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REVIEW ARTICLE

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Less is more: Caloric regulation of neurogenesis and adult brain function

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Calorie intake is essential for regulating normal physiological processes and is fundamental to maintaining life. Indeed, both extremes of calorie intake result in increased morbidity and mortality. In this review, we discuss the effect of calorie intake on adult brain function, with an emphasis on the beneficial effects of mild calorie restriction. Recent findings relating to the regenerative and protective effects of the gastrointestinal hormone, ghrelin, suggest that it may underlie the beneficial effects of calorie restriction. We discuss the putative cellular mechanisms underlying the action of ghrelin and their possible role in supporting healthy brain ageing.

KEYWORDS
acyl-ghrelin, adult hippocampal neurogenesis, calorie restriction, diet-induced obesity

1 | INTRODUCTION

The energy status of an organism is tightly controlled around a set-point by hormonal and neural systems. These neuroendocrine checks and balances promote homeostasis, thereby ensuring an appropriate energy supply for the energetic demands of metabolism, physical activity, growth and repair.

Disturbances to this homeostasis result in multiple physiological impairments, including age-related conditions such as type 2 diabetes mellitus and obesity. Perhaps less well recognised are the consequences of metabolic dysfunction on neuronal health and cognition. Diet-induced obesity (DIO) is a risk factor for neurodegenerative diseases such as Parkinson's disease (PD) and dementia. Conversely, calorie restriction (CR) (ie, an approximate 30% reduction in calorie intake) protects against neurodegeneration and promotes cognitive function (Figure 1). Indeed, there is a growing body of work supporting the CR paradigm as a tool for promoting lifespan and, perhaps more importantly, healthspan.[1] However, the physiological mechanisms underlying these effects are not fully understood. Here, we review literature that identifies calorie intake as an important regulator of nerve cell function, with important implications for neurodegenerative disorders. Moreover, we describe recent research showing that CR promotes new neurone formation (neurogenesis) and neuroprotection in the adult brain and discuss the possible mechanisms that underpin this effect.

2 | CALORIE RESTRICTION REGULATES BRAIN FUNCTION

Calorie restriction, in the absence of malnutrition, has beneficial effects on brain function, including reducing the incidence of age-related neurodegenerative disease,[2] eliciting anti-depressant behaviour[3] and improving memory function in rodents.[4] In nonhuman primates, prolonged CR in adulthood decreases the incidence of age-related disease, including measures of brain atrophy and glucose regulation.[5-7] Translation of the carefully controlled CR experiments from preclinical models to humans is problematic. In particular, it is difficult to re-capitulate experimental controls such as genetic background and home environment (diet, temperature and lighting) that are standard in biomedical laboratory research. Nonetheless, in adult humans, a 3-month period of CR improved verbal memory function that correlated with decreases in plasma fasting insulin levels.[8]

The physiological mechanism(s) underlying these effects are not fully understood. However, research using rodents has demonstrated an important role for the cAMP response element binding protein (CREB) transcription factor in regulating the neuronal response to CR. In particular, CR activates CREB in the hippocampus, a brain region essential in the formation and maintenance of episodic memory.[9] CREB is known to regulate the expression of several genes; nevertheless, the precise targets that are responsible for enhanced memory function have not been fully clarified. One CREB target implicated in neuronal

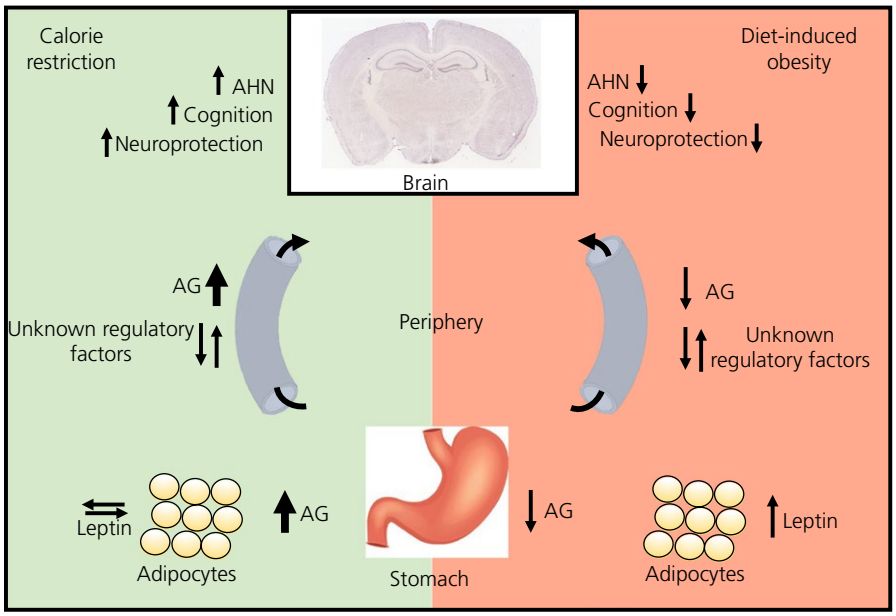

FIGURE 1 Schematic representation of physiological adaptations to calorie restriction (CR) and diet-induced obesity (DIO). The adipose-derived hormone, leptin, is reduced during CR, whereas acyl-ghrelin (AG) release from the stomach is increased and is associated with enhanced adult hippocampal neurogenesis (AHN), cognition and neuroprotection. Intriguingly, the opposite occurs during DIO because plasma acyl-ghrelin decreases in conjunction with a detrimental effect on AHN, cognition and neuroprotection. These observations suggest that ghrelin is a metabolic signal that is increased during times of energy need to promote brain function and support survival

plasticity and spatial memory function is the NAD^+-dependent histone de-acetylase, Sirtuin 1 (SIRT1). Fusco et al.[9] show that hippocampal CREB expression is increased by CR and is needed for subsequent increases in SIRT1 expression and memory function. Similarly, CR increased hippocampal SIRT1 activity, delayed neurone loss and maintained memory function in a mouse model of neurodegeneration.[2] Furthermore, administration of a SIRT1 activator (SRT3657) re-capitulated the beneficial effects of CR on neurone loss and memory function in this model.[2] CR also increases hippocampal levels of another CREB target, brain-derived neurotrophic factor (BDNF), in adult mice.[10] BDNF has well described neuroprotective and memory enhancing effects, suggesting that it may play an important role in the beneficial effects of CR. These molecular changes are associated with multiple cellular processes that may account for the beneficial effects of CR on brain function, and perhaps the most widely studied are autophagy and mitochondrial function.

2.1 | CR and autophagy

Autophagy is a process of removing and recycling damaged organelles and/or proteins within a cell. It is essential for protecting the cell from harmful oxidation products, maintaining cellular nutrient homeostasis and supplying an energy source during stress conditions. A loss of autophagy components can have detrimental effects on the ability to sustain life[11,12] and defects in autophagy have been linked to numerous neurodegenerative disorders, including Alzheimer's disease (AD),[13,14] PD,[15] Huntington's disease (HD),[16] prion diseases[17] and lysosomal storage diseases.[18] Indeed, the autophagy pathway is notably down-regulated and negatively correlates with AD progression.[19,20] More specifically, autophagy is particularly important in the maintenance of healthy neurones with defects linked to neuronal death.[17,21-23] Thus, the molecular pathways involved in autophagic regulation are considered as important therapeutic targets for a wide range of neurodegenerative diseases.

Calorie restriction is a form of mild stress that is known to induce autophagy.[24] However, the regulation of autophagy is organ-dependent and can vary significantly in response to starvation or high-calorie diets.[25,26] Short-term food deprivation (24-48 hours) increases the formation and/or accumulation of autophagosomes, which is indicative of augmented autophagy, in mouse cortical neurones[26] and neuronal cell lines (hypothalamic GT1-7 cells) (deprived of nutrients for 1-4 hours).[27] Evidence for some of the cellular pathways that are involved can be drawn from published literature (Figure 2).

Cellular mechanisms involved in CR-induced autophagy in neuronal cells are likely to involve Ca^{2+}/calmodulin-dependent protein kinase (CAMK) and AMP-activated protein kinase (AMPK). In an AD cell model (using SH-SY5Y cells), the formation of amyloid-β (AB)-induced autophagosomes was dependent on CAMK and AMPK.[28] This is supported by research in neutrophils[29] and multiple cell line models where the withdrawal of essential amino acids or serum starvation is reported to mediate autophagy via a CAMK-AMPK mechanism.[29,30] Also, leucine-rich repeat kinase 2 (LRRK2), a gene associated with PD, affects AMPK by interacting with CAMK, leading to an increase in autophagosomes.[31] Taken together, these findings support the theory that altering autophagy may be an effective form of treatment for neurodegenerative disease.[32]

Importantly, neurones are protected from degradation following increases in AMPK-mediated autophagy during CR.[33] AMPK can have multiple effects that lead to the induction of autophagy. It can directly activate autophagy-related proteins, including Unc-51 like autophagy activating kinase (ULK),[28,34] or indirectly by inhibiting mechanistic target of rapamycin (mTOR)[35] and/or activate SIRTs.[36]

Nutrient deprivation reduced mTOR activity (which is also likely to be CAMK-dependent[31]) and ultimately, increased autophagy in neurones.[26] CR is reported to decrease mTOR signalling, increasing the autophagic degradation of proteins and attenuating the symptoms of age-related neurodegenerative diseases.[37,38] More specifically, a comparison of hippocampi from control and AD patients revealed increased

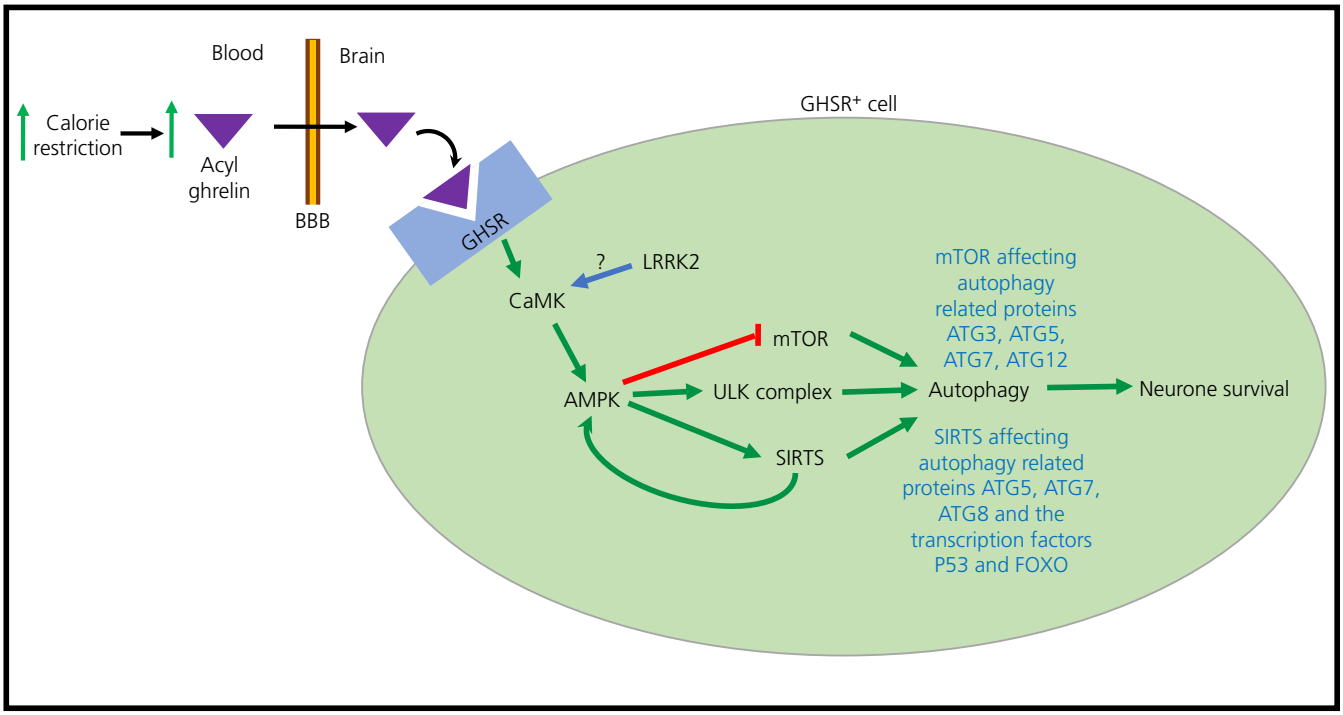

FIGURE 2 Schematic representation of putative cellular mechanisms triggered by calorie restriction (CR)-mediated autophagy leading to neuroprotection. CR increases circulating acyl-ghrelin, which crosses the blood-brain barrier (BBB) and binds growth hormone secretagogue receptor (GHSR), leading to activation of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) and AMP-activated protein kinase (AMPK)-mediated autophagy by up-regulating sirtuins (SIRTS) or the formation of the Unc-51 like autophagy activating kinase (ULK) complex. In addition, AMPK may inhibit mechanistic target of rapamycin (mTOR) to induce autophagy. ATG, autophagy-related; LRRK2, leucine-rich repeat kinase 2

levels of mTOR pathway proteins in patients with severe AD.[39] Also, in a murine model of AD lacking the mTOR gene, there is an increase in several autophagy-related (ATG) proteins, including ATG3, ATG5, ATG7 and ATG12.[40] Furthermore, in an in vitro PD model, over-expression of A53T α-synuclein up-regulates mTOR and impairs autophagy.[41]

Mice with mutations in the tubular sclerosis complex 1 (TSC1) or TSC2 genes (mutations within the mTOR pathway leading to its hyperactivity[35]) have decreased rates of autophagy in both the hippocampus and cortex.[42] However, these mice have a distinct neurodegenerative phenotype compared to mice that are deficient in the autophagosome components,[43,44] which suggests that there is an mTOR-independent pathway that can regulate autophagy. AMPK may be central to this mTOR-independent mechanism. These reports certainly suggest a link between reduced mTOR signalling in response to CR, autophagy and neurodegenerative disorders, which requires further investigation.

There is growing evidence implicating CR-induced SIRTs in the regulation of autophagy and neuroprotection. For example, the pro-longevity effect of CR-induced autophagy is SIRT1-dependent[45] and SIRT1 activated autophagy is neuroprotective in a prion disease primary cell model.[46] Furthermore, AB has been shown to suppress AMPK signalling in neuronal cells, leading to the down-regulation of SIRT1 and autophagy.[47] In neurones, the molecular mechanisms underpinning CR and SIRT regulated autophagy are not well understood. However, evidence from an alternative model supports the idea that a ketogenic style diet is likely to induce autophagy in a SIRT1-dependent manner.[48] Also, CR and AMPK are already known to increase up-regulation and activity of SIRTs.[36,49] Moreover, oligonol, a compound known to induce SIRTs, causes an increase in microtubule-associated protein 1 light chain 3 (LC-3) (a marker of autophagy) and phosphorylation of AMPK.[50] However, the interactions between AMPK and SIRTs are complex because SIRT1 can also increase the activity of AMPK,[51] suggesting that a positive-feedback loop may exist to either prolong or potentiate the activation of autophagy. SIRTs are reported to activate autophagy through their histone deacetylase activity. Indeed, several downstream targets of SIRTs regulate autophagy, including ATG5, ATG7 and ATG8,[45,52] as well as the transcription factors forkhead box (FOXO) and P53.[46,53,54] To add further complexity to interaction between AMPK and SIRTs, both can affect p53.[53,55] However, the detailed mechanisms linking CR-AMPK-SIRT and the autophagy pathway have yet to be investigated in the context of neuronal cells.

2.2 | CR and mitochondrial health

Mitochondria are dynamic organelles that produce energy in the form of ATP and assist in synaptic transmission.[56] Cycles of oxidative phosphorylation are required to generate ATP that is necessary for cellular function. However, the generation of ATP also results in the formation of reactive oxygen species (ROS) that cause cellular damage. Mitochondria change their shape in response to oxidative damage or changes in cellular energy demands through a process known as fission and fusion, which are key in maintaining mitochondrial health.[57,58] Sustaining the presence of healthy mitochondria may

attenuate the decrease in age and disease-related brain function. Indeed, the increased presence of abnormal "donut" shaped mitochondria has been linked to decreased age-related cognitive function in monkeys[59] and mitochondrial damage is likely to contribute to the pathophysiology of AD, PD and HD.[60-65] Furthermore, several proteins linked to mitochondrial function are mutated in familial forms of PD, including α-synuclein, PTEN-induced putative kinase 1 (PINK1), parkin, LRRK2, protein deglycase DJ-1 and high temperature requirement protein A2 (HTRA2).[66] Together, these studies suggest damage to multiple elements of the mitochondria, including proteins, lipids, DNA and extra-mitochondrial structures.[67] Thus, it is logical to suggest that treatments promoting a healthy mitochondrial shape and function, or increasing the removal of dysfunctional mitochondria, may be beneficial in slowing cognitive decline and neurodegeneration.

The rate of fission and fusion is continually altered in response to the fluctuating energy demands of the cell.[68] For example, low glucose conditions increase AMPK phosphorylation and fusion, whereas the absence of glucose leads to fission.[69,70] During mild stress, such as energy deficiency, mitochondria fuse (forming a tubular network) to maximise energy production. However, severe stress, such as starvation, increases the production of ROS, which damages DNA, proteins and lipids, and also impairs ATP production, resulting in mitochondrial fission. Intriguingly, fission was recently described as an AMPK-dependent process,[71] suggesting a mechanism by which AMPK may regulate both mitochondrial fission and fusion in response to fluctuations in energy supply and demand. Further research is needed to determine whether CR-mediated neuroprotection that is AMPK-dependent[72] regulates mitochondrial fission and fusion.

A malfunction at several points within the bioenergetics process within mitochondria could contribute to cellular damage and age-related neurodegenerative disorders. This includes reduced mitochondrial biogenesis[73] and ATP production,[74] deficient calcium buffering,[75] augmented production of free radicals and oxidative stress,[66,73,76] and reduced mitochondrial uncoupling (leading to a build up of H+ ions).[77-79] CR is reported to stop the age-related reduction of base excision repair in brain tissue.[80] However, base excision repair mechanisms are unchanged in the mitochondria of CR mouse brains,[81] suggesting that the beneficial effects of CR are not the result of an increase in the repair of mitochondrial DNA. CR contributes to the improvement of multiple aspects of mitochondrial health considered important for neuroprotection.[82] Also, it appears that CR-mediated mechanisms could ameliorate the age-associated dysfunction in cellular bioenergetics. Dysfunctional mitochondria in response to ageing and high-calorie diet is partly a result of the depletion of NAD+.[49] The addition of nicotinamide riboside (ie, a precursor of NAD+) to muscle stem cells can restore NAD+ and improve mitochondrial function in a process that is AMPK- and SIRT1-dependent.[49,83]

Insufficient clearance of dysfunctional mitochondria has been associated with PD[84] and other neurodegenerative disorders.[85] This process, termed mitophagy (a specific form of autophagy), is also affected by diet. Notably, mitophagy is defective in the kidneys of mice fed high-calorie diets where there is a significant increase in PINK1, a gene associated with PD that is an indicator of mitochondrial damage leading to the initiation of mitophagy.[86] Conversely, there is a reduction of PINK1 in CR mice, indicating that CR may attenuate mitochondrial damage. CR-mediated autophagy that is dependent on SIRT1 increases mitophagy,[87] whereas a loss of SIRT2 function leads to a dysregulation in mitophagy.[88] AMPK is also implicated in mitophagy via the activation of Ulk1.[34]

CR decreases levels of ROS in the rat brain[89] and reduces the amount of oxidative damage to mitochondrial DNA in liver.[90,91] Notably, inhibition of mTOR during CR reduces mitochondrial ROS in the brain.[92] CR can also reduce the mitochondrial membrane potential, which further attenuates oxidative damage.[93] CR may decrease the presence of ROS in neurones by increasing levels of the ROS scavengers cytochrome-C oxidase and citrate synthase.[94] In support of this, a SIRT1 activating compound, oligonol, increases expression of superoxide dismutase 2, a mitochondrial antioxidant.[50]

Although the multiple pathways and processes described above combine to determine the response of the cell to CR, it is still not clear how cells detect changes in whole organism energy balance. However, there is increased understanding of the role that circulating hormones play in mediating adaptations to changes in the environment. One such factor is the orexigenic gastrointestinal hormone, acyl-ghrelin. Acyl-ghrelin is synthesised in X/A-like cells of the gastric mucosa and travels to the brain via the circulation. Notably, its level in blood is elevated during CR.[3,72] Circulating ghrelin is transported across the blood-brain barrier (BBB) via a regulated process that is not fully understood[95] and then binds to the growth hormone secretagogue receptor (GHSR) within several brain regions, including the hippocampus.[96] GHSR is necessary for the anxiolytic effect of CR and exogenous treatment with acyl-ghrelin reduces anxiety behaviour[3] and also improves performance in spatial learning tasks.[96,97] Notably, the GHSR1a agonist, MK-0677, promotes the accumulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on excitatory hippocampal synapses and enhances long-term potentiation, a physical correlate of memory function.[98] Because acyl-ghrelin is the only known gut hormone that is increased in the blood during CR (others such as glucagon-like peptide-1 and cholecystokinin are elevated by feeding to induce satiety), it is uniquely positioned to relay CR-related information to the brain. These data suggest that acyl-ghrelin acts directly on hippocampal neurones to support mnemonic processes and may be important in regulating central adaptations to changes in energy balance.

Acyl-ghrelin mediates the neuroprotective effects of CR in an AMPK-dependent manner in an experimental model of PD.[72] Moreover, because AMPK and SIRTs increase mitochondrial biogenesis by regulating the activity of proliferator-activated receptor γ coactivator 1α (PGC-1α), acyl-ghrelin may have a similar effect.[99–101] More specifically, PGC-1α activation in response to acyl-ghrelin is AMPK-dependent,[102,103] and PGC-1α null mice are more prone to neuronal damage in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of PD.[73]

An increasing number of studies show that acyl-ghrelin also regulates the autophagic pathway. For example, in liver cells of CR acyl-ghrelin deficient (*Goat*$^{-/-}$) mice, there is a reduction in the

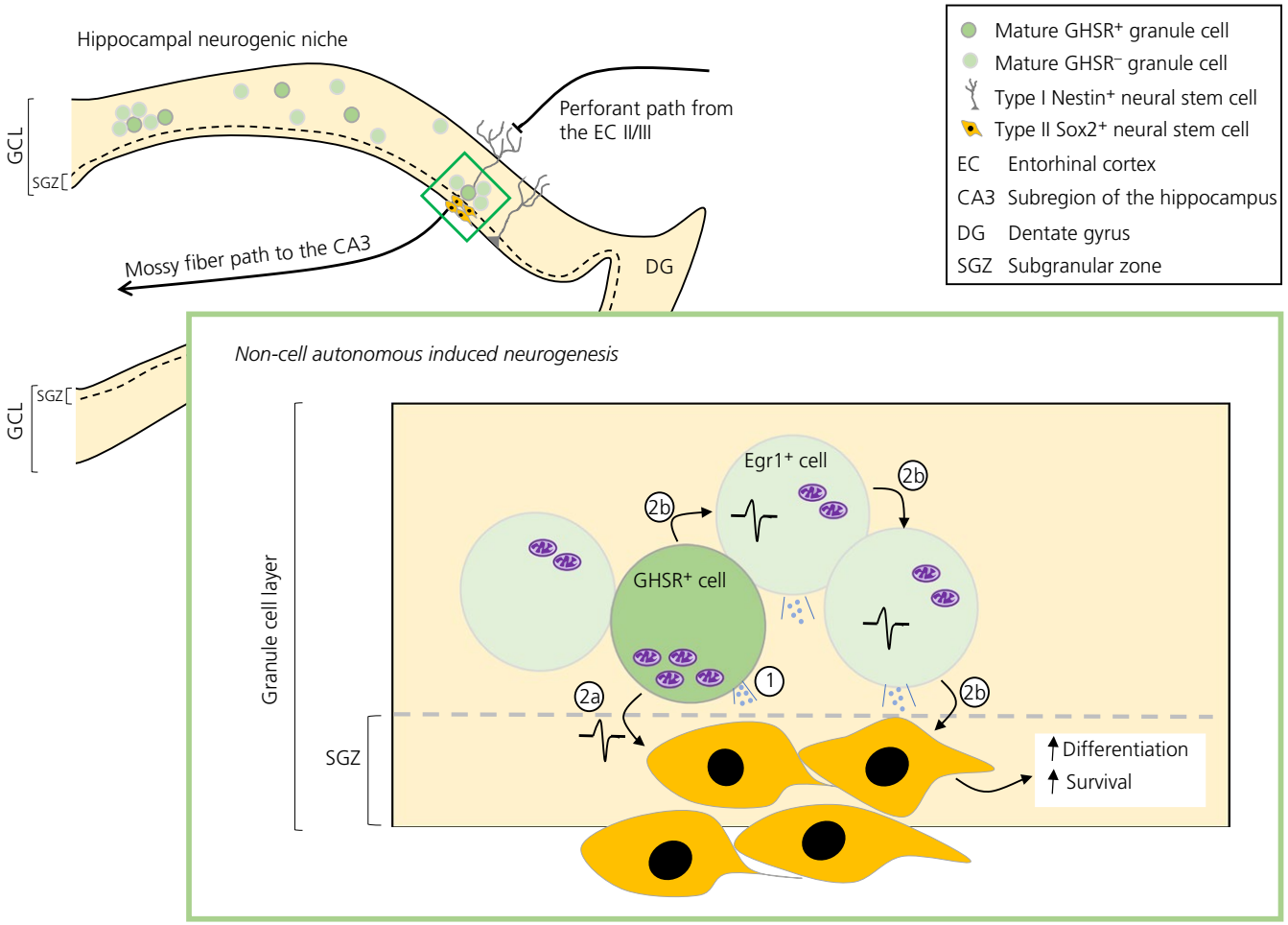

FIGURE 3 Schematic of putative cell-extrinsic mechanisms of acyl-ghrelin-mediated adult hippocampal neurogenesis (AHN). Because ghrelin-receptor, growth hormone secretagogue receptor (GHSR), is expressed in mature granule neurones of the dentate gyrus (DG) but not in neural stem/progenitor cells (NSPCs), we suggest acyl-ghrelin-mediated AHN is supported by: (1) induction of AHN following the release of soluble neurogenic and/or neurotrophic factors (ie, brain-derived neurotrophic factor [BDNF], glial cell-derived neurotrophic factor [GDNF] and fibroblast growth factor [FGF]; (2a) direct activity-induced AHN by which stimulation of $GSHR^+$ DG neurones results in depolarisation and activation of neighbouring type II NSPCs; or (2b) indirect activity-induced AHN by which stimulation of $GHSR^+$ DG cells results in activation of neighbouring DG cells (as indicated by expression of the immediate early gene, early growth response 1 [Egr1] and subsequent regulation of type II NSPCs via [1] and/or [2a]). GCL, granular cell layer

autophagosome protein LC3-II, demonstrating a decreased rate of autophagy.[104] The activity of various lysosomal enzymes, which are required for the breakdown of autophagosomes, is raised in the blood plasma of rabbits treated with acyl-ghrelin.[105] Also, acyl-ghrelin activates autophagy in carcinoma cells.[19] Importantly, a recent study provides evidence indicating that the protective properties of acyl-ghrelin within the brain may be mediated via autophagy. In AD-like cells, acyl-ghrelin decreased the activity of cathepsin B,[106] a key autophagy gene, whereas CR stimulates autophagy by increasing ghrelin protein in rat cortical neurones.[107] Notably, the anti-ageing effects of CR are dependent on SIRT1 activation via the ghrelin receptor,[108] suggesting a possible role for autophagy and/or mitophagy. Studies in various cell types indicate that CR-induced autophagy may occur via activation of the energy-sensing mTOR and AMPK pathways.[72,109] However, the exact range of cellular mechanisms induced by CR and acyl-ghrelin is largely unknown, particularly in the context of the aged brain.

It is important to note that the CR-mediated increase in acyl-ghrelin may also increase the expression of the other two peptides, unacylated-ghrelin and obestatin, that are encoded by the ghrelin gene. Unacylated-ghrelin, which represents approximately 80% of circulating ghrelin, can inhibit acyl-ghrelin signalling. For example, elevated unacylated-ghrelin prevents acyl-ghrelin-induced orexigenesis[110,111] and growth hormone (GH)-secretion.[112] On the other hand, unacylated-ghrelin does not share the protective effect of acyl-ghrelin in a mouse model of PD.[113] Intriguingly, the post-translational acylation of ghrelin by the enzyme, ghrelin-O-acyl transferase,[114] may provide a novel mechanism for tissue specific regulation of acyl-ghrelin levels.[115] In addition, although the role of the obestatin peptide remains unclear, it is distinct from acyl-ghrelin in certain contexts. For example, obestatin has an opposing effect on ingestive behaviour[116] and does not modulate GH secretion.[117] However, both peptides have beneficial effects on memory[118,119] and reduce toxicity to AB.[120] Given that obestatin does not cross the BBB,[121] its central mechanism

of action is likely to be distinct from that of acyl-ghrelin. Nonetheless, further studies are warranted to investigate the role these two peptides may play in CR-mediated effects within the brain.

3 | CALORIE RESTRICTION REGULATES ADULT HIPPOCAMPAL NEUROGENESIS

One process implicated in regulating anxiolytic and mnemonic behaviour is adult hippocampal neurogenesis (AHN). This is a form of ongoing plasticity that occurs throughout life involving the birth, differentiation and the maturation of new neurones in the adult mammalian dentate gyrus (DG). Decreased neurogenesis has been implicated in the pathogenesis of anxiety and depression,[122] as well as cognitive impairment[123] and dementia.[124,125] Recently, it was shown that AHN is essential for distinguishing similar but distinct contexts as a result of the laying down of non-overlapping memory traces;[126–129] this form of cognition, termed pattern separation, is impaired in anxiety disorders[130] and cognitive decline.[123] Notably, factors such as exercise[131,132] and environmental enrichment positively modulate the rate of AHN and performance in pattern separation-dependent cognitive tasks[126] and anxiety-related tests.[133] The molecular mechanisms underlying these effects are not well understood; however, recent research has identified the myokine, cathepsin B, as a causal factor in exercise-mediated AHN. Cathepsin B is secreted by skeletal muscle and crosses the BBB, resulting in increased levels of BDNF and doublecortin (a protein transiently expressed by immature neurones) and improved performance on a spatial memory task. Notably, plasma levels of cathepsin B were elevated following treadmill exercise in the mouse, rhesus monkeys and humans, and were also correlated with cognition in humans.[134] These findings demonstrate the importance of circulating factors to the regulation of neurogenesis and memory function.

A 3-month period of alternate day fasting, which resulted in less total calories being consumed, enhanced the level of hippocampal BDNF and promoted the survival of newborn cells in the hippocampus (Figure 3).[10] However, until recently, it was unclear whether these newborn cells matured into differentiated neurones or whether they integrated with hippocampal circuitry to modulate mnemonic processes. One factor that is increased in the circulation during CR is acyl-ghrelin and, as noted above, it readily crosses the BBB and binds to its receptor in the hippocampus.[96] Indeed, increasing circulating levels of acyl-ghrelin by exogenous administration was demonstrated to increase hippocampal cell proliferation[135] and adult ghrelin deficient mice showed reduced rates of new neurone differentiation that were restored to wild-type levels following acyl-ghrelin treatment.[136] These data demonstrate that supraphysiological doses of acyl-ghrelin improve aspects of hippocampal neurogenesis. However, more recently, we showed that 2 weeks of daily acyl-ghrelin injections, at a dose similar to plasma concentrations after a 24-hour fast, enhanced AHN and pattern separation memory performance when assessed 4 weeks after the first injection.[137] This treatment regime did not have a detrimental effect on the number of new progenitor cells or new astrocytes in the hippocampal niche and this suggests that acyl-ghrelin may mediate, at least in part, the neurogenic and cognitive enhancing effects of CR.

Subsequently, we demonstrated that the cognate receptor for ghrelin, GHSR, is expressed in mature granule cells of the DG.[127] Furthermore, elevating peripheral acyl-ghrelin, either by injection or CR, increases expression of the zinc finger transcription factor, early growth response 1 (Egr-1) in the DG.[138] Egr-1 is an immediate-early gene involved in mitogenesis and differentiation that has recently been implicated in increasing AHN in mice. Indeed, Egr-1 expression in mature DG neurones resulted in increased proliferation and neural differentiation of adult hippocampal stem cells (Figure 3).[139] Using a 2-week CR paradigm, paired with a 5-bromo-2-deoxyuridine pulse-chase approach, we showed that CR increased the subsequent generation of adult born mature neurones in a GHSR-dependent manner. CR did not affect on-going hippocampal cell proliferation,[140] but promoted neuronal differentiation and survival of newborn neurones.[138] Furthermore, the increase in hippocampal plasticity was accompanied by enhanced remote contextual fear memory, a mnemonic process associated with AHN.[141] Taken together, these results show that GHSRs mediate the beneficial effects of CR on AHN and memory, and suggest that circulating acyl-ghrelin enhance AHN via a neural stem cell (NSC)-extrinsic mechanism, possibly mediated by activation of Egr-1-dependent genes in mature DG neurones.

In support of these findings, a more prolonged CR, using a 3-month alternate day feeding paradigm, increased the survival of new hippocampal cells in wild-type but not ghrelin-KO mice, suggesting that ghrelin-signalling is important for the cell survival of new adult born cells. This effect was not likely mediated by BDNF because CR elevated BDNF levels in the hippocampus of both wild-type and ghrelin-KO mice.[142] Acyl-ghrelin regulates other factors involved in the neuronal responses to nutrient availability, including CREB,[143] SIRT1[144] and AMPK.[102] Indeed, acyl-ghrelin is essential for the neuroprotective action of CR via the induction of AMPK signaling in mid-brain dopamine neurones.[72] Notably, activity of hippocampal AMPK is increased in response to CR,[145] although it is unclear whether AMPK, a well described cellular nutrient sensor, contributes to CR and/or acyl-ghrelin-mediated AHN. Despite emerging evidence for acyl-ghrelin regulating CR pathways, including autophagy, mitochondria and ROS, the molecular and cellular processes regulating the hippocampal neurogenic niche and enhanced cognition, remain unknown.

Autophagy is up-regulated during cell differentiation in neuroblastoma cells and its inhibition prevents the differentiation of neurones, a process that is also regulated by mTOR.[146] Several autophagy-related proteins, including ambra1, beclin 1, ATG5, and calpain, have recently been implicated in regulating AHN.[147–149] Because CR and acyl-ghrelin regulate both autophagy and AHN, albeit in different brain regions, it is reasonable to suggest that CR and acyl-ghrelin-mediated regulation of AHN may be influenced by autophagy.

Similarly, mitochondrial dynamics are linked to neurogenesis via the process of fission/fusion[150] and blocking mitochondrial function prevents the differentiation and maturation of human mid-brain neurones.[151] Furthermore, loss of the mitochondrial fusion protein, MFN1/2,

disrupts mitochondrial dynamics and prevents the self-renewal capacity of neuronal stem cells, and also causes cognitive defects in mice.[150,152]

Although the damaging effects of ROS are well known, it is becoming evident that the level of ROS may also be important in regulating neurogenesis.[151,153] Indeed, mitochondrial bioenergetics regulate ROS upstream of nuclear factor-like 2, a key developmental gene for neural stem cells.[150]

Each of these factors may well contribute to CR/acyl-ghrelin-mediated AHN.[138] However, if we are to develop CR-mimetics with the aim of effectively treat age-related cognitive decline or neurodegeneration, then careful clarification of the cellular and molecular mechanisms underlying this paradigm is essential. Indeed, consideration needs to be given to what effect CR and/or chronic AG-treatment has over a lifetime. For example, does it prematurely deplete the NSC pool and exacerbate cognitive decline or does it replenish the NSC pool, preferentially remove senescent NSCs and slow age-related cognitive decline? Because acyl-ghrelin levels are reduced in ageing,[154] can restoring acyl-ghrelin levels rejuvenate neurogenesis and cognitive function in ageing and is there therapeutic potential for attenuating neurodegeneration? Research into these questions is now warranted.

4 | DIET-INDUCED OBESITY REGULATES BRAIN FUNCTION AND ADULT HIPPOCAMPAL NEUROGENESIS

Neurodegenerative disorders often display coexisting metabolic dysfunction, and there are several converging lines of evidence linking altered metabolism with an increased risk of developing AD and dementia.[155] Notably, a high-fat diet[156] and obesity[157] are associated with reduced circulating levels of acyl-ghrelin in rats and humans, respectively,[158] and DIO is known to cause ghrelin resistance in the hypothalamus and in reward processes,[159–162] thereby reducing the efficacy of acyl-ghrelin. In addition, DIO is associated with impaired memory function in rodents[163] and with an increased risk of dementia in humans.[164–166] Whether or not ghrelin resistance occurs in the hippocampus during DIO remains to be determined.

Moreover, because diets high in fat reduce neurogenesis,[167] it is tempting to speculate that a circulating factor may contribute to this effect. The adipose-derived hormone, leptin, is increased in obesity and therefore represents a candidate molecule for mediating a reduction in AHN. However, chronic 14-day administration of leptin significantly increased hippocampal cell proliferation without influencing neuronal differentiation or new cell survival.[168] It is unclear whether the leptin-mediated increase in dividing stem/progenitor cells affects cognitive function in any way. This apparently paradoxical finding excludes leptin as a likely factor mediating the obesity-induced reduction in neurogenesis; however, there is a possibility that long-term leptin treatment may deplete the neural progenitor pool and exacerbate age-related cognitive decline. Another caveat is that leptin regulation of AHN may be dependent on leptin receptor sensitivity and therefore its effect should be studied under CR and DIO conditions.

One intriguing possibility is that circulating acyl-ghrelin may regulate changes in AHN brought about during both CR and DIO. This hypothesis is supported by the well described reduction in circulating acyl-ghrelin by high-fat diet[158,169] and ghrelin levels are significantly reduced in human obesity.[157] In addition, the number of dividing hippocampal stem/progenitor cells and the rate of new neurone differentiation is reported as being reduced in ghrelin KO mice.[136] It is unclear whether the genetic ablation of ghrelin results in alteration of other aspects of the hippocampal neurogenic niche (ie, stem cell number, ratio of type I and type II stem cells, neurogenesis-dependent memory function). These questions need to be addressed, particularly in the context of DIO across the lifespan.

Interestingly, data have emerged suggesting the presence of a hypothalamic neurogenic niche, that is responsive to DIO, and may regulate energy balance (170). Given the role of circulating ghrelin in regulating orexigenic neurones in this region,[102] studies are needed to investigate whether the peptide modulates new neurone formation in the hypothalamus. Furthermore, other stem cell niches (eg, skeletal muscle) that are regulated by CR[171] should be examined to determine the extent to which acyl-ghrelin regulates stem cell dynamics and tissue function.

These data suggest an important mechanism by which elevating or reducing acyl-ghrelin in the circulation may improve or impair cognitive function, respectively, via the control of neurogenesis and/or neuroprotection. With established 'omic' technologies, it is possible to characterise the molecular events induced by acyl-ghrelin in distinct neuronal populations within the brain. These studies are now warranted to clarify the underlying mechanisms that hold promise for identifying modifiable lifestyle factors and novel therapeutic targets that might exert beneficial effects on the brain.

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