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COMMENTARY

NUTRITION AND THE BIOLOGY OF HUMAN AGEING: COGNITIVE DECLINE/FOOD INTAKE & CALORIC RESTRICTION

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In the last session of the symposium an extensive body of evidence was presented on "Aging, energetic challenges and neuronal vulnerability". One key message was brought forward by Mark Mattson: Throughout adult life it is important to challenge nerve cells from an energetic standpoint in analogy to exercise and muscle cells, which makes the muscle cells stronger.

With a look at the predicted age distribution pyramid, it is apparent that by 2050 there will be a doubling of the number of individuals above the age of 65 and therefore at a high risk for Alzheimer's disease (AD) and Parkinson's disease (PD) (1).

The resulting question therefore becomes: Why do some people suffer degeneration and others not?

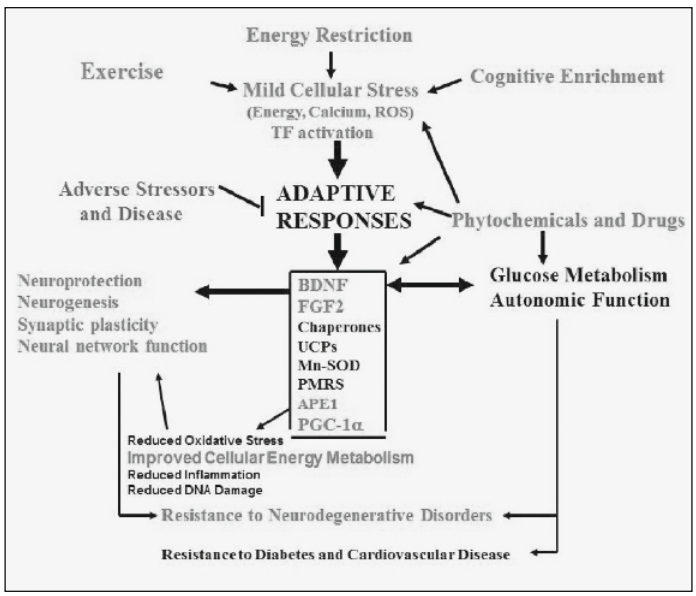
Current understanding implies that once symptomatic for AD and once neuronal networks are destroyed it may not be possible to reverse this process. Therefore, prevention is important, and it is anticipated that a delay in onset of AD by 5 years would result in 50% less people getting AD before they die.

To elucidate the mechanism of AD a mouse model has been developed, which shows both age dependent amyloid plaque formation and synaptic dysfunction (2). From a dietary standpoint these mice show accelerated cognitive impairment when fed in an ad libitum paradigm as compared to a feeding scheme of intermittent fasting or caloric restriction (3).

Prior to using drugs the effects of diet and lifestyle are key to prevention and in particular dietary moderation, exercise and cognitive stimulation can suppress the neurodegenerative process with aging.

Overall a model is proposed in which intermittent challenges to neurons, including regular exercise, energy restriction and cognitive enrichment impose mild cellular energetic and oxidative stress, which in turn induces a number of adaptive responses by increasing the expression of a variety of regulatory proteins. Among those upregulated proteins are neurotropic factors such as BDNF, FGF2 and GDNF which enhance synaptic plasticity and can protect neurons against oxidative and metabolic stress. In addition, protein chaperones such as HSP-70 and GRP-78, and antioxidant enzymes such as manganese SOD and HO-1 are induced by intermittent fasting. In the nucleus, levels of the DNA repair enzyme APE1 is increased as part of the stress response (4). Moreover, the transcriptional coactivator PGC-1α is induced by energetic challenges, which leads to an increase of mitochondrial biogenesis and enhanced formation and maintenance of synapses in the brain1 (see figure 1).

Figure 1



Looking at a brain region uniformly affected by AD – the hippocampus – reveals parts of the complex physiology involved with the mild cellular stress response. The hippocampus integrates inputs from our different senses and it is essential for integration of inputs in time (vision, hearing, touch, etc.). With AD the hippocampus gets smaller as nerve cells are degenerating.

Environmental stimulation such as exercise, energy restriction, etc.) seems to improve synapse plasticity in the hippocampus. Moreover, dietary energy restriction and exercise stimulates adult hippocampal neurogenesis (from stem cells).

Finally, "The fasting cure" a 1911 book by Upton Sinclair shows 277 "case studies" of fasting demonstrating the anecdotal benefits of fasting.

Based on this and summarizing the current understanding (5) a number of "priceless prescriptions" have been proposed for overweight people – combinations of exercise, energy restriction and cognitive challenges.

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Following discussion on the overall cellular adaptive responses to stress an additional angle on stress responses has been put forward by Andrew Dillin with a view on stress responses in the mitochondrial electron transport chain (ETC).

As the principal energy sources for cells mitochondrial integrity implicitly affects aging and in particular integrity of electron transport chain function can modulate aging in a cell non-autonomous fashion.

Mitochondria are the cornerstone for aging research community dating back to the Metabolic theory of aging also known as the ROS theory of aging (6).

Strikingly, certain perturbations in mitochondrial function lead to increase in lifespan! This is unexpected and found in yeast, C. elegans, flies and mice (7). A genome-wide RNAi Screen in worms revealed that knocking down selected nuclear encoded subunits of the mitochondrial ETC almost doubles the lifespan. However, the increased lifespan comes at a price and the animals suffer various problems during development, they are slow moving, and display perturbed reproduction.

Knocking down individual components in the ETC by RNAi leads to stoichiometric imbalance of ETC complexes and instability, mainly resulting in a huge protein folding challenge for the organelle. The depletion of almost any subunit of the ETC complexes leads to the falling apart of the whole complex i.e. a proteotoxic challenge to the mitochondria.

To elucidate the underlying mechanism it was hypothesized that a rescue pathway - the mitochondrial unfolded protein response – is at work, where upon a proteotoxic challenge, a signaling cascade involving protease ClpP is activated to prevent unfolding.

All observed perturbations in the ETC that lead to lifespan extension leads to the mitochondrial unfolded protein response.

Pushing these findings to the next level it turned out that perturbation of mitochondrial function i.e. subunit misfolding and the induced UPR in one cell type can be transmitted and communicated to other cells.

When asking the question if the observed lifespan extension can be induced in any tissue, it turned out that in C. elegans only neuronal or intestinal mitochondria impart prolonged lifespan to the organism. By some means these tissues seem to convey the message to the entire organism. Indeed, intestinal and neuronal tissues seem to be talking to each other through a putative novel mitokine pathway.

Taken together the mitochondrial ETC longevity pathway thus is marked by four corner stones: it functions cell non-autonomously; it specifically induces the UPRmt; the timing requirements overlap with the UPRmt and it requires the UPRmt.

Understanding the role of the mitochondrial unfolded protein response not only provides insight into C. elegans longevity, the response is also observed in specific mouse strains (Auwerx, unpublished). Moreover, in analogy to the mitochondrial unfolded protein stress response also an ER unfolded protein response (UPRER) has been described; however, the signaling pathway appears to be different from the mitochondrial one.

Finally, from an evolutionary standpoint it might at first sight seem unclear why a cell communicates its proteome folding status across other cell types i.e. why would metazoans evolve cell non-autonomous UPRs? However, upon shifting the view from a "selfish cell" to the overall health of a given tissue or even an entire organism the non-autonomous UPR might play a key role in the coordination of limiting resources vs. an uncoordinated competition for those limiting resources.

Jeffrey Friedman finished the session with a presentation on Leptin and the homeostatic system regulating body weight. While conceptually obesity could be related to a lack of willpower, to lifestyle and environment or to biology and genes, a Mendelian analysis makes a strong case for a key role of biology. Just looking at heritability it turns out that obesity is almost as heritable as body height thus ranking higher and more heritable than Schizophrenia, Diabetes or Hypertension – to name a few.

In 1994 leptin was discovered as the basis for the mouse ob/ob phenotype, which is based upon a mutation in the leptin gene (10). In the ob/ob mice treatment with leptin induces substantial weight loss and reduction of food intake returning the animal to a normal phenotype. The cloning of the ob gene and identification of leptin has uncovered a new endocrine system regulating body weight. Overall, this system detects changes in nutritional state and in turn regulates other physiologic systems.

Human patients with leptin mutations have been identified and significant similarities were observed with the ob/ob mouse (8, 9). In the specific case of the obese child studied by O'Rahilly, leptin replacement led to drastic reduction in obesity. Before leptin administration the child ate 2000 kcal in one meal, afterwards 180 kcal demonstrating that food intake control is severely impaired without leptin (9).

Biologically leptin is part of an intricate homeostatic system to regulate the nutritional state of the body. Leptin levels correlate with fat mass, and its level communicates information about the nutritional state of the organism; low leptin signals starvation and a high leptin level signals that there is excess fat. Without leptin the body gains weight because the brain thinks the body is starving and appetite is stimulated. In wild populations and in humans living as hunter gatherers fat mass appears to be under evolutionary selection. For example, 25,000 years ago in populations of hunters and gatherers leanness induced an increased risk of starvation whereas obesity increased the risk of predator attacks. The leptin system maintains homeostatic control of fat mass and balances the relative risks of being too obese or too lean. Whether selection remains in modern times is debated, as there is no risk for obese people of predator attacks, and the consequences of obesity do not generally develop until later in life after reproduction has been completed. Nonetheless there still do appear to be negative evolutionary consequences of obesity leading to

selective disadvantages of being obese in modern times. To name a few: In many parts of the world gestational diabetes is leading to large babies and cephalo-pelvic disproportion. In addition, it is not infrequent that health consequences are observed in morbidly obese people even of reproductive age.

Thus the leptin system protects the organism from maladaptive consequences of low body weight on the one hand and it prevents excessive adiposity on the other hand. As mentioned low leptin levels signal starvation and are associated with many physiologic abnormalities and paradoxically therefore, the pleiotropic abnormalities observed in leptin deficient obese subjects are those, which are typically associated with a starvation response rather than obesity (11). Indeed several diseases with low leptin levels have been identified and leptin treatment has been shown to ameliorate the severity of these diseases.

For example, in patients with lipodystrophy, i.e. the congenital or acquired loss of adipose tissue, the reduced fat mass leads to decreased leptin levels. Patients develop severe insulin resistance, diabetes and a fatty liver and leptin treatment markedly improves this condition.

In another state of leptin deficiency abnormality, a low leptin level is linked to a cessation of reproduction as hypothesized by Rose Frisch who proposed that a critical, minimum amount of body fat is necessary for, and directly influences, female reproduction. Anorexic females are often infertile suffering from hypothalamic amenorrhea whereas girls with higher BMI enter earlier into puberty (12). These patients have very low leptin levels, and recombinant leptin has been shown to restore reproductive function and correct the related abnormalities these patients manifest (13). Moreover, leptin significantly improves bone mineral density in lean hypoleptinemic women, independently of effects on reproductive capacity. This is important because patients with hypothalamic amenorrhea often develop premature osteoporosis.

The leptin endocrine system also is linked with changes in immune function. Strikingly, starvation and leptin deficiency cause the same changes in the immune system such as shifting from Th1 to Th2 immunity with susceptibility to bacterial infection and alterations in other immune cell populations. Thus, a high rate of death from bacterial pneumonia has been observed in pedigrees of patients with leptin mutations.. In this case as well, leptin treatment normalizes the immune abnormalities of leptin deficiency and starvation.

Finally, leptin treatment also extends life span in starved animals suggesting that a drop in leptin during starvation could increase mortality thus eliminating the most compromised individuals that might spread disease from a population.

What about leptin in overweight and obesity? As it turns out, a substantial fraction of morbid obesity is the result of Mendelian defects in the neural circuit that is modulated by leptin. Most obese patients are not leptin deficient, and have increased levels of leptin suggesting that they are leptin resistant. When it was found that most obese patients are hyperleptinemic the question was then whether it would help to give extra leptin to obese if they are already resistant? Roth et al (14) found that leptin administration leads to modest effects on body weight in a subset of obese individuals. However, the majority are leptin resistant but appear to respond to a Leptin-Amylin combination using agents such as pramlintide - an amylin analog. Therefore the question becomes: What is the molecular basis of leptin resistance?

A few hallmarks of leptin signaling have been established in recent years (see e.g. 15): In the arcuate nucleus of the hypothalamus at least two groups of leptin responsive neurons are involved in the signaling pathway. One subset of cells (AGRP cells) expresses the leptin receptor and the peptide NPY and when these neurons fire an animal eats more. Another subset of cells (POMC cells) expresses the leptin receptor and the peptide α-MSH and when these neurons fire feeding is inhibited. Leptin works in part by inhibiting the NPY neurons and by activating the α-MSH neurons.

An important open question is: Where do the wide variety of stimuli such as leptin, taste, smell, vision, emotion and other signals get read out in the brain to stimulate or inhibit feeding? As such feeding is a complex, motivational behavior depending upon the integration of smell, vision, emotion, volition and also low or high leptin. The neural processes controlling this integration are unknown and research is underway in the framework of a multilayered program in the Friedman laboratory. The key objective of this program is to localize sites of neural activation, to identify candidate neurons in these regions and finally regulate their level of activity using optogenetics or other methods to elucidate their possible function to regulate food intake.

For this a novel technology has been developed – "Phospho-Trap" (16), which allows one to obtain gene expression profiles from neurons, or other cell types, that have been activated or inhibited in response to a stimulus. Briefly, mTOR activation, which is co-localized with the known neuronal activity marker c-fos, activates the S6-kinase, which in turn phosphorylates S6, a ribosomal subunit. Thus, neurons that have been activated have a higher proportion of phosphorylated ribosomes and neurons that have been inactivated have a lower proportion. By immune-precipitating ribosomes using a phospho-specific S6 antibody it is possible to capture specific RNAs (genetic markers) from cells that have been activated and deplete RNAs from cells that have been inactivated. In this manner, cells that have responded to a stimulus can be identified and studied further. To identify the molecular basis for the hypothalamic response to fasting the approach validated AgRP and Npy as the most substantially enriched genes. In addition, the technique identified a variety of additional novel genes involved in the anorexigenic and the orexigenic hypothalamic response.

This afternoon's session was largely concerned with energy metabolism in brain function in aging with the hopes of potentially identifying a role for nutrition in optimal cognitive

function and longevity. Mitochondrial function appears to play a key role in fostering this outcome. Although the brain can only use glucose and ketone bodies as fuels, the amount of fuel provided either in surfeit or in chronic deprivation, the level of energy expenditure including that from activity, the frequency of dietary intake, or the way energy is metabolized by mitochondria may profoundly influence the quality and duration of optimal cognitive function. Work by Dr. Mattson's group showed that either a 30% caloric restriction or alternate day fasting improved cognitive function in a transgenic model of Alzheimer's disease in mice (3). Preliminary human studies have shown that intermittent energy and carbohydrate restriction improved markers of oxidative stress and inflammation, which are presumed mediators of neuronal degeneration, in patients with moderate asthma (17) or obesity (18). Diets such as these, restricted in energy and particularly of carbohydrate, lead to ketone body production, since ketogenesis is primarily determined by low insulin levels and elevated free fatty acid levels, with elevated glucagon levels playing a modulating role. Ketogenic diets have profound effects to treat drug-resistant epilepsy and have been used since 1921 for this purpose (19). Substantial experimental evidence has developed for a number of other neurodegenerative disorders including Alzheimer's, Parkinson's disease, and post-ischemic and traumatic brain injury (20). Unlike total and to a similar extent intermittent modified fasting where ketogenesis is pronounced due to the total or severe energy deprivation and weight loss is inevitable, ketogenic diets as first-line therapy for epilepsy and perhaps in the future for other neurodegenerative conditions, are intended to provide sufficient energy to maintain weight in adults and allow for growth in children. This is accomplished by restricting dietary carbohydrate to 8g/day (3 % of energy) in the classic ketogenic diet, to 50 g/day (20%) in the Medium Chain Triglyceride diet, 10 g/day (5%) in the Modified Atkins Diet, and to 40 g/day (27%) in the Low Glycemic Index Diet, the latter three diets used to improve compliance (19). The mechanism of action of ketone bodies are not completely known, but they are known to improve mitochondrial bioenergetics through improvement in antioxidant status and redox state (21).

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References

1. Stranahan, A.M. & Mattson,M.P. Recruiting adaptive cellular stress responses for successful brain ageing. Nat. Rev. Neurosci. 2012;13, 209-216
2. Oddo, S. et al. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 2003;39, 409-421
3. Halagappa, V.K. et al. Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. Neurobiol. Dis. 2007;26, 212-220
4. Yang, J.L., Tadokoro,T., Keijzers,G., Mattson,M.P., & Bohr,V.A. Neurons efficiently repair glutamate-induced oxidative DNA damage by a process involving CREB-mediated up-regulation of apurinic endonuclease 1. J. Biol. Chem. 2010;285, 28191-28199
5. Mattson, M.P. Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. Cell Metab 2012;16, 706-722
6. Harman, D. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 1956;11, 298-300
7. Durieux, J., Wolff,S., & Dillin,A. The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell 2011;144, 79-91
8. Ingalls, A.M., Dickie,M.M., & Snell G.D. Obese, a new mutation in the house mouse. J. Hered. 1950;41, 317-318
9. Farooqi, I.S. et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J. Clin. Invest 2002;110, 1093-1103
10. Zhang, Y. et al. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372, 425-432
11. Ahima, R.S. et al. Role of leptin in the neuroendocrine response to fasting. Nature 1996;382, 250-252
12. Frisch, R.E. Weighing the evidence. Lancet 1985;2, 952
13. Welt, C.K. et al. Recombinant human leptin in women with hypothalamic amenorrhea. N. Engl. J. Med. 2004;351, 987-997
14. Roth, J.D. et al. Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc. Natl. Acad. Sci. U. S. A 2008;105, 7257-7262
15. Barsh, G.S. & Schwartz,M.W. Genetic approaches to studying energy balance: perception and integration. Nat. Rev. Genet. 2002;3, 589-600
16. Knight, Z.A. et al. Molecular profiling of activated neurons by phosphorylated ribosome capture. Cell 2012;151, 1126-1137
17. Johnson, J.B. et al. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radic. Biol. Med. 2007;42, 665-674
18. Harvie, M.N. et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. Int. J. Obes. (Lond) 2011;35, 714-727
19. Kossoff, E.H. & Hartman,A.L. Ketogenic diets: new advances for metabolism-based therapies. Curr. Opin. Neurol. 2012;25, 173-178
20. Hartman, A.L. Neuroprotection in metabolism-based therapy. Epilepsy Res. 2012;100, 286-294
21. Milder, J. & Patel,M. Modulation of oxidative stress and mitochondrial function by the ketogenic diet. Epilepsy Res. 2012;100, 295-303