. Sci. Med. Sci.* **61**, 444–460.

Henderson S. T., Vogel J. L., Barr L. J., Garvin F., Jones J. J. and Costantini L. C. (2009) Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. *Nutr. Metab. (Lond).* **6**, 31.

Hertz L. (1989) Is Alzheimer's disease an anterograde degeneration, originating in the brainstem, and disrupting metabolic and functional interactions between neurons and glial cells?. *Brain Res. Brain Res. Rev.* **14**, 335–353.

Hertz L. (2013) The glutamate-glutamine (GABA) cycle: importance of late postnatal development and potential reciprocal interactions between biosynthesis and degradation. *Front. Endocrinol.* **4**, 59.

Hertz L. and Dienel G. A. (2002) Energy metabolism in the brain. *Int. Rev. Neurobiol.* **51**, 1–102.

Hertz L., Yu A. C., Kala G. and Schousboe A. (2000) Neuronal-astrocytic and cytosolic-mitochondrial metabolite trafficking during brain activation, hyperammonemia and energy deprivation. *Neurochem. Int.* **37**, 83–102.

Hertz L., Lovatt D., Goldman S. A. and Nedergaard M. (2010) Adrenoceptors in brain: cellular gene expression and effects on astrocytic metabolism and [Ca(2+)]i. *Neurochem. Int.* **57**, 411–420.

Hertz L., Xu J., Song D., Du T., Li B., Yan E. and Peng L. (2015) Astrocytic glycogenolysis: mechanisms and functions. *Metab. Brain Dis.* **30**, 317–333.

Ho L., Fukuchi K. and Younkin S. G. (1996) The alternatively spliced Kunitz protease inhibitor domain alters amyloid beta protein precursor processing and amyloid beta protein production in cultured cells. *J. Biol. Chem.* **271**, 30929–30934.

Hölscher C. (2014) First clinical data of the neuroprotective effects of nasal insulin application in patients with Alzheimer's disease. *Alzheimers Dement.* **10**, S33–S37.

Hoyer S. (1991) Abnormalities of glucose metabolism in Alzheimer's disease. *Ann. N. Y. Acad. Sci.* **640**, 53–58.

Hoyer S. (1998) Is sporadic Alzheimer disease the brain type of non-insulin dependent diabetes mellitus? A challenging hypothesis. *J. Neural. Transm.* **105**, 415–422.

Humpel C. and Hochstrasser T. (2011) Cerebrospinal fluid and blood biomarkers in Alzheimer's disease. *World J. Psychiatry* **1**, 8–18.

Hyman B. T., Phelps C. H., Beach T. G. *et al.* (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement.* **8**, 1–13.

Ikemoto A., Bole D. G. and Ueda T. (2003) Glycolysis and glutamate accumulation into synaptic vesicles—role of glyceraldehyde phosphate dehydrogenase and 3-phosphogylcerate kinase. *J. Biol. Chem.* **278**, 5929–5940.

Imura Y., Morizawa Y., Komatsu R., Shibata K., Shinozaki Y., Kasai H., Moriishi K., Moriyama Y. and Koizumi S. (2013) Microglia release ATP by exocytosis. *Glia* **61**, 1320–1330.

Isopi E., Granzotto A., Corona C. *et al.* (2014) Pyruvate prevents the development of age-dependent cognitive deficits in a mouse model of Alzheimer's disease without reducing amyloid and tau pathology. *Neurobiol. Dis.* doi: 10.1016/j.nbd.2014.11.013. [Epub ahead of print].

Jantaratnotai N., Ryu J. K., Kim S. U. and McLarnon J. G. (2003) Amyloid beta peptide-induced corpus callosum damage and glial activation in vivo. *NeuroReport* **14**, 1429–1433.

Kashiwaya Y., Bergman C., Lee J. H., Wan R., King M. T., Mughal M. R., Okun E., Clarke K., Mattson M. P. and Veech R. L. (2013) A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. *Neurobiol. Aging* **34**, 1530–1539.

Koen J. D. and Yonelinas A. P. (2014) The effects of healthy aging, amnestic mild cognitive impairment, and Alzheimer's disease on recollection and familiarity: a meta-analytic review. *Neuropsychol. Rev.* **24**, 332–354.

Kossoff E. H., Bosarge J. L., Miranda M. J., Wiemer-Kruel A., Kang H. C. and Kim H. D. (2010) Will seizure control improve by switching from the modified Atkins diet to the traditional ketogenic diet? *Epilepsia* **51**, 2496–2499.

Laffel L. (1999) Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab. Res. Rev.* **15**, 412–426.

LaManna J. C., Harik S. I., Light A. I. and Rosenthal M. (1981) Norepinephrine depletion alters cerebral oxidative metabolism in the "active" state. *Brain Res.* **204**, 87–101.

Lambert M. P., Barlow A. K., Chromy B. A. *et al.* (1998) Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc. Natl Acad. Sci. USA* **95**, 6448–6453.

Lee Y. J., Galoforo S. S., Berns C. M., Chen J. C., Davis B. H., Sim J. E., Corry P. M. and Spitz D. R. (1998) Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J. Biol. Chem.* **273**, 5294–5299.

Lehmann M., Ghosh P. M., Madison C., Karydas A., Coppola G., O'Neil J. P., Huang Y., Miller B. L., Jagust W. J. and Rabinovici G. D. (2014) Greater medial temporal hypometabolism and lower cortical amyloid burden in ApoE4-positive AD patients. *J. Neurol. Neurosurg. Psychiatry* **85**, 266–273.

Leke R., Bak L. K., Iversen P., Sørensen M., Keiding S., Vilstrup H., Ott P., Portela L. V., Schousboe A. and Waagepetersen H. S. (2011) Synthesis of neurotransmitter GABA via the neuronal tricarboxylic acid cycle is elevated in rats with liver cirrhosis consistent with a high GABAergic tone in chronic hepatic encephalopathy. *J. Neurochem.* **117**, 824–832.

de Leon M. J., Ferris S. H., George A. E. *et al.* (1983) Positron emission tomographic studies of aging and Alzheimer disease. *AJNR Am. J. Neuroradiol.* **4**, 568–571.

Lim D., Ronco V., Grolla A. A., Verkhratsky A. and Genazzani A. A. (2014) Glial calcium signalling in Alzheimer's disease. *Rev. Physiol. Biochem. Pharmacol.* **167**, 45–65.

Lin A. P., Shic F., Enriquez C. and Ross B. D. (2003) Reduced glutamate neurotransmission in patients with Alzheimer's disease: an in vivo (13)C magnetic resonance spectroscopy study. *MAGMA* **16**, 29–42.

Liu Y. M. and Wang H. S. (2013) Medium-chain triglyceride ketogenic diet, an effective treatment for drug-resistant epilepsy and a comparison with other ketogenic diets. *Biomed. J.* **36**, 9–15.

Lund T. M., Risa O., Sonnewald U., Schousboe A. and Waagepetersen H. S. (2009) Availability of neurotransmitter glutamate is diminished when beta-hydroxybutyrate replaces glucose in cultured neurons. *J. Neurochem.* **110**, 80–91.

Magistretti P. J., Morrison J. H., Shoemaker W. J., Sapin V. and Bloom F. E. (1981) Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: a possible regulatory mechanism for the local control of energy metabolism. *Proc. Natl Acad. Sci. USA* **78**, 6535–6539.

Marien M. R., Colpaert F. C. and Rosenquist A. C. (2004) Noradrenergic mechanisms in neurodegenerative diseases: a theory. *Brain Res. Rev.* **45**, 38–78.

McGeer E. G., McGeer P. L., Akiyama H. and Harrop R. (1989) Cortical glutaminase, beta-glucuronidase and glucose utilization in Alzheimer's disease. *Can. J. Neurol. Sci.* **16**, 511–515.

McLean C. A., Cherny R. A., Fraser F. W., Fuller S. J., Smith M. J., Beyreuther K., Bush A. I. and Masters C. L. (1999) Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* **46**, 860–866.

Mesulam M., Shaw P., Mash D. and Weintraub S. (2004) Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. *Ann. Neurol.* **55**, 815–828.

Miners J. S., Baig S., Palmer J., Palmer L. E., Kehoe P. G. and Love S. (2008) Abeta-degrading enzymes in Alzheimer's disease. *Brain Pathol.* **18**, 240–252.

Mishra N., Sasmal D. and Singh K. K. (2013) Attenuating Abeta1-42-induced toxicity by a novel acetylcholinesterase inhibitor. *Neuroscience* **250**, 309–319.

Molokanova E., Akhtar M. W., Sanz-Blasco S., Tu S., Pina-Crespo J. C., McKercher S. R. and Lipton S. A. (2014) Differential effects of synaptic and extrasynaptic NMDA receptors on Abeta-induced nitric oxide production in cerebrocortical neurons. *J. Neurosci.* **34**, 5023–5028.

Moore R. Y. and Bloom F. E. (1979) Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annu. Rev. Neurosci.* **2**, 113–168.

Morris G., Anderson G., Dean O., Berk M., Galecki P., Martin-Subero M. and Maes M. (2014) The glutathione system: a new drug target in neuroimmune disorders. *Mol. Neurobiol.* **50**, 1059–1084.

Mosconi L., Brys M., Switalski R., Mistur R., Glodzik L., Pirraglia E., Tsui W., De Santi S. and de Leon M. J. (2007) Maternal family history of Alzheimer's disease predisposes to reduced brain glucose metabolism. *Proc. Natl Acad. Sci. USA* **104**, 19067–19072.

Mosconi L., Pupi A. and De Leon M. J. (2008) Brain glucose hypometabolism and oxidative stress in preclinical Alzheimer's disease. *Ann. N. Y. Acad. Sci.* **1147**, 180–195.

Mosconi L., Murray J., Tsui W. H. *et al.* (2014) Brain imaging of cognitively normal individuals with 2 parents affected by late-onset AD. *Neurology* **82**, 752–760.

Newport M. T., VanItallie T. B., Kashiwaya Y., King M. T. and Veech R. L. (2015) A new way to produce hyperketonemia: use of ketone ester in a case of Alzheimer's disease. *Alzheimers Dement.* **11**, 99–103.

Nilsen L. H., Witter M. P. and Sonnewald U. (2014) Neuronal and astrocytic metabolism in a transgenic rat model of Alzheimer's disease. *J. Cereb. Blood Flow Metab.* **34**, 906–914.

O'Donnell J., Zeppenfeld D., McConnell E., Pena S. and Nedergaard M. (2012) Norepinephrine: a neuromodulator that boosts the function of multiple cell types to optimize CNS performance. *Neurochem. Res.* **37**, 2496–2512.

Owen O. E., Morgan A. P., Kemp H. G., Sullivan J. M., Herrera M. G. and Cahill G. F., Jr (1967) Brain metabolism during fasting. *J. Clin. Invest.* **46**, 1589–1595.

Palaiologos G., Hertz L. and Schousboe A. (1989) Role of aspartate aminotransferase and mitochondrial dicarboxylate transport for release of endogenously and exogenously supplied neurotransmitter in glutamatergic neurons. *Neurochem. Res.* **14**, 359–366.

Pastorino J. G. and Hoek J. B. (2008) Regulation of hexokinase binding to VDAC. *J. Bioenerg. Biomembr.* **40**, 171–182.

Patel A. B., de Graaf R. A., Rothman D. L. and Behar K. L. (2015) Effects of GAT1 inhibtion by tiagabine on brain glutamate and GABA metabolism in the anesthetized rat in vivo. *J. Neurosci. Res.* doi: 10.1002/jnr.23548. [Epub ahead of print].

Pirttimaki T. M., Codadu N. K., Awni A., Pratik P., Nagel D. A., Hill E. J., Dineley K. T. and Parri H. R. (2013) alpha7 Nicotinic receptor-mediated astrocytic gliotransmitter release: Abeta effects in a preclinical Alzheimer's mouse model. *PLoS ONE* **8**, e81828.

Polakis P. G. and Wilson J. E. (1985) An intact hydrophobic N-terminal sequence is critical for binding of rat brain hexokinase to mitochondria. *Arch. Biochem. Biophys.* **236**, 328–337.

Prebil M., Vardjan N., Jensen J., Zorec R. and Kreft M. (2011) Dynamic monitoring of cytosolic glucose in single astrocytes. *Glia* **59**, 903–913.

Rasool C. G., Svendsen C. N. and Selkoe D. J. (1986) Neurofibrillary degeneration of cholinergic and noncholinergic neurons of the basal forebrain in Alzheimer's disease. *Ann. Neurol.* **20**, 482–488.

Readnower R. D., Sauerbeck A. D. and Sullivan P. G. (2011) Mitochondria, amyloid beta, and Alzheimer's disease. *Int. J. Alzheimers Dis.* **2011**, 104545.

Reger M. A., Henderson S. T., Hale C., Cholerton B., Baker L. D., Watson G. S., Hyde K., Chapman D. and Craft S. (2004) Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiol. Aging* **25**, 311–314.

Reiman E. M., Chen K., Alexander G. E., Caselli R. J., Bandy D., Osborne D., Saunders A. M. and Hardy J. (2005) Correlations between apolipoprotein E epsilon4 gene dose and brain-imaging measurements of regional hypometabolism. *Proc. Natl Acad. Sci. USA* **102**, 8299–8302.

Rothman D. L., De Feyter H. M., de Graaf R. A., Mason G. F. and Behar K. L. (2011) 13C MRS studies of neuroenergetics and neurotransmitter cycling in humans. *NMR Biomed.* **24**, 943–957.

Sailasuta N., Harris K., Tran T. and Ross B. (2011) Minimally invasive biomarker confirms glial activation present in Alzheimer's disease: a preliminary study. *Neuropsychiatr. Dis. Treat.* **7**, 495–499.

Sanganahalli B. G., Herman P., Behar K. L., Blumenfeld H., Rothman D. L. and Hyder F. (2013) Functional MRI and neural responses in a rat model of Alzheimer's disease. *NeuroImage* **79**, 404–411.

Saper C. B., German D. C. and White C. L., 3rd (1985) Neuronal pathology in the nucleus basalis and associated cell groups in senile dementia of the Alzheimer's type: possible role in cell loss. *Neurology* **35**, 1089–1095.

Savaki H. E., Kadekaro M., McCulloch J. and Sokoloff L. (1982) The central noradrenergic system in the rat: metabolic mapping with alpha-adrenergic blocking agents. *Brain Res.* **234**, 65–79.

Schmidt C., Lepsverdize E., Chi S. L., Das A. M., Pizzo S. V., Dityatev A. and Schachner M. (2008) Amyloid precursor protein and amyloid beta-peptide bind to ATP synthase and regulate its activity at the surface of neural cells. *Mol. Psychiatry* **13**, 953–969.

Schousboe A., Bak L. K. and Waagepetersen H. S. (2013) Astrocytic control of biosynthesis and turnover of the neurotransmitters glutamate and GABA. *Front. Endocrinol.* **4**, 102.

Seidel J. L., Faideau M., Aiba I., Pannasch U., Escartin C., Rouach N., Bonvento G. and Shuttleworth C. W. (2015) Ciliary neurotrophic factor (CNTF) activation of astrocytes decreases spreading depolarization susceptibility and increases potassium clearance. *Glia* **63**, 91–103.

Seki Y., Sato K., Kono T., Abe H. and Akiba Y. (2003) Broiler chickens (Ross strain) lack insulin-responsive glucose transporter GLUT4 and have GLUT8 cDNA. *Gen. Comp. Endocrinol.* **133**, 80–87.

Sharma S. and Jain P. (2014) The modified Atkins diet in refractory epilepsy. *Epilepsy Res. Treat.* **2014**, 404202.

Sheu K. F., Kim Y. T., Blass J. P. and Weksler M. E. (1985) An immunochemical study of the pyruvate dehydrogenase deficit in Alzheimer's disease brain. *Ann. Neurol.* **17**, 444–449.

Shilling M., Matt L., Rubin E., Visitacion M. P., Haller N. A., Grey S. F. and Woolverton C. J. (2013) Antimicrobial effects of virgin coconut oil and its medium-chain fatty acids on Clostridium difficile. *J. Med. Food* **16**, 1079–1085.

Shoshan-Barmatz V., Ben-Hail D., Admoni L., Krelin Y. and Tripathi S. S. (2014) The mitochondrial voltage-dependent anion channel 1 in tumor cells. *Biochim. Biophys. Acta* (Biomembranes) doi: 10.1002/jnr.23548. [Epub ahead of print].

Sibson N. R., Dhankhar A., Mason G. F., Rothman D. L., Behar K. L. and Shulman R. G. (1998) Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proc. Natl Acad. Sci. USA* **95**, 316–321.

Siman R., Card J. P., Nelson R. B. and Davis L. G. (1989) Expression of beta-amyloid precursor protein in reactive astrocytes following neuronal damage. *Neuron* **3**, 275–285.

Simic G., Stanic G., Mladinov M., Jovanov-Milosevic N., Kostovic I. and Hof P. R. (2009) Does Alzheimer's disease begin in the brainstem? *Neuropathol. Appl. Neurobiol.* **35**, 532–554.

Spencer D. (2014) Seizures and epileptiform activity in early Alzheimer disease: how hard should we be looking? *Epilepsy Curr.* **14**, 73–75.

Subbarao K. V. and Hertz L. (1990) Noradrenaline induced stimulation of oxidative metabolism in astrocytes but not in neurons in primary cultures. *Brain Res.* **527**, 346–349.

Subbarao K. V. and Hertz L. (1991) Stimulation of energy metabolism by alpha-adrenergic agonists in primary cultures of astrocytes. *J. Neurosci. Res.* **28**, 399–405.

Subbarao K. V., Richardson J. S. and Ang L. C. (1990) Autopsy samples of Alzheimer's cortex show increased peroxidation in vitro. *J. Neurochem.* **55**, 342–345.

Sun L., Shukair S., Naik T. J., Moazed F. and Ardehali H. (2008) Glucose phosphorylation and mitochondrial binding are required for the protective effects of hexokinases I and II. *Mol. Cell. Biol.* **28**, 1007–1017.

Takahashi J., Shibata T., Sasaki M., Kudo M., Yanezawa H., Obara S., Kudo K., Ito K., Yamashita F. and Terayama Y. (2015) Detection of changes in the locus coeruleus in patients with mild cognitive impairment and Alzheimer's disease: high-resolution fast spin-echo T1-weighted imaging. *Geriatr. Gerontol. Int.* **15**, 334–340.

Takata N., Mishima T., Hisatsune C., Nagai T., Ebisui E., Mikoshiba K. and Hirase H. (2011) Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity in vivo. *J. Neurosci.* **31**, 18155–18165.

Takita M., Mikuni M. and Takahashi K. (1992) Habituation of lactate release responding to stressful stimuli in rat prefrontal cortex in vivo. *Am. J. Physiol.* **263**, R722–R727.

Talantova M., Sanz-Blasco S., Zhang X. *et al.* (2013) Abeta induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proc. Natl Acad. Sci. USA* **110**, E2518–E2527.

Töpper R., Gehrmann J., Banati R., Schwarz M., Block F., Noth J. and Kreutzberg G. W. (1995) Rapid appearance of beta-amyloid precursor protein immunoreactivity in glial cells following excitotoxic brain injury. *Acta Neuropathol.* **89**, 23–28.

Vanitallie T. B. (2013) Preclinical sporadic Alzheimer's disease: target for personalized diagnosis and preventive intervention. *Metabolism* **62**(Suppl 1), S30–S33.

Vanitallie T. B., Nonas C., Di Rocco A., Boyar K., Hyams K. and Heymsfield S. B. (2005) Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study. *Neurology* **64**, 728–730.

Vannucci S. J., Rutherford T., Wilkie M. B., Simpson I. A. and Lauder J. M. (2000) Prenatal expression of the GLUT4 glucose transporter in the mouse. *Dev. Neurosci.* **22**, 274–282.

Vardjan N., Kreft M. and Zorec R. (2014) Regulated exocytosis in astrocytes is as slow as the metabolic availability of gliotransmitters: focus on glutamate and ATP. *Adv. Neurobiol.* **11**, 81–101.

Veech R. L., Chance B., Kashiwaya Y., Lardy H. A. and Cahill G. F., Jr. (2001) Ketone bodies, potential therapeutic uses. *IUBMB Life* **51**, 241–247.

Verkhratsky A., Nedergaard M. and Hertz L. (2015) Why are Astrocytes Important? *Neurochem. Res.* **40**, 389–401.

Ververken D., Van Veldhoven P., Proost C., Carton H. and De Wulf H. (1982) On the role of calcium ions in the regulation of glycogenolysis in mouse brain cortical slices. *J. Neurochem.* **38**, 1286–1295.

Vining E. P., Freeman J. M., Ballaban-Gil K., Camfield C. S., Camfield P. R., Holmes G. L., Shinnar S., Shuman R., Trevathan E. and Wheless J. W. (1998) A multicenter study of the efficacy of the ketogenic diet. *Arch. Neurol.* **55**, 1433–1437.

Vyssokikh M. Y. and Brdiczka D. (2003) The function of complexes between the outer mitochondrial membrane pore (VDAC) and the adenine nucleotide translocase in regulation of energy metabolism and apoptosis. *Acta Biochim. Pol.* **50**, 389–404.

Waagepetersen H. S., Sonnewald U., Gegelashvili G., Larsson O. M. and Schousboe A. (2001) Metabolic distinction between vesicular and cytosolic GABA in cultured GABAergic neurons using 13C magnetic resonance spectroscopy. *J. Neurosci. Res.* **63**, 347–355.

Walls A. B., Nilsen L. H., Eyjolfsson E. M., Vestergaard H. T., Hansen S. L., Schousboe A., Sonnewald U. and Waagepetersen H. S. (2010) GAD65 is essential for synthesis of GABA destined for tonic inhibition regulating epileptiform activity. *J. Neurochem.* **115**, 1398–1408.

Walls A. B., Eyjolfsson E. M., Smeland O. B., Nilsen L. H., Schousboe I., Schousboe A., Sonnewald U. and Waagepetersen H. S. (2011) Knockout of GAD65 has major impact on synaptic GABA synthesized from astrocyte-derived glutamine. *J. Cereb. Blood Flow Metab.* **31**, 494–503.

Watson G. S. and Craft S. (2004) Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. *Eur. J. Pharmacol.* **490**, 97–113.

Weinshenker D. (2008) Functional consequences of locus coeruleus degeneration in Alzheimer's disease. *Curr. Alzheimer Res.* **5**, 342–345.

Wu S. Z., Bodles A. M., Porter M. M., Griffin W. S., Basile A. S. and Barger S. W. (2004) Induction of serine racemase expression and D-serine release from microglia by amyloid beta-peptide. *J. Neuroinflammation* **1**, 2.

Xing S. L., Chen B., Shen D. Z. and Zhu C. Q. (2012) β-Amyloid peptide binds and regulates ectopic ATP synthase α-chain on neural surface. *Int. J. Neurosci.* **122**, 290–297.

Zhang Y. W. and Xu H. (2007) Molecular and cellular mechanisms for Alzheimer's disease: understanding APP metabolism. *Curr. Mol. Med.* **7**, 687–696.

Zhou Y. and Danbolt N. C. (2013) GABA and glutamate transporters in brain. *Front. Endocrinol.* **4**, 165.

Zilberter M., Ivanov A., Ziyatdinova S. *et al.* (2013) Dietary energy substrates reverse early neuronal hyperactivity in a mouse model of Alzheimer's disease. *J. Neurochem.* **125**, 157–171.

Zweig R. M., Ross C. A., Hedreen J. C., Steele C., Cardillo J. E., Whitehouse P. J., Folstein M. F. and Price D. L. (1988) The neuropathology of aminergic nuclei in Alzheimer's disease. *Ann. Neurol.* **24**, 233–242.