● PERSPECTIVE

Ghrelin is the metabolic link connecting calorie restriction to neuroprotection

Parkinson's disease (PD) is the second most common neurodegenerative disease whereby the number of diagnosed patients rises by 3–4% each year creating an ever-expanding social, medical and financial burden. Symptoms such as rigidity, postural instability and bradykinesia are due to diminished levels of dopamine within the brain. More specifically dopamine cell bodies are located in the substantia nigra (SN) and dopamine is released in the striatum. Motor dysfunction becomes evident with a 70–80% loss of dopamine in the striatum. The cause of PD is currently unknown and hence most treatments are aimed at minimizing symptoms not preventing the cause. However, it is known that metabolic status, more specifically calorie restriction is neuroprotective in PD (Bayliss and Andrews, 2013).

Calorie restriction (CR), or reducing calories without causing malnutrition is beneficial in a number of pathological conditions including diabetes, cancer and neurodegeneration. Primates with a chronic overall 30% reduction in food intake were found to be resistant to the MPTP model of PD (Maswood et al., 2004). This study highlights that CR is neuroprotective however, the difficulty to adhere to CR necessitates an alternate method to recapitulate the neuroprotective benefits of CR. Evidence from cells treated with serum from CR rats suggests that a hormonal factor improves mitochondrial function and cell viability (Lopez-Lluch et al., 2006). One hormone that is elevated with prolonged fasting and promptly falls post-prandially is ghrelin. Ghrelin plays a role in maintaining steady blood glucose levels during fasting and also has many other non-metabolic functions including enhanced learning and memory (Diano et al., 2006) and neuroprotection in PD (Andrews et al., 2009; Bayliss et al., 2016). For this reason we hypothesized that CR elicits neuroprotective actions in PD by elevating ghrelin levels in the bloodstream.

To address this hypothesis we used mice unable to produce the hormone ghrelin, reduced their calorie intake by 30% and exposed them to the MPTP model of PD. MPTP is used to selectively target dopaminergic neurons and inhibit complex I of the electron transport chain. This results in selective destruction of dopamine neurons thus mimicking the human condition. In normal mice (denoted Ghrelin WT) CR diminished the loss of dopamine neurons, enhanced dopamine turnover and reduced gliosis after MPTP. However, in mice lacking ghrelin (Ghrelin KO) CR had no neuroprotective effects. This data strongly implicates ghrelin as a metabolic hormone responsible for the neuroprotective actions of CR. Although this is the first study to show ghrelin mediated neuroprotective effects of CR in a mouse model of PD, it supports other research linking ghrelin with minimizing the negative consequences of CR. A recent study by Macfarlane (McFarlane et al., 2014) showed that adult ablation of ghrelin secreting cells did not affect blood glucose, body weight or food intake during ad libitum fed conditions. Only

when mice were calorie-restricted was an effect apparent in blood glucose regulation. This study in conjunction with ours provides support that ghrelin acts as a metabolic signal during CR to maintain adequate physiological and neurological function.

The next step was to identify the downstream targets of ghrelin. In the hypothalamus ghrelin increases AMPK activity (Andrews et al., 2008); whether or not ghrelin increases AMPK in the SN was unknown. AMPK is a sensor of cellular energy and enhances mitochondrial function in response to cellular stress, such as CR. We found elevated AMPK activity in the striatum in response to CR in Ghrelin WT mice, which was absent in Ghrelin KO mice indicating a connection between ghrelin and AMPK. Hence we hypothesised that ghrelin is neuroprotective in PD via increased AMPK activity. To address this we measured AMPK levels in both cultured dopaminergic neurons and mice given a single dose of ghrelin. We found a robust increase in response to either ghrelin or a ghrelin agonist in cultured dopaminergic neurons. Acute *in vivo* injection of ghrelin increased AMPK activity in the SN but not the striatum in C57bl6 mice. This is the first *in vivo* study linking ghrelin with AMPK activity in the midbrain. We believe the discrepancy between area specific activation of AMPK in response to CR or ghrelin injection is due to time dependent activation. Chronically high ghrelin levels, as seen in CR Ghrelin WT mice activates the ghrelin receptor, GHSR, to activate AMPK and send it to areas of high energy demand, *i.e.* the striatum where dopamine is released. The single dose of ghrelin had insufficient time to bind to GHSR, activate AMPK and propagate to the striatum, hence only an elevation in the SN was observed. In further support of this argument GHSR is abundantly expressed on the dopamine cell bodies in the SN with little or no expression in the striatum (Zigman et al., 2006; Mani et al., 2014).

Collectively our data has shown that AMPK in SN dopaminergic neurons is a molecular target of ghrelin during CR. To determine if AMPK was essential for the neuroprotective actions of ghrelin we generated a novel mouse line where AMPKβ1 and β2 was successfully deleted in DAT expressing neurons (AMPK KO). These mice were unable to activate AMPK selectively in dopaminergic neurons. We injected both AMPK WT and KO mice daily with ghrelin over 2 weeks and exposed them to MPTP. Mice with functional AMPK activity (denoted AMPK WT) given ghrelin and MPTP had a diminished loss of dopaminergic cell number, enhanced dopamine turnover and reduced gliosis compared to the mice given saline. The protective actions of ghrelin were ablated in AMPK KO mice. As a proof of principle AMPK WT had elevated AMPK activity in response to ghrelin and the stressor MPTP whereas AMPK KO mice did not respond. This result clearly establishes AMPK as a critical molecular mechanism mediating the neuroprotective actions of ghrelin on dopaminergic neurons. Collectively these studies indicate a direct pathway between CR, ghrelin and AMPK in dopamine neurons to elicit neuroprotective properties in a mouse model of PD (**Figure 1**).

This fundamental breakthrough in PD research now allows further studies looking into AMPK activators in dopaminergic neurons to recreate CR without the need to minimise caloric consumption. Indeed AMPK activators such as the CR-mimetic Resveratrol and Metformin elicit neuroprotective
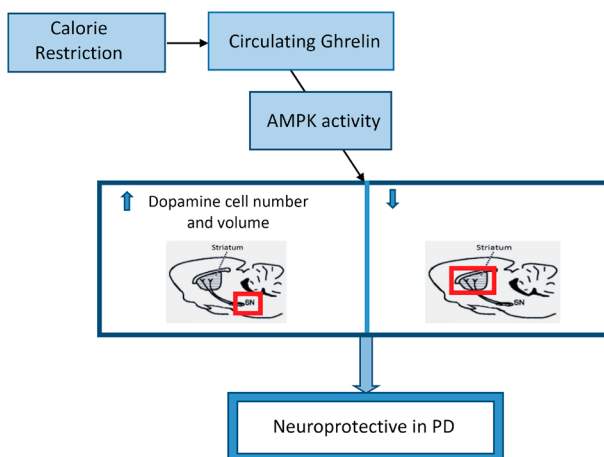
Figure 1 Calorie restriction results in ghrelin mediated AMPK activation, ultimately leading to neuroprotection in Parkinson's disease (PD).
AMPK: AMP-activated protein kinase.

actions in models of PD. As AMPK activity diminishes with age (Reznick et al., 2007) and ghrelin's function diminishes with age (Englander et al., 2004) we propose that the ability of CR to maintain AMPK activity in a ghrelin-dependent manner may restrict age-related decline in the dopaminergic neurons. In further support of this argument PD patients also have reduced postprandial ghrelin levels (Unger et al., 2010). As a therapeutic option elevated AMPK activity is an ideal target. AMPK activators such as Resveratrol and Metformin are both orally administered and well tolerated in the general public. However, our recent works show that the neuroprotective actions of metformin are independent from AMPK activity in DAT neurons. Future research should focus on combination therapy with already approved PD therapeutics with AMPK activators to both minimise symptoms and treat disease progression.

In conclusion, CR is perhaps the most reproducible strategy to increase mean and maximal lifespan while promoting healthy ageing. However, the exact mechanism for how this occurs is currently unknown. It is thought to involve altered stress pathways, changes in signalling pathways as well as alterations in metabolic hormones such as ghrelin and insulin. We believe that the minor stress placed on the system due to CR promotes mitochondrial health creating a direct beneficial effect in PD. However the poor compliance within society to adhere to CR necessitates an alternative approach to recapitulate the benefits without having to minimise the calories. We have discovered a novel pathway whereby CR enhances circulating ghrelin to play a neuroprotective role in the dopaminergic neurons *via* enhanced AMPK activity. Future research should focus on exploiting this pathway in order to recreate the beneficial effects of CR without needing to adhere to strict dietary regimes. This study is not only limited to neuroprotective actions in PD but also could have the potential to minimise other neurodegenerative disease and enhance overall healthy ageing.

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