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Dietary restriction supports peripheral nerve health by enhancing endogenous protein quality control mechanisms

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Synopsis

The peripheral nervous system (PNS) comprises of an extensive network of connections that convey information between the central nervous system (CNS) and peripheral organs. Long myelinated nerve fibers are particularly susceptible to age-related changes, as maintenance of the insulating glial membrane requires extensive synthesis and processing of many proteins. In rodent models, peripheral demyelination caused by genetic risk factors or by normal aging are attenuated by intermittent fasting (IF) or calorie restriction (CR) supporting a role for dietary intervention in preserving neural function. This review will summarize recent studies examining mechanisms by which life-long CR or extended IF supports peripheral nerve health.

Peripheral nerves are vulnerable to aging-related degeneration

Peripheral nerves are responsible for bringing information to and from the body and the central nervous system (CNS). The neuronal cell bodies of most peripheral nerves are located within the CNS or clustered in ganglia along the spinal cord, and extend long axons that innervate distant targets such as the skin and muscles. To protect these long projections the axons are wrapped in multiple layers of glial membranes called myelin. In the peripheral nervous system (PNS) Schwann cells provide this wrapping, with each glial cell forming a single internodal myelin segment. Non-myelinating Schwann cells are also present in the PNS and these cells ensheath presynaptic nerve terminals, which are the sites for communication between neurons and target cells.

As seen in other tissues, the cells of the PNS, including Schwann cells, are susceptible to age-associated insults such as DNA damage, oxidative stress, perturbed energy homeostasis, and accumulation of damaged proteins. Furthermore, since it is believed that neurons with long processes are particularly prone to aging-related impairment (Mattson and Magnus, 2006), the extensive lengths of peripheral axons makes these cells exceptionally vulnerable. Indeed, aging-related peripheral neuropathy that is not associated with a specific underlying disease, such as diabetes or a known hereditary risk factor, is estimated to affect as much as 22% of people aged 60–74 years, and up to 58% of people aged 85 years and older (Mold et al., 2004). Neurological deficits caused by altered peripheral nerve function include slowed nerve conduction velocity, difficulties with walking and balance, decreased muscle strength

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and sensory discrimination (Ceballos et al., 1999). It is likely that the combined effects of environmental insults and genetic factors, as well as dietary and life style choices contribute to age-associated changes in peripheral nerve function.

Morphological changes in peripheral nerves with aging are detectable both within the cell bodies of neurons and Schwann cells, as well as along axons. Similar to other tissues, aging in the PNS is associated with increased generation of reactive oxygen species as a by-product of cellular respiration, which can impact DNA, membranes, lipids, and proteins. On light microscopic examination, peripheral nerves of aged rodents show segmental demyelination, myelin thinning, axonal degeneration and regeneration, Schwann cell proliferation and axonal swelling (Grover-Johnson and Spencer, 1981). Within the neuronal cell bodies, decreased efficiency in degradative mechanisms over time is manifested as an accumulation of oxidized proteins and lipids in granules known as lipofuscin (Gray and Woulfe, 2005). These structures are pathological signatures of aged cells, also called age pigment, and originate from cumulative inefficiency of multiple protective, repair and clearance mechanisms (Terman and Brunk, 2004). Oxidized lipids and proteins are also detected within Schwann cells of aged peripheral nerves (Opalach et al., 2010) along with other abnormalities such as infolded myelin and collagen pockets (Grover-Johnson and Spencer, 1981). Associated with the gross morphological alterations, subcellular compartments are also affected and include widening of the nodes of Ranvier (Rangaraju et al., 2009), which are critical for efficient signal propagation along axons. Alterations in subcellular axonal cytoskeletal elements and decreased efficiency in axonal transport with aging have also been described (Stokin and Goldstein, 2006), which can impair retrograde transport and deprive the neurons of target-derived trophic support.

The described morphological changes are most pronounced in distal nerve segments which are in close proximity to the contact sites with the target cells, such as skeletal muscle. The junction between a peripheral axon and a skeletal muscle cell is a specialized synapse, the neuromuscular junction (NMJ). In the neuromuscular research community it is well-recognized that aging impacts the morphological and functional aspects of NMJs. Abnormalities in the morphology of NMJs include axon terminal thinning and sprouting, widening of the synaptic cleft and fragmentation of the postsynaptic sites (Valdez et al., 2010). These changes are progressive and begin to occur around 18 months of age in normal mice (Valdez et al., 2010). In an attempt to reverse or slow these events, mice were subjected to one month of exercise (wheel running) beginning at 22 months, which led to the reversal of some synaptic abnormalities. In the same study, the authors examined NMJs from 24 month old life-long calorie restricted animals and found that the morphological alterations at the presynaptic terminals and postsynaptic motor end plate due to aging were significantly blunted (Valdez et al., 2010). The mechanism for these pronounced effects of reduced calorie diet on NMJ structure is unclear, but it could involve improvements in electrophysiological activity. In agreement with this possibility, a recent study demonstrated that maintaining drosophila on a low calorie diet is associated with an increase in synaptic vesicle release by motor neurons and improved vigor, and better motor functions (Rawson et al., 2012). These studies provide evidence for the concept that reducing calorie intake enhances neuronal processes, including synaptic activity (Martin et al., 2006).

While early signs of disease and degeneration in aging are often reported at distal sites of the PNS, Schwann cells along the entire length of the nerve are susceptible to aging-associated deterioration. Starting at the level of gene activation, recent studies have identified transcriptional alterations in aged Schwann cells, with the most significant changes occurring in genes encoding proteins that regulate lipid metabolism or the inflammatory response (Verdier et al., 2012). Indeed, damage to myelin is associated with an elevated immune response, and infiltration of circulating macrophages was observed in nerves of

aged, as well as neuropathic rodents (Misko et al., 2002; Opalach et al., 2010). Subcellular pathways in Schwann cells that are affected with age include cell cycle-associated proteins, cytosolic chaperones and components of the autophagy-lysosomal pathway (Rangaraju et al., 2009). These deficiencies affect protein homeostasis and negatively impact the quality of myelin, as detected by morphological as well as biochemical analyses. Alterations in these pathways result in reduced ability of Schwann cells to process functionally important proteins (Fortun et al., 2005), while accumulating damaged or misfolded molecules (Opalach et al., 2010), a feature observed in multiple organisms (Grune et al., 2004).

Protein homeostatic mechanisms are critical in myelinating Schwann cells

To prevent the accumulation of misfolded/damaged proteins within the cytosol, mammalian cells utilize three mechanisms: 1) degradation by the ubiquitin-proteasome system (UPS); 2) suppression of aggregate formation by heat shock proteins (HSPs), also known as chaperones, and 3) clearance of abnormal cytosolic contents through autophagy. In agreement with findings in non-neural cells, our studies suggest that these three pathways are similarly critical for Schwann cell function, including myelin formation and maintenance, as well as for supporting axonal biology (Fortun et al., 2005; Opalach et al., 2010; Rangaraju et al., 2009). Moreover, these cellular processes are vulnerable to inefficiencies with aging and disease states. In nerves of aged rats, or mice with genetic defects linked to hereditary neuropathies, we found that impairment in these mechanisms lead to the accumulation of protein aggregates and nerve demyelination (Fortun et al., 2003; Fortun et al., 2006; Rangaraju et al., 2009).

The UPS is the primary mechanism for the removal and degradation of short-lived proteins, as well as for incomplete and/or otherwise damaged polypeptides. Decreased activity in UPS-mediated proteolysis with aging is observed in many cell types including neurons (Keller et al., 2000), where UPS function is necessary for maintaining activity-dependent plasticity through dendritic membrane remodeling (Hamilton et al., 2012). Schwann cells are similarly sensitive to changes in UPS function as this pathway is responsible for maintaining the levels of essential myelin proteins, including myelin basic protein (MBP) and peripheral myelin protein 22 (PMP22) (Akaishi et al., 1996; Notterpek et al., 1999). This is a particular challenge for Schwann cells, as dosage-sensitive, hydrophobic proteins such as PMP22 have high propensity to aggregate, which in turn can decrease the activity of the UPS (Fortun et al., 2005). In addition, the proteasome regulates cytosolic and receptor-protein kinases by terminating their activity through ubiquitination and degradation. Normal myelin formation and myelin repair involve a number of protein kinases including AKT, Erbs, and the Src-family of tyrosine kinases, whose activities are terminated through removal by the proteasome (Lu and Hunter, 2009). Thus, decreased UPS function may lead to irregular kinase activity and alter temporally regulated cell signaling events that are required for myelin maintenance and repair during aging.

During the course of our studies of peripheral nerve biology in normally aged rats and in neuropathic mice, we have detected an increase in protein aggregation and an associated decrease in UPS activity, as revealed by elevated levels of undegraded poly-ubiquitinated (pUb) substrates (Fortun et al., 2006; Fortun et al., 2005; Opalach et al., 2010). These findings are consistent with recent work that revealed a role for proteasome function in preventing age-related metabolic disorders, including obesity and hepatic steatosis (Tomaru et al., 2012). Abnormal cytosolic aggregates can absorb chaperones and other essential cellular proteins, and exert toxicity by the inability of affected cells to respond to subsequent stresses. Therefore, approaches to enhance proteasome function in aged cells and in protein misfolding diseases are of great interest, however are proving to be a challenge. Therefore, we and others have rather focused on preventing the accumulation of undegraded/damaged

molecules either by dietary intervention (Opalach et al., 2010; Rangaraju et al., 2009) or by enhancing the function of chaperones and/or the autophagy-lysosomal pathway through pharmacological modulation (Fortun et al., 2007; Madorsky et al., 2009; Rangaraju et al., 2008; Rangaraju et al., 2010).

Because of the ability of HSPs to aid protein processing and prevent misfolding, the chaperone pathway provides a viable approach to slow age-associated changes within cells. Indeed, activation of chaperones has been shown to be protective in a variety of organisms and cell types, and multiple mechanisms by which increased levels of chaperones promote protein quality control have been identified (Hartl et al., 2011). In neurons, increasing HSPs through pharmacological activation is neuroprotective by reducing harmful protein aggregation in diseases such as Huntington's, Parkinson's and Amyotrophic Laterosclerosis (Neef et al., 2010). Chaperones are also critical in myelinating glial cells in the CNS, as genetic inactivation of HSPs through deletion of the transcription factor heat shock factor 1 (HSF1), results in demyelination (Homma et al., 2007). In agreement with this finding, chaperones are necessary for myelination by Schwann cells, as they assist newly-synthesized proteins to achieve proper folding conformations. This role is particularly essential during membrane expansion and for the processing of hydrophobic myelin proteins such as PMP22 that have a high propensity for misfolding (Fortun et al., 2007). Upon misfolding, HSPs can prevent aggregate formation by refolding and/or promoting clearance by the UPS and/or the autophagy-lysosomal pathway. In Schwann cells from neuropathic mice with PMP22 mutations, chaperone activation is associated with an improvement in the processing of PMP22 through the secretory pathway and increased myelin synthesis (Rangaraju et al., 2008). This data supports the notion that activating HSPs improves subcellular conditions that promote the folding and processing of myelin-associated proteins.

During normal aging, the availability of HSPs is generally reduced and culture studies with Schwann cells indicate that the efficiency of activating the chaperone response upon heat shock (20 min, at 45°C) (Rangaraju et al., 2009) or pharmacologic treatment (unpublished results) is muted. Still, in cultures from young neuropathic animals the activation of chaperones through pharmacological inhibition of HSP90 has been proven to be effective in enhancing the ability of Schwann cells to form myelin (Rangaraju et al., 2008). Current studies in our laboratory are aimed at determining if the positive results from the *in vitro* studies will translate to functional improvement in affected mice. One challenge for the development of effective small molecule therapies for the PNS concerns the delivery of the compounds in high enough quantities to distal target sites. Therefore, dietary restriction provides an attractive alternative for proof-of-concept studies as both life-long calorie restriction (CR) and intermittent fasting (IF) have been shown to be effective inducers of chaperones (Martin et al., 2006). Although the molecular mechanisms that are modulated by CR or IF are complex, a major cellular stress response involves the upregulation of the chaperone, HSP70 (Martin et al., 2006). Indeed, increased expression of HSP70 has been consistently observed in various tissues from calorie-restricted animals, including in peripheral nerves of rats (Rangaraju et al., 2009).

When the UPS and/or chaperones are unable to prevent protein aggregation, such undegraded proteasome substrates can be removed from cells by autophagy. Macroautophagy refers to a bulk degradation pathway that sequesters cytosolic substrates into double membrane autophagosomes and subsequently delivers them to lysosomes for proteolysis (Mizushima, 2007). In addition, proteins with specific amino acid recognition motifs maybe selectively channeled from the cytoplasm directly into lysosomes by chaperone-mediated autophagy (Kaushik and Cuervo, 2012). More recently, an emerging body of evidence has identified receptor proteins that recognize both ubiquitin and

autophagy proteins for ubiquitin-mediated autophagy clearance, as an alternative mechanism for the degradation of UPS substrates (Kraft et al., 2010). Still, with increasing age, progressive accumulation of damaged proteins and lipids is observed within Schwann cells and nerves, likely as a reflection of declining efficiencies in UPS and autophagic degradation (Opalach et al., 2010).

In general, the essential role of autophagy for neural health has been clearly demonstrated in mice by genetic suppression of key autophagy genes Atg5 or Atg7 (Hara et al., 2006; Komatsu et al., 2006). These studies showed that maintaining protein homeostasis via autophagy is essential for neuronal survival as loss of autophagy led to accumulation of polyubiquitinated inclusions and severe neurodegeneration, followed by death within 6–8 weeks after birth. In affected mice, the loss of autophagy caused pronounced neuropathic phenotypes with motor deficits, ataxia, tremors, and limb clasping starting at 2 weeks of age (Hara et al., 2006; Komatsu et al., 2006). This developmental period in rodents corresponds with robust peripheral myelin synthesis and strongly supports a critical role for autophagy in peripheral nerve biology. More recently, a direct role for autophagic protein turnover in Schwann cell myelination has been demonstrated *in vitro* using rapamycin (Rangaraju et al., 2010), which is a macrolide antibiotic that activates autophagy by inhibition of the mammalian target of rapamycin (mTOR). Significantly, the beneficial effects of rapamycin relied on the expression of a key autophagy gene Atg12, thus verifying the important role for this pathway in myelin synthesis by Schwann cells. One mechanism by which autophagy may facilitate myelin synthesis by Schwann cells from neuropathic mice is by clearing the cytosol of misfolded proteins to create a more conducive environment for processing of newly-synthesized proteins (Fortun et al., 2003; Fortun et al., 2007). Unexpectedly, rapamycin treatment also aided myelin formation by normal Schwann cells, which may have involved enhanced protein delivery to the plasma membrane (Rangaraju and Notterpek, 2011). These *in vitro* findings in cultures from normal mice are in agreement with the above mentioned genetic ablation studies on autophagy and its critical role in peripheral nerve development and function (Hara et al., 2006; Komatsu et al., 2006). Conversely, with aging, a decrease in autophagy-lysosomal mechanisms can increase the cellular toxicity of damaged proteins, disrupt glial biology and impact overall nerve health. In Schwann cells isolated from 25 month-old rats, the expression of essential proteins for autophagy activation and autophagosome-lysosome (completion) fusion events were significantly reduced (Rangaraju et al., 2009). The molecular changes underlying these impairments are likely complex and involve transcriptional, translational as well as post-translational events. Therefore, effective approaches to restore autophagy in aged cells will require targeting a number of repair and protective mechanisms.

Calorie restriction delays aging-associated peripheral nerve degeneration

The anti-aging effects of CR have been studied in a variety of species and multiple tissues, including the nervous system (Martin et al., 2006). Starvation, or calorie restriction are effective inducers of degradative mechanisms, particularly autophagy (Mizushima, 2007). Upon nutrient deprivation, autophagy activation is a robust response in a wide range of tissues including liver, muscle and heart (Mizushima et al., 2004), which provides raw materials through degradative mechanisms and supports vital functions. Therefore, autophagic activity may be central to the protective effects of reduced caloric intake. CR may also reverse the negative consequences of dietary fats, which are known to cause dysfunction in chaperone-mediated autophagy (Rodriquez-Navarro et al, 2012). For neurons, the activation of autophagy in response to nutrient deprivation has been demonstrated most notably in hypothalamic neurons, where starvation-induced autophagy regulates food intake and energy balance (Kaushik et al., 2011). In addition, the downstream effects of CR also illicit adaptive response to energy deficits and increased mitochondrial

function leading to decreased oxidative stress. Although the precise mechanisms for neuroprotection are not fully understood, CR has been shown to mitigate or reverse many deleterious effects of aging on CNS functions, including learning and memory. For example, in aged rats, life-long CR prevented memory deterioration (Pitsikas and Algeri, 1992); and deficits in hippocampal long-term potentiation and NMDA receptor expression (Eckles-Smith et al., 2000), which represent the cellular building blocks of learning and memory.

Recent studies demonstrate that the effects of CR are extended to the PNS and offer protection against aging-related insults. In a large collaborative study, we asked whether lifelong CR in male Fisher 344 × Brown Norway rats can support the activity of subcellular protein quality control mechanisms and slow aging-related biological changes in myelinated peripheral nerves (Opalach et al., 2010; Rangaraju et al., 2009). The rats in this study were either given food *ad libitum* (AL) or maintained on a calorie restricted diet (60% of AL food intake) starting at 4 months of age. Sciatic nerves were analyzed at four time points, including 8-, 18-, 29- and 38-months of age. As predicted from previous studies that report numerous alterations in axonal biology during aging (Stokin and Goldstein, 2006), peripheral nerves in normally aged AL-fed rats displayed degenerative features including reduction in myelin proteins, increased dispersion of axonal nodal ion channels and changes in the expression of key neurofilament proteins. These degenerative changes were associated with the de-differentiation of Schwann cells and an increase in immune response. In comparison, peripheral nerves from CR-rats displayed remarkably preserved nerve architecture and minimal indication of degenerative events, such that the levels of myelin proteins and axonal constituents were similar to younger nerves (Rangaraju et al., 2009).

To investigate the contribution of protein quality control mechanisms in preserving nerve health in the intervention group, we analyzed the expression of chaperones, autophagy-related proteins as well as indicators of proteasomal degradation (Opalach et al., 2010; Rangaraju et al., 2009). In aged AL-fed rodents, we detected an increase in the steady-state expression of heat shock response regulatory proteins, particularly HSP90, a binding partner of HSF1. Somewhat unexpected is the finding that the levels of individual chaperones did not appear to change much within the same nerve lysates; however their localization within the cells may have been affected. Similarly, in samples from AL-fed rats we found an age-associated gradual increase in lysosomal and autophagic proteins, yet the nerves contained high levels of polyubiquitinated UPS substrates and lipofuscin-like aggregates (Opalach et al., 2010). Significantly, the nerves from diet restricted rats showed leveled expression of the studied molecules up to 29 months of age, and even at 38-months the changes were muted. These results strongly suggest that life-long CR is effective in slowing the intracellular buildup of misfolded or damaged proteins in myelinated nerves by supporting the efficiency of protein quality control mechanisms. In the same study we reported alterations in the response of cultured Schwann cells from 25 month-old AL-fed rats to starvation and heat shock challenges, which support an age-associated decline in the efficiency of these pathways. In agreement, recent studies in liver cells have shown that aging or dietary fats cause malfunction in the UPS (Tomaru et al., 2012) or chaperone-mediated autophagy (Rodriguez-Navarro et al., 2012). How dietary fats affect protein degradative mechanisms in peripheral nerves is unknown, however exogenous lipids are critical for myelination (Salvati et al., 2002). For translational consideration, these studies also revealed that analyses of steady–state protein levels are not necessarily a good indication of pathway activity, certainly not in the case of the chaperone and the autophagy-lysosomal systems. Future studies will determine if Schwann cells isolated from aged or neuropathic rodent nerves after long-term CR have an increased capacity to respond to stressors.

Intermittent fasting alleviates neuropathic phenotype and demyelination in a mouse model of hereditary neuropathy

Approaches to enhance endogenous cellular protective mechanisms may offer benefits to affected individuals with aging-related or genetically-linked neurodegenerative disorders. Even though it might not be feasible for human applications, cycles of intermittent fasting and feeding are known to induce mild stress on the organism, and enhance the activity of protective mechanisms (Martin et al., 2006). While on this feeding schedule, rodents consume about the same amount of calories in a 48 hour period as animals on an AL diet, therefore their overall calorie intake is not reduced (Martin et al., 2006). As a proof-of-principle experiment, we assigned a cohort of Trembler J neuropathic mice to either AL or IF diet at two months of age at which point affected mice show pronounced neuropathic features, including trembling and reduced ability to stay on the rotarod (Madorsky et al., 2009). Trembler J mice carry a point mutation in PMP22 and are used as a model of hereditary demyelinating neuropathy of Charcot-Marie-Tooth disease type IA (Fortun et al., 2003). Intermittently fed neuropathic mice received food AL on every other day and consumed about the same amount of total chow as AL-fed littermates over a 48 hour period. After five months on the IF regimen, neuropathic mice showed significant improvement in performance on the rotarod, and increased forepaw grip strength relative to their AL-fed counterparts (Madorsky et al., 2009). This data on functional improvement in neuropathic mice is in agreement with a more recent study in a spinal cord injury model where IF promoted post-injury motor recovery (Jeong et al., 2011).

The beneficial effects observed in the motor function of the IF-fed neuropathic mice were attributed to an overall improvement in nerve morphology and preservation of axonal integrity and myelin proteins (Madorsky et al., 2009). Besides the increased expression of myelin proteins, we detected elevated levels of autophagic and lysosomal proteins in sciatic nerves from IF mice. The enhanced expression of degradative molecules was paralleled with a reduction in the pathologic accumulation of PMP22-aggregates and in poly-ubiquitinated proteasome substrates. While we did not directly measure UPS activity, the reduction in the levels of poly-ubiquitinated proteins suggests an improvement in proteasomal function. Similar to the findings in nerves of CR-fed aged rats (Opalach et al., 2010), samples from diet restricted neuropathic mice showed attenuation of secondary degenerative events; including macrophage infiltration and Schwann cell proliferation. These results indicate that IF can slow degenerative events originating from the expression of a mutant myelin protein and thereby attenuate loss of axonal and neuromuscular function.

Additional considerations for slowing age-associated deficits in the PNS

As mentioned above, for neural tissue health maintenance with age, an interaction between degradative and protective protein quality control pathways becomes increasingly important. In mice with genetic defects linked to neuropathies, compensatory repair mechanisms and activation of alternative degradation pathways appear to help Schwann cell function and slow degenerative changes (Madorsky et al., 2009). While we detected similar improvements in peripheral nerve structure in the CR and the IF study, it is unknown if a five month long IF regimen could reverse age-associated degenerative events in middle-aged or older rodents. It is also unknown if an extended CR diet would slow the progression of neuropathies and other hereditary neurodegenerative disorders. While our studies have focused on examining the effects of these dietary interventions on protein homeostatic pathways, we found additional, overlapping mechanisms between the two approaches. Similar to the CR intervention in aged rats (Opalach et al., 2010), the IF regimen in neuropathic mice also muted the nerve inflammatory response and supported the maintenance of the myelinated Schwann cell phenotype (Madorsky et al., 2009). One could

ask why it took five months of IF to see a significant improvement in the motor performance of neuropathic mice. Could shorter periods of IF throughout the lifespan, or intervention started at a later age, slow hereditary nerve degeneration? While these are important questions for better understanding the underlying mechanism for the observed improvements, such studies will require significant investment in resources.

With regards to additional mechanisms that are known to be affected by aging, peripheral nerves of aged AL-fed rats contained elevated levels of oxidized proteins and lipids, as well as pro-inflammatory mediators (Opalach et al., 2010). As in the case of the myelin and protein quality control markers, these indicators of unhealthy nerves were muted in samples from aged-CR rats. Therefore, our studies suggest that a life-long CR intervention preserves nerve health by minimizing damage and sustaining the activity of protective mechanisms. In turn, healthy nerves support the conduction of electrical impulses along the axons, maintain strong connections with target muscles, and thereby support neuromuscular function. Indeed, studies from the same group of rodents indicate that CR significantly attenuated the reduction in muscle strength in all age groups (Xu et al., 2008). The authors of this study also reported a reduction in age-related iron accumulation within skeletal muscle tissue, yet providing additional data on the global effects of this intervention. As we found no evidence for oxidative damage in the nerves of 10-month old neuropathic mice, we did not examine this pathway in the IF study. In the nerves of hereditary neuropathic mice, the genetic insult accelerates age-associated degenerative events, likely initiating from the accumulation of the misfolded PMP22 in Schwann cells (Fortun et al., 2003; Fortun et al., 2005). However, CR has also been shown to facilitate maintenance of motor neuron activity in aged organisms (Rawson et al., 2012), which will also contribute to preserving peripheral nerve health. Consistently, in a recent elegant study using transgenic mice where axon terminals were fluorescently labeled, Valdez and colleagues uncovered specific subcellular improvements in the architecture of neuromuscular synapses in response to life-long CR intervention, which was accompanied by protection against motor neuron loss and muscle turnover (Valdez et al., 2010). Therefore, the observed beneficial effects of CR on aging-related changes in neuromuscular performance involve several organ systems and cell types, including neurons, myelinating Schwann cells, and skeletal muscle cells. While there are many unanswered questions related to the mechanisms by which dietary modulation support the function of peripheral nerves, our results show that at least in part it involves restoration of protein homeostasis through increasing the efficacy of endogenous protein quality control pathways.

As IF or life-long CR are not readily suitable for clinical applications, approaches to mimic the effects of diet restriction are of great interest. Resveratrol, a polyphenol plant product is perhaps the most studied mimetic for CR (Chung et al., 2012). Resveratrol has a number of biological effects that could potentially protect against age-related degenerative changes and appears to mimic the effects of CR by inhibiting cAMP phosphodiesterases (Chung et al., 2012). While the peripheral nerves of resveratrol-fed aged animals have not been studied, based on its neuroprotective and anti-inflammatory effects, resveratrol has been tested in diabetic and toxin-induced neuropathy models. For example, in a 3-nitropropionic acid-evoked peripheral neuropathy model, pretreatment of the rats with resveratrol was neuroprotective and prevented functional deficits (Binienda et al., 2010). Rapamycin is another compound that has the potential to mimic the anti-inflammatory and autophagy-inducing effects of CR and IF, but its effect on preventing age-associated peripheral nerve degeneration has not been explored. In vitro studies in neuropathic models however revealed a positive effect of autophagic activation in restoring myelination (Rangaraju et al., 2010). While small molecule therapies are attractive possibilities for mimicking the beneficial effects of CR, the potential contribution of muscle-derived factors should not be underestimated as target muscles directly influence peripheral nerve biology. Indeed,

exercise provides known benefits to peripheral nerve function and a number of rehabilitation programs are now being implemented to slow degenerative changes in neuropathic patients (Maggi et al., 2011).

Conclusion

Approaches to enhance endogenous repair and protective mechanisms that can support healthy neuromuscular function are of great interest in aging, as well as for hereditary neurodegeneration. The studies cited above strongly support the hypothesis that dietary modulations through life-long CR or extended IF can delay the progression of aging or neuropathy-related alterations in peripheral nerves. The beneficial effects in both approaches appear to have involved the chaperone and the autophagy-lysosomal pathways validating these mechanisms for therapy development. Conceivably, a combined approach of mild dietary modulation along with a pharmacological small molecule therapy targeting chaperones and/or autophagic-lysosomal pathway could provide a suitable option in humans. In either scenario there might be a critical time point by which such intervention should be initiated to obtain maximal benefits. Finally, since peripheral nerves rely on muscle cells for trophic support, combined interventions that involve increased physical activity should be considered for added benefits.

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Highlights

This mini-review will summarize recent findings from rodent models of aging and hereditary neurodegeneration concerning the impact of dietary modulation on peripheral nerve health. Specifically, three main aspects are highlighted:

- Peripheral nerves are vulnerable to aging-related degeneration
- Protein homeostatic mechanisms are critical in myelinating Schwann cells
- Dietary restriction delays normal aging- and genetically- linked peripheral nerve damage