

Protein Cycling Diet

A Defence Against the Diseases of Aging

by Ron Mignery

1. Introduction

Protein cycling is intended as a way for you to live longer and healthier with minimal interference with your normal routine and diet. It is based on well-established observations that animals and people who have endured periods of famine have extended life spans compared to those who have not and on more recent observations that periodic protein restriction alone can accomplish the same thing.

When the cells of the body are denied nutrients, they consume parts of themselves in a controlled process called autophagy. In a manner reminiscent of urban renewal, a part of the cell is fenced off and everything inside is demolished. The ruins are then salvaged to meet the ongoing needs of the cell. The lost volume of the cell is then rebuilt when the restriction ends. The net benefit of the full cycle is that the old stuff of the cell is replaced with the new. The old stuff may have become degraded and non-functional with time, and its replacement likely rejuvenates the cell.

There are a number of diseases that are characterized by the build-up of degraded substances in the cell. Among them are Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). A process that reverses the build-up holds particular promise to prevent or to delay the onsets of any of these so-called neurodegenerative diseases. Hopefully the protein cycling diet does just that.

The diet plan described in this book involves periodically restricting your protein intake and adding common herbs and spices to your diet that are known to promote autophagy. The remainder of the book:

- elaborates on the material already presented
- discusses issues to consider before starting the diet plan
- lists the protein content of common food items and how to calculate your total protein intake
- discusses how specific diseases relate to autophagy induction
- suggests low protein substitutes for high protein food items
- suggests a number of diet schedules you might want to follow
- provides a link to the on-line forum for discussing the protein cycling diet

Since I will be mentioning Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease often in the remainder of this book, I will use the following abbreviations to conserve space and apostrophes:

- Alzheimer's disease AD
- Parkinson's disease PD
- Amyotrophic lateral sclerosis ALS
- Huntington's disease HD

Personal Note

I came to this subject through a tragedy in a close friend's family. Her former husband, the father of her children, was diagnosed with Huntington's disease, a horrendous degenerative neurological disease that typically develops in mid-life and seemingly combines the worst aspects of Alzheimer's and Parkinson's. Further the disease is genetic, and worse yet, genetically dominant, meaning that the chance that none of her children and grandchildren would eventually develop the disease was statistically quite small. As a PhD biologist it came to me to search the literature to see what could be done to deal with the disease apart from the efforts of her ex-husband's physicians. I had earlier researched protein-related diseases (proteopathies) for a friend who developed amyloidosis and for the mother of another friend who developed ALS. I found that HD seemed to have much in common with those two diseases.

Those that inherit the Huntington's gene invariably develop the disease if they live long enough though the age of onset varies widely. With this in mind, I wanted to find a safe and unobtrusive way to delay the onset as much as possible in otherwise healthy people. A survey of research in HD and related diseases eventually led me to the conclusion that promotion of cellular autophagy would achieve my goal.

In subsequent years since the first publication, additional information from the research community now implies a role for autophagy in heart disease, stroke, diabetes, infections, osteoporosis, arthritis and just about any disease you can name. This is not too surprising considering how fundamental autophagy is to all multi-cellular life. It does now however give me a selfish interest in the subject since my family history makes me susceptible to heart diseases.

None of the ideas presented here are original to me. I have only gathered ideas and observations from the real workers in the field and attempted to present them in a form intelligible to the layman. To keep things simple, I have rounded a lot of numbers, usually in a conservative direction. This approach prevents me from fully presenting and acknowledging the work of scientists in the text and I give my apologies now to all who may feel slighted or are unhappy with how I have represented their work. The bibliography is heavily weighted to review articles rather than research presentations as such articles are less apt to be behind a pay wall and are generally more readable for a lay audience. Some excellent reviews of the subject of autophagy and neurodegenerative diseases are now available that covers the subject in far more detail than what I present here¹.

Caveat

The effectiveness of the diet strategy presented here is as yet unproven. The frequency, duration and extent of protein deprivation optimal for inducing autophagy has not been measured. Those that chose to follow it are pioneers and do so at their own risk. Those with health problems should not follow it without consulting their physician. All should understand the scientific rationale of the diet and review and consider any objections that may be raised in response to its publication. They should stop if problems develop. A web site for this book <http://proteincycling.blogspot.com/> has been created to answer questions, make clarifications, correct mistakes, report developments, and present user and reviewer feedback. This site might be reviewed before starting the diet.

2. A Brief Overview of Cell Biology

To understand subsequent chapters, the reader needs some comprehension of the structure and chemistry of the human cell and of the irregular terminology with which I have chosen to model it. Keep in mind that life is an organic process and, as such, produces exceptions to any generalizations one makes about it, including mine. Also in a complex organic process, you can probably find anything you look for. Numbers must be attached to any theories or findings to view them in perspective and distinguish the significant from the insignificant.

Matter is, of course, composed of atoms. Atoms can be bound to each other in clumps called molecules. Cells are composed of molecules of atoms of the elements oxygen, carbon, hydrogen, nitrogen, calcium, sulfur, phosphorous, and a few others. Oxygen alone comprises over 60% of the human body by weight.

At minimum a cell is a generally microscopic drop of liquid termed cytoplasm enclosed in a membrane. Most importantly it is alive, meaning that it constructs and repairs itself and reproduces its own kind. It achieves all this ultimately by manufacturing molecules called proteins. Basically a cell is a protein factory. It contains DNA, a long polymer of four types of molecules called nucleotides. The cell constructs polymers from 20 types of molecules called amino acids in sequences that mirror the sequence of nucleotides in the DNA, three nucleotides corresponding to one amino acid (with some redundancy). The amino acid polymers so produced are the proteins that form the bulk of the cell and catalyze its chemical processes. They then convert molecules drawn from the cell's environment into the other chemical types (fats, sugars, etc) of which the cell is composed.

The cytoplasm is, of course, much more than just a drop of liquid. Cells have motion and hold and change shape, and solid structures must be constructed to support these activities and to move things around. The cell lays down a virtual railroad composed of filaments called microtubules. Upon these rails, specialized locomotive proteins drag other proteins and even larger structures to their destinations within the cell. Each protein displays a ticket (a particular amino acid sequence usually) that identifies its destination.

The cell is the smallest autonomous unit of all life-forms. Even the largest whale was at one time a single cell. All free-living life-forms have bodies

composed of cells. Most have only one cell in their body. A single cell is generally invisible to the naked eye so any visible plant or animal must be composed of many cells. For instance estimates for the number of cells in the human body range between 10 and 100 trillion (million million)! For the typical adult body, the range is 50 to 75 trillion².

A meter is a little more than a yard in length. A typical bacterial cell is about 3 to 5 millionths of a meter (micrometers) in diameter. For a globular human cell the diameter is about 50 micrometers³, at least 10 times greater. This means that the volume of a human cell can be over a thousand times greater than a bacteria's volume ($10 \times 10 \times 10$).

Some human cells are much larger than average. A nerve cell though only 50 micrometers tall and wide can be as much as a meter long. That could be 20,000 times the volume of a globular cell and 20 million times the volume of a bacterial cell.

Independent life forms come in two flavors cell-wise: bacteria and everybody else. As noted bacteria cells are small and typically contain their DNA in a ring floating in the cytoplasm of the cell. They are unicellular. Despite their size bacteria form the vast bulk of living mass on Earth.

The cells of everybody else including yeast, fruit flies, mice and humans are, as mentioned, much larger. The DNA is kept in a separate membrane-bound compartment of the cell called the nucleus in special structures called chromosomes. The cells even contain so-called organelles called mitochondria that evolutionarily are themselves captive bacteria with their own membranes, DNA and protein-synthesis machinery.

In subsequent chapters we will focus on nerve cells and mitochondria and this might be a good point at which to ponder the idea that a single nerve cell could harbor hundreds of thousands of them.

The mechanics of the cell seem extremely cumbersome and inefficient from our macro perspective. Mostly protein molecules wait around for the right molecule to bump into them at just the right angle. In the complex way that proteins are made, for example, it seems it would take forever for one little chain. Yet ribosomes spit out polypeptides at the rate of 20 amino acids per second⁴. You have always to keep in mind that, when you move from the macro to the micro, time speeds up and what takes a day to work out in our macro models may happen in the cell in a microsecond.

Oil and Water

When oil and water are shaken together, they quickly separate when the shaking stops. The oil being less dense rises to the top. This happens because the water molecule, though its net electrical charge is zero, is slightly positively charged on one end and negatively charged on the other. Opposite charges attract and water molecules align to satisfy this attraction. Oil molecules do not have this charge separation and are squeezed out of the water semi-crystal formed by these so-called hydrogen bonds.

The electrical force, like gravity, operates 24/7, and though ever so slight, organizes life forms as much as gravity, though ever so slight, organizes the cosmos. Molecules in the cell that have charge separation can likewise attract water and remain in the aqueous water phase. Molecules without charge separation are pushed aside and accumulate in oil droplet ghettos in the cell.

Micelles

Some large molecules have charge separation on one end but lack it on the other. They can move within the cell on their own to end up on the surface of an oil droplet with their so-called hydrophilic (water-loving) end in the aqueous phase and their so-called hydrophobic (water hating) end in the oil (lipid) phase forming structures called micelles like this:

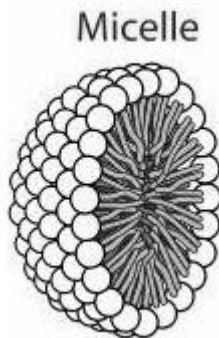


Fig. 1 (public domain image)

Except for in some specialized fat storage cells, most oil-type molecules in the cell also have aqueous ends. There is very little purely hydrophobic oil to

surround and so, as more of these molecules are recruited into the micelle, it tends to flatten out into a sheet like this:

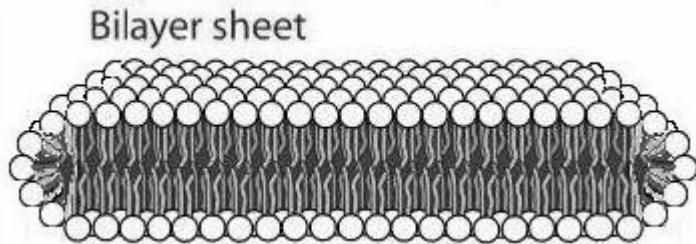


Fig. 2 (public domain image)

This sheet can wrap around itself as shown in two dimensions as follows:

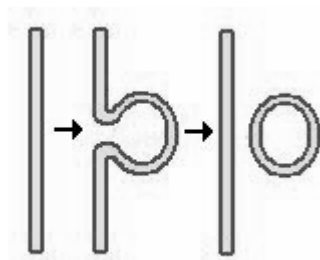


Fig. 3

This forms the so called liposome with an aqueous phase totally enclosed by a lipid membrane:

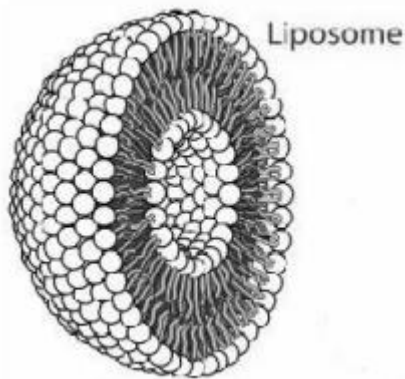


Fig. 4 (public domain image)

Note that these structures tend to self-assemble and, with a little guidance, can be made to form functional 'organelles' for life purposes. For example proteins or protein complexes with the right arrangement of hydrophilic and hydrophobic parts can embed themselves in the liposome and pump water and other selected molecules into the liposome to enlarge and to specialize it. Or they may pump hydrogen ions into it to create an acid interior.

In actuality most liposome structures in the cell bud off from existing liposome structures rather than arising de novo as outlined here, but the dynamics remain the same.

Liposome structures within cells are called 'vesicles'. Terms for specialized vesicles include 'vacuoles', 'peroxisomes', 'endosomes', 'phagosomes', 'autophagosomes', 'lysosomes' and arguably 'mitochondria', 'nuclei' and the cell itself.

The cell itself is like a vesicle containing vesicles, some of which contain their own vesicles. Protein structures embedded in the vesicle walls control the flow of materials in and out of the vesicles and, in this way, the cell is divided into compartments and the compartments specialize to serve different cell functions.

Endocytosis and Exocytosis

Liposomes can be formed from the outer membrane of the cell as well as from internal membranes. When this happens, the process is called

‘endocytosis’. It is used by cells to take in nutrients or to clean up extra cellular materials. The reverse of endocytosis, ‘exocytosis’, is used to dump liposome contents outside of the cell.

Proteins

This separation of oil and water not only acts between molecules but within large molecules as well, in particular, proteins. Proteins are long single-chain polymers of small molecules called amino acids. 21 different types of amino acids are used to make human proteins, some of which are hydrophobic and others hydrophilic. The same forces as organize micelles organize the protein to produce its functional configuration. Generally for soluble proteins, hydrophilic amino acids occupy the outside of the protein that is exposed to the cytoplasm. Proteins that embed in membranes have hydrophobic amino acids where the protein interfaces with the lipids.

Acid Denaturation

Hydrophobic amino-acids generally occupy the core of most protein molecules and give them their structure. Acids can be a threat to this structure. By definition, solutions are ‘acid’ in proportion as hydrogen ions are abundant. Positively charged hydrogen ions, just protons with no electron shell, are effectively so small compared to other molecules that they can navigate into the core of proteins and disrupt the charge arrangements between the atoms of the protein. This usually causes the protein to change its configuration irreversibly to a non-functional form, a process called denaturation. A similar thing happens when you boil an egg. It cannot be un-boiled. The body uses acid to denature proteins at two levels, in the stomach and in a cell vesicle called the lysosome. In both there are proteins that are specifically designed to be unaffected by the acid and continue to serve their intended functions. Indeed they cannot function without it. There can also be accidental aggregates of protein that can resist this denaturation as well. Some of these aggregates and the diseases they may cause are a central topic of this narrative.

Aggregates and Condensate

When any two or more proteins form an attachment, it can be said that they have formed an aggregate. Now there any number of aggregate forms. Some are soluble, others insoluble, Some contain water and are like gels. Others

have excluded water and can appear as solid objects. In some, the constituent molecules are free to come and go. In others, they are permanently bound. In this book, I am referring to soluble labile aggregates as 'aggregates' and insoluble fixed aggregates as 'condensates'. This distinction is important since the aberrant proteins that underlie neurodegenerative diseases are toxic in solution or in soluble labile aggregates but inert in condensates. The argument as to whether or not the aggregation is responsible for the disease often depends on this distinction.

3. Protein Folding

As mentioned proteins form the bulk of the cell and catalyze its chemical processes. Their production is its central activity of all cells. Proteins are long single-chain polymers of small molecules called amino acids. 20 different types of amino acids are used to make proteins. The type and order of each amino acid in the protein chain strictly correspond to the type and order of nucleotide sequences in the cell's DNA.

Of the 20 types of amino acids required for protein synthesis, the human body can make 11 from other substances. 9 types of amino acids however must be obtained from digestion of the proteins in the diet.

Protein chains are built up by adding one amino acid at a time. The chain so built then goes through a process called folding to assume the shape necessary to its function. Actually it's more like a string wadding up into a ball. A protein is like a string of magnetic beads. The different segments of the string interact depending on the shape and charge of each bead (in this case each amino-acid) and the environment within which the folding occurs. Presumably segments of the string pair with other segments with high specificity like a three-dimensional jig-saw puzzle. This proceeds in the juvenile protein until the protein achieves the configuration suited to its function. Subsequent enzymatic modification to the juvenile protein may then further stabilize its final configuration.

Mis-folding can occur whenever anything goes wrong for whatever reason. It is thought that mis-folding of proteins may be involved in progressive neurodegenerative diseases like AD, PD, ALS and HD.

Mis-folded proteins may expose amino-acid sequences that are normally paired internally with other complementing sequences. A second protein molecule of the same type may then pair its complementing sequences with the exposed sequence of the mis-folded protein. It's now unpaired sequence may then trap a third instance of the protein and so on forming an aggregate in the cell that grows inexorably with time. In the final stages when the cell can no longer prevent oxidative damage, the aggregate may condense to form an insoluble indigestible condensate, the so-called inclusion body characteristic of these diseases. The aggregate may grow so large that it leaves no room for other vital cell functions. Or as the aggregate grows, more and more of the protein species of which it is composed may be unable to escape its expanding grasp. If

that protein is needed elsewhere by the cell, its apparent deficiency could thereby weaken or kill the cell. Or the aggregate may trap the other protein species with which it normally interacts and make them unavailable.

While one molecule is folding another molecule of the same type can interact with it. This is possible because the other molecule also has the same complementary segments needed for precision pairing as the first. In the right circumstances this can result in the adult protein causing the juvenile protein also to mis-fold.

This interaction has been hypothesized as an explanation of how the prion diseases, scrapie, BSE and CJD, might operate. In these diseases, the infectious agent, the prion, is a protein. Presumably the mis-folded infecting protein interacts with the same or similar protein in the host and causes it to mis-fold. The newly mis-folded protein then persuades others of its kind to mis-fold in an exponential progression that, though slow, can ultimately mis-fold all instances of the protein.

Logically this might require that adult mis-folded protein be more likely than functional protein to combine with juvenile protein or that the mis-folded protein be degraded more slowly. Perhaps by consequence of having its pairing segments exposed, the mis-folded protein out-competes the functional in influencing juvenile folding. And perhaps by a tendency to aggregate, the mis-folded protein is protected from degradation.

This mis-folding cascade may be the slow but inexorable process in the prion diseases that can take decades to develop. The population of mis-folded protein doubles continually until there is enough to cause symptoms. Even if this involves hundreds of doublings, it is only the last that really matters where as much protein is mis-folded as in all the previous doublings combined. This is how the symptoms can suddenly appear decades after the initial infection event.

Perhaps this mis-folding cascade mechanism operates in some degree on other protein species, not just the prion-associated protein, and may account for some of the decline in cell vigor associated with aging and neurodegenerative diseases. If so, a mechanism to clear out aggregates would benefit cell longevity.

4. Longevity

On the physical plane at least, it seems necessary that we each inhabit an individual of the species *homo sapiens*, a variety of ape that fortunately is related by birth to all other forms of life on Earth. I say fortunately because observations on our relatives, chimps, mice, fruit flies, yeast, algae, etc., therefore become relevant to our own situation since we have all inherited nearly identical cellular structures and mechanisms. Without this relationship, biology would become an impenetrable mystery.

Humans are unusually long-lived for a highly-active, tightly-organized, medium-sized animal. Longevity has its benefits for all organisms in that it allows a species to survive unusual seasons of scarcity. It does however has its costs. First the species must support the additional genes and structures for the mechanisms that promote longevity. Secondly, with fewer generations in a time period, a species risks being out-evolved by others with shorter generation times.

Humans (and perhaps a number of other species) have evolved an additional emergent benefit of longevity we can call culture that more than compensates for the costs. By culture I mean the institutions that allow the experiences of one generation to be transmitted to the next.

As to culture as here defined, snakes have none. Some birds have some where songs and feeding habits of one generation may be taught to the young. Whales perhaps more than we know. Humans however go way beyond any other species in this regard where the accumulated knowledge of hundreds of generations may be transmitted to the next generation. Language itself is a component of this culture.

Longevity is a prerequisite of culture. First, to support the transmission of knowledge, the parent's life must significantly overlap the child's. Even further, to get through the lean years, the parent's life may need to overlap multiple seasons of child bearing. Second, the child also needs an extended childhood for learning. Consider that the human childhood alone exceeds in length the entire lifespan of cats and dogs for instance. Even when child-bearing ends, the parent must live on for at least the length of the childhood of its youngest.

Actually humans can live for over two generations beyond the end of child bearing. This implies that grand parenting and even great-grand parenting contribute significantly to the survival of our species.

The other species with lifespans comparable to humans are either immense, loosely organized, or live where time passes more slowly.

The immense animals (whales, elephants, etc.) need long lives to give time for the young to achieve the large size they need to survive as adults. These same animals have even been able to develop some level of culture thanks to that longevity.

For species loosely organized (trees, fungi, etc.) cells of one part are not necessarily dependent on the survival of other cells and parts. Cells that die can be replaced by new cells. The north and south sides of trees, for example, need each other for little more than mechanical support and usually either can survive the death of the other. Indeed the perception of a tree as an individual of a species is more in the mind of the viewer than in physical reality.

Finally time passes more slowly for some species than for others. Where temperatures are lower, chemical processes are slower. Where the organism needs little energy input and output, entire systems can be shut down until needed. The net result is the long lifespan typical of turtles and similar conservative animals.

At a cellular level what strategies have life forms developed to achieve longevity? Firstly why is any strategy needed at all? Why should a cell ever die? Of course the answer is that any complex mechanism is subject to damage over time. This is especially true on the surface of our planet where certain of us life-forms have been pouring their waste products into the atmosphere for billions of years. Blue-green algae – I am talking about you and the free oxygen you belch out. Actually (by necessity) we humans have grown quite fond of oxygen and now find we cannot live without it. Its handling does however produce damaging by-products in our cells, the so-called reactive oxygen species (ROS). The cells make extraordinary efforts to contain the by-products but some damage occurs regardless. And there are other sources of damage as well. Unless the cell takes steps to correct the damage, the cell will eventually die.

So how does a cell correct damage? The most basic strategy is to divide before the damage occurs. After division if one cell is damaged and dies the other undamaged cell still lives. In a sense every live cell on Earth is as old as life on earth, some two billion or so years, the end product of countless cell divisions where the daughter cells, though two, are the same flesh as the parent cell and can claim its identity. Of course over the ages the DNA of the cell has changed through mutation and exchange (or even union) with other cells but never has

any cell currently alive died. The vast majority of cells now alive will of course soon die but some few will live on to replace them. Damaged cells or those otherwise so destined will die, but the undamaged lines can survive forever. This kind of longevity can be seen in the cells of rapidly dividing tissues such as found in the liver and the lining of the intestines where little difference can be seen between those of an octogenarian and a teenager. It also suffices for the longevity seen in loosely organized life-forms such as trees and fungi and, of course, for all one cell organisms.

It is not the form of longevity targeted by this book, however, and I have no more to say about it except in regards to mitochondria. Nor is the longevity of turtles and the cold-blooded of interest. We will now focus on human longevity.

In multi-cellular organisms such as humans there are some tissues where the cells stop dividing altogether after the tissue has formed. These cells must live for the entire life span of the individual if their function is not to be lost. Included in this category are the nerve cells and muscle cells that together constitute most of the mass of the body.

Muscle cells achieve longevity by fusing together so that a single muscle cell has many nuclei and thus many copies of the DNA that directs the cell. If some of the DNA fails, the cell still has good copies to keep going. Also the energy production task for muscles that involves oxygen and its toxic ROS by-products is somewhat off-loaded to the liver which maintains its youth by the cell division strategy mentioned earlier. Even when muscle cells die, muscle stem cells can, within limits, replace them.

Nerve cells get special treatment from the other cells of the body. Special nurse cells wrap around them or separate them from the blood stream to keep away potential toxins and to provide nutrients and mechanical protection.

Despite the coddling nerve cells get, they are nevertheless vulnerable to damage from their own internal processes. Nerve cells are high energy users and burn a lot of sugars in oxygen to support their function. This process produces potentially damaging ROS by-products that must be cleaned up by the cell. These by-products and other consequences of time cause the cumulative damage that eventually kills the cell and produces the neurodegenerative diseases characteristic of human aging.

5. Cellular Maintenance and Repair

Death is of course inevitable, but, given the choice, most of us would rather die with the nervous system intact. Happily there are things one can do to retard the accumulation of damage (and, unhappily, things to advance it). The protein cycling diet supports one particular mechanism of cell repair, autophagy, but there are others that bear mention.

As mentioned it is the reactive oxygen species (ROS) produced by the oxygen-consuming energy-generating processes of the cell that likely do the most damage. The body produces or incorporates substances called anti-oxidants both within and without the cell to minimize their effects. One substance, ascorbate (vitamin C), is produced by most animals but not by humans and other members of the primate order of mammals. Because ascorbate was likely so plentiful in the natural diet of early primates, the necessary genes to complete its synthesis were lost and never regained. As a result we continue to require that the anti-oxidant, ascorbate, be obtained from the diet. Similarly the anti-oxidant tocopherol (vitamin E) must also be obtained from the diet. Many of the anti-oxidants produced by the body contain the element sulfur and sulfur in some available form must also be obtained from the diet.

The scientific studies on which the protein cycling diet are based are generally done with animals on diets deficient in only the substance under test. In consequence of modern dietary practices however, many people are border-line deficient in essential vitamins and minerals. To help the chances of getting the same results as the studies, daily supplementation with a full multi-vitamin and mineral tablet is recommended when on any diet. In particular ascorbate (vitamin C), tocopherol (vitamin E) and some source of sulfur (cysteine, SAMe, MSM, onions, etc.) should be provided. Other common border-line deficiencies in western industrial diets include omega-3 fatty acids, folic acid and magnesium and again supplementation is recommended.

Also one should refrain from substances that diminish anti-oxidant capacity such as foods high in iron and copper and acetaminophen (paracetamol).

Anti-oxidants may also prevent the accumulation of lipofuscin, a lipid-containing cellular condensate thought responsible for a number of neurodegenerative diseases called lipofuscinoses⁵.

Perhaps the most important task of a cell to prolong its life is to protect its DNA, the blue-prints from which everything else is made. Higher cells keep their DNA locked up in a double-walled bubble called the nucleus. It is guarded by nuclear pores that only allow entrance to proteins that display certain sequences of amino-acids, the so-called nuclear location signals.

Within the nucleus a number of enzyme systems work tirelessly to repair any damage to the DNA itself. Also autophagy, a cell recycling process, has been seen to operate even on the nucleus to dispose of its garbage as will be extensively discussed later.

Cells perform much of their oxygen dependent metabolism within the mitochondria. A cell may have tens, hundreds or thousands of them. They contain their own DNA (mDNA) and protein synthesis system and must reproduce within the cell like the bacteria that evolutionarily they once were. Their DNA, like bacterial DNA, is not enclosed in a nucleus and is likely subject to more damage than if it were. Indeed most of the genes for the proteins of the mitochondria are in the host cell's nucleus. Apparently the few proteins still encoded by the mitochondrial DNA are so difficult to transport that they justify the retention of a separate protein synthesis system in the mitochondria just so that the proteins can be assembled in place.

When its DNA is damaged, the mitochondrion will likely then be unable to reproduce or at least to reproduce as efficiently as before. The damage to the mDNA is then effectively removed as the damaged mitochondria are out-reproduced by the undamaged. Note that the cell must be growing or replacing itself before mitochondrial reproduction is needed. This point will also be discussed later in the context of autophagy.

The cell has a mechanism to selectively destroy individual proteins that are degraded – chaperone-mediated autophagy. When many proteins break down, they expose special amino-acid sequences that other proteins called chaperones can recognize. The chaperones then escort the degraded protein (and any object to which they may be attached) to a site in the cell where their amino-acids can be recycled. Note that this is not the form of autophagy that the protein cycling diet seeks to promote.

Protein turnover is a tightly-regulated essential cell function. It was once thought that after the cell had expended all the energy and effort required to build a large protein that it would be unreasonable that the cell would then destroy it before it had destroyed itself. Unreasonable but true nevertheless.

Proteins actually signal their appropriate lifespans to the cell by the amino acid species on one end of the chain for times ranging between about 20 minutes to 20 hours accordingly. Nearly 100% of the amino acids of proteins can be recycled. Nevertheless losses from growth, reproduction, skin shedding and the digestive process are unavoidable and must be recovered from the diet.

There are proteins in the cell whose job is to attach chemical tags (ubiquitin) to any proteins they bump into. There are other protein complexes called proteasomes that chew up any proteins that have a lot of these tags attached. Without going into the mathematics, the net effect is that the proteins of the cell statistically have a fairly well determined life-span and are replaced when old whether they are degraded or not.

Proteins that form aggregates however may be able to escape the proteasomes even though they are extensively tagged with ubiquitin. The aggregate proves literally too large to fit into the proteasome's mouth. These aggregates may however be dealt with by the forms of autophagy, macroautophagy and microautophagy, to be discussed in the next chapter and whose promotion is the purpose of the protein cycling diet.

6. Autophagy

Hard times exist for all life-forms. The population of any species grows exponentially until the amount of what it feeds on matches what is available to feed it. Anything that drops the amount available, depletion, drought, what have you, results in starvation, and thus episodes of starvation become inevitable. The ability to survive starvation must be developed and retained if a species is not to go quickly extinct.

Some species simply shut down for the duration and wait for the good times to return. Others draw on reserves built up in times of surplus. Humans have body fat as reserve for energy but have no comparable tissue dedicated to protein storage. Instead each cell must consume some part of itself to survive. Cells from yeast to humans have a complex mechanism to deal with the lean times called autophagy⁶.

Cells form membrane-bound compartments within themselves for specific activities. One common compartment so formed is called the lysosome. It contains enzymes in an acid environment that can degrade lipids, carbohydrates, nucleic acids, and proteins and is where the cell recycles its degraded or surplus parts.

In chaperone-mediated autophagy as mentioned in an earlier chapter, chaperone proteins escort degraded proteins to the surface of the lysosome where protein complexes embedded in the its membrane transport them into the body of the lysosome for digestion.

Lysosomes also participate in two other forms of autophagy, microautophagy and macroautophagy that operate in bulk rather than one molecule at a time.

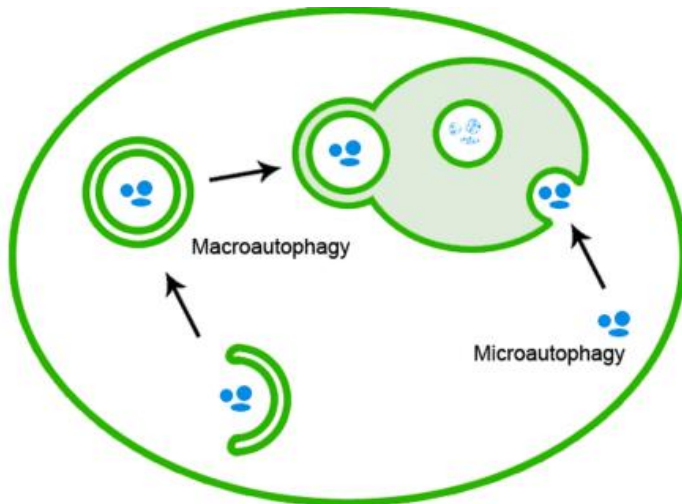


Fig. 5 (public domain image)

In microautophagy the membrane of the lysosome invaginates to suck in some portion of the cytoplasm around it including whatever might be floating in it. The pocket so formed then pinches off at its opening to form a new vesicle within the lysosome vesicle. The new vesicle and its contents are then digested. This process has even been seen to pinch off portions of the mitochondria and even of the nucleus¹⁰⁹.

In macroautophagy the lysosome merges with another membrane-bound vesicle and releases its digestive enzymes into it. Among the candidates for merger may be a so-called autophagosome which is itself formed for just this purpose.

The autophagosome begins as an empty membrane-bound vesicle (of mysterious origins⁷) that, like the lysosome itself in microautophagy, invaginates to enclose some portion of the cytoplasm and its contents forming a filled vesicle within an otherwise empty vesicle. (Note that an autophagosome can be rather large and encompass entire organelles such as mitochondria and peroxisomes.) The empty vesicle is then filled by merger with lysosomes that then digest the internal vesicle.

The net effect of the two processes is the same, some bulk of the cytoplasm is selectively or non-selectively enclosed and its contents are recycled by lysosomes.

Note that micro-autophagy provides a means to recycle the lysosome membrane. How the internal enzymes of the lysosome get recycled is a bit of a mystery. Perhaps as they degrade, they lose their acid resistance and are then digested by their fellow enzymes. The ultimate fate of the entire aging lysosome is usually to merge back into the vesicle factory of the cell, the endoplasmic reticulum (ER), from which it likely came. The ER then dumps any undigested lysosome contents so received into the cytoplasm by a special mechanism known as ERAD and the proteins are then recycled by the usual pathways.

There is also some evidence that lysosomes can merge with the outer cell membrane as well (exocytosis) and dump their contents outside the cell⁸. This may account for the extracellular plaques observed in AD.

And finally, perhaps sometimes an aged lysosome simply loses its acidity, shrivels and disintegrates.

Though also used by the cell for other purposes, non-selective bulk autophagy of the micro and macro varieties provides the mechanism for a cell to feed on itself in times of nutrient scarcity. Perhaps as a by-product it also clears out aggregates and other detritus that the other forms of cell trash removal miss. This is the very idea behind the protein cycling diet, to promote autophagy to reduce the aggregates that lead to neurological diseases and other lesser nuisances of aging. This might then explain why animals and people who have endured periods of famine have extended life spans compared to those who have not.

Macroautophagy is not entirely non-selective. There is evidence that degraded mitochondria and protein aggregates are actively transported to autophagosomes for engulfment and that their very presence can initiate autophagosome formation⁹.

7. Famine and Longevity

In the 1930s it was observed that feeding laboratory rats a severely reduced calorie diet otherwise sufficient in other nutrients resulted in life spans of up to twice as long as otherwise expected¹⁰. Similar results have since been produced in a range of other animals from yeast and fruit flies to primates and, to some degree, with just reduced protein¹¹. Though the phenomenon has not yet been demonstrated in humans (they are such difficult lab animals!), many people have independently adopted the practice of calorie restriction in some form as part of research efforts or from their own faith in the subject.

Practitioners of calorie restricted (CR) diets experience improved cholesterol, glucose, and blood pressure profiles and that alone should extend average life-span especially since desirable weight loss usually results. The goal of many in the movement is, however, to extend maximum life-span.

Why should CR extend maximum life-span? Is it simply that metabolism and thus time itself is slowed down? That seems unlikely for a warm-blooded organism whose temperature holds to 37°C regardless of diet.

It seems more likely to me that CR promotes autophagy. By living at the edge of starvation, the insulin-signaled autophagy response is likely triggered every time a meal is delayed.

One form of CR involves eating only on alternate days (ADCR). All the benefits of 'chronic' CR are also seen with this approach. It has also shown benefit in animal models of HD¹², PD¹³, AD¹⁴, diabetes¹⁵, cancer¹⁶ and asthma¹⁷. I will discuss it in more detail in the next chapters.

ADCR seems even more likely than chronic CR to promote autophagy as the trigger is pulled on a regular basis. It is puzzling though why a single day would produce any effect on available calories since the liver maintains a readily-available store of about 500g of glycogen, a starch, representing a reserve of 2000 calories, enough for a whole day. It seems more likely that it is the lack of amino acids from protein that is inducing the autophagy by a pathway independent of the insulin signaling pathway.

Autophagy has been directly implicated in CR at least in roundworms¹⁸. Autophagy can be microscopically observed in the living worm when calories are restricted or when induced by a drug. Either CR or the autophagy-inducing drug (rapamycin) extends life span but the two together are not additive. This

strongly implies that the life-span extending benefit of CR in worms is from autophagy promotion alone.

A study in fruit flies¹⁹ has shown that an alternate day protein no-protein diet extends the lifespan as much as a ADCR diet. This and the worm study together (along with many other studies) strongly imply to me that protein restriction promotes autophagy.

This then is the progression for the scientific rationale for the protein cycling diet:

- ADCR extends life
- ADCR promotes autophagy
- Autophagy extends life
- Protein cycling promotes autophagy

Therefore, if Socrates is a man, protein cycling extends life.

8. The Natural State

One question that arises from CR studies is why the cell would not practice autophagy independent of diet if the benefits were so substantial? A good question, one that should be asked of any simple panacea. To answer the question, we need to consider the 'natural state' within which human evolution has operated.

Consider the case with ascorbate (vitamin C). Humans and other primates lack the ability to synthesize it and as a result are prone to scurvy and other problems when it is in short supply. It is thought that primates in the 'natural state' lost the necessary gene, the so-called 'thrifty gene' hypothesis²⁰, since they were primarily tropical and ate lots of fruit and the loss of the gene had no immediate down side. There was no selection pressure to retain it and we now suffer the consequences.

Consider then the benefits of exercise to human health. Again in the 'natural state' vigorous exercise was probably a given for all individuals. There was no selection pressure to provide its benefit in its absence.

Similarly there was probably little selection pressure to promote autophagy independent of diet since being on the edge of starvation is so common in the natural state. Animals generally reproduce until their numbers match the available food supply, which is more-or-less the definition of being on the edge of starvation. In the natural state, an animal crosses that edge many times in its life, inducing autophagy each time.

Nor would they be eating 'balanced' meals. When a fruit was ripe, they would eat that until it was gone. Similarly with any other scavenged food source. There would be times of low protein intake as well as times of high with days or weeks between. Even when fed to satiation, our genetic ancestors could have been protein cycling.

We cosmopolitan humans have 'progressed' beyond the natural state and must consciously compensate by exercising, taking vitamins, and dieting. protein cycling may then be just one more activity to add to that list.

Before we managed fire, our days were governed by the sun. In the tropics, the sun is down for 12 hours every day. Without fire, there is little to do in the dark but sleep. So our ancestors likely fasted at least 12 hours every night even if they ate continually throughout the day. A 12 hours fast with our ancestors' diet may have been sufficient to induce autophagy and perhaps they

were already protein cycling. Even today the conventional American diet is three meals a day in one 12 hour interval and two 6 hour intervals. The English language even recognizes the overnight as a fast and calls the morning meal 'breakfast.' On a low protein diet, the overnight interval may be sufficient to induce autophagy and perhaps protein cycling is routine for most people of the world. Indeed the traditional breakfast in many cultures is notably lacking in protein and perhaps there are 18 hours of protein fasting when that is the case. In parts of the Amazon, for instance, breakfast is often just coffee and a piece of casabe, a no-protein bread made from cassava. The 'traditional' breakfast in most countries is coffee or tea and bread with sugar in some form. Again protein is notably lacking. Interestingly, caffeine and similar substances, as found in coffee or tea, are also promoters of autophagy. They inhibit the same enzyme known as 'mammalian target of rapamycin' (mTOR) just as does rapamycin, the standard research drug for inducing autophagy²¹.

Perhaps the health problems peculiar to modern culture, the increasing incidence of AD and especially of diabetes²², are a consequence of the extinguishing of the overnight protein fast. Many people now eat protein-laden snacks well into the evening and have 'balanced' high protein breakfasts the next morning. This, together with a high protein diet generally, may be enough so that routine autophagy that the cell may depend on for aggregate clearance fails to occur. If this speculation were true, and it is just a speculation, then protein cycling could be achieved merely by not snacking in the evening and eating a low protein breakfast with coffee or tea!

9. Mortality and Morbidity

Modern governments for all sorts of mundane reasons now collect and publish data on the health of their populations. Mortality data identifies the causes of death within a population. Here is some selected data from a publication of the United States for the year 2005²³.

Cause of death	All ages	25-34	35-44	45-54	55-64	65-74	75-84	85+
All causes	2,448,017	41,925	84,785	183,530	275,301	398,355	686,665	703,169
Parkinson's disease	19,544	4	7	66	434	2,414	9,294	7,322
Alzheimer's disease	71,899	2	10	80	648	3,813	23,139	43,906
Cancers	559,312	3,601	14,566	50,405	99,240	138,446	166,421	83,455
<i>colon, rectum and anus</i>	53,252	305	1,297	4,343	8,153	11,792	16,254	11,054
<i>pancreas</i>	32,760	64	565	2,744	5,984	8,288	10,147	4,962
<i>trachea, bronchus and lung</i>	159,292	133	2,323	12,624	31,363	48,390	49,032	15,404
<i>breast</i>	41,491	356	2,497	6,232	8,646	8,113	9,382	6,252
<i>prostate</i>	28,905	3	24	395	2,154	5,764	11,666	8,897
<i>lymph and blood</i>	55,028	811	1,498	3,687	7,623	12,487	18,109	9,671
<i>All others</i>	62,851	701	1,827	5,558	10,422	14,019	18,480	10,848
Diabetes mellitus	75,119	617	2,045	5,691	11,301	16,183	23,136	15,903
Cardiovascular diseases	856,030	4,041	15,852	46,928	79,896	124,366	256,362	326,066
<i>Heart</i>	652,091	3,249	12,688	38,103	65,208	96,729	190,693	243,504
<i>Kidney</i>	24,902	84	406	1,166	1,954	3,306	7,256	10,703
<i>Stroke</i>	143,579	546	2,260	6,381	10,028	18,839	46,859	58,183
<i>Atherosclerosis</i>	11,841	6	31	193	440	1,008	3,387	6,773
<i>Other circulatory</i>	23,617	156	467	1,085	2,266	4,484	8,167	6,903
Influenza and pneumonia	63,001	354	934	2,183	3,422	6,623	18,563	30,267
Chronic lower respiratory	130,933	258	890	3,977	12,747	29,910	50,333	32,473
Inhalation pneumonia	7,279	66	172	476	833	1,741	5,558	8,352
Other respiratory system	9,466	6,955	27,056	181	474	1,360	2,676	5,373
Liver disease and cirrhosis	5,781	1,002	27,530	311	2,688	7,517	7,126	5,066
Kidney disease	14,403	14,693	43,901	285	742	2,028	4,141	7,320
Septicemia	10,626	9,544	34,136	311	840	2,211	3,912	6,073
All other diseases	56,700	79,449	217,632	3,159	8,118	17,122	21,108	27,236
Accidents (unintentional injuries)	117,809	13,997	16,919	18,339	10,853	8,632	13,854	14,243
Intentional self-harm (suicide)	32,637	4,990	6,550	6,991	4,210	2,344	2,200	860

We see in that year that, for those aged 65 to 74, deaths from cardiovascular diseases were over 20 times that from AD and PD. The same for deaths from cancer. Twice as many die of diabetes. Even for those 85 and older, cardiovascular diseases still account for over six times as many as those from the common neurodegenerative diseases. Of course this table is for the primary cause of death and a neurodegenerative disease may underlie something like accidents, suicide, septicemia, inhalation pneumonitis, etc. Nevertheless cancer and cardiovascular disease overwhelm neurodegenerative diseases as a cause of death. Further hypertension, cardiovascular disease and diabetes are themselves correlated with dementia and parkinsonism, the main two reasons for protein cycling in the first place.

In view of this does it make sense to adjust a diet to prevent neurodegenerative diseases when cancer, diabetes and heart disease are much greater threats and are themselves responsive to dietary manipulation? Clearly we would not want a diet that increases cardio, diabetic or cancer risks. Likewise, we would not want a diet that puts reducing minor threats ahead of major threats. If you have the discipline to follow protein cycling indefinitely, you should certainly have the discipline to reduce weight if indicated (or increase it).

There are, however, at least two good reasons why we might want to prevent neurodegenerative diseases before all others: heredity and morbidity.

The death data in the chart is for the entire U.S. population and reflects average risk. Many of us, however, are at much higher risks. Perhaps our ancestors have suffered AD or PD at much higher rates than average. Or more directly perhaps we have tested positive for HD and know we will eventually develop the disease. AD and PD as well as ALS and HD all have familial forms where a particular gene defect has been identified and perhaps we know we have a 50% or greater risk based on heredity and a poor choice of parentage or a 100% risk based on genetic testing..

Morbidity is the measure of the degree to which a disease reduces the quality of life. Its measure is subjective and necessarily less precise than the measure of mortality but can be the more important measure to some. Given the inevitability of death, we might prefer to go with at least our minds intact without the horror of dying a little bit every day for years before the big finale. That is not to say that cancer, diabetes and heart disease are more pleasant

though their duration is often shorter; perhaps it is just a matter of personal taste.

Even if our prevention goal is targeted to a neurodegenerative disease, we must consider the affects of our actions on the far more common diseases of aging. Heart disease, diabetes and even cancer rates all increase with obesity. To begin protein cycling while significantly overweight would be ignoring the elephant in the room so to speak. You might say it would be penny wise and pound foolish.

Of course a weight reduction plan could be combined with protein cycling. Calories could be counted and contained and a regular exercise regime followed. Exercise might then be scheduled for when you are on the protein positive half of the cycle when your energy levels are at their highest.

The body mass index (BMI) has been developed as a simple age and sex neutral measure of ideal weight as 19 to 25 kg per square meter of body surface area (skin). Anything above 25 kg is overweight and below 19 underweight. Medical evidence suggests that all adult body weights within this range are reasonably equally healthy. Outside this range, health risks may occur. Above this range, the rates of heart disease, diabetes and cancer all increase dramatically. Of course measuring body surface area is impractical and the area is generally estimated from height. Numerous web sites provide calculators where you can get your individual BMI range.

The amount of abdominal fat as reflected in waist size has also been identified as a measure of risk apart from BMI from recent studies. Males should have a waist less than 40 inches (37 inches for Asian males) and females should have a waist less than 35 inches (31 inches for Asian females). The ratio of waist to hip should not exceed 0.9 for males or 0.8 for females.

If you have the discipline and need to reduce weight quickly, the alternate day calorie restriction diet (ADCR) may be better suited. It avoids all calories, not just protein calories, in the fasting phase and should induce autophagy as much as or more than the protein cycling diet I am proposing. The protein cycling diet is in fact modeled on the ADCR as a less obtrusive way to achieve the same results. I will discuss it later in its own chapter.

As an example, a 170 lb male adult consuming 2700 calories per day could conserve 8100 calories per week on a ADCR diet if fasting every Monday, Wednesday and Friday and eating normally otherwise. Since there are 3500 calories in a pound of fat, he would expect to lose a little over two pounds a

week He could thus rapidly reduce his weight to his ideal BMI range and waist size while getting the benefits of autophagy as well. When he reaches his weight target, he could switch to just restricting protein Monday, Wednesday, and Friday and keep his weight constant.

Aside from weight reduction there is preliminary evidence that calorie restriction and the ADCR diet and, by implication, protein cycling may work against cancer²⁴, asthma and diabetes²⁵ as well. In fact much of the current activity in anti-cancer drugs centers on autophagy promoters²⁶. Autophagy induced by protein cycling may even work against some infectious diseases. The tuberculosis bacterium, for instance, hides in an autophagosome within the cell and produces a substance that inhibits lysosomes from merging with the autophagosome and completing autophagy. CR autophagy induction has been shown to override this inhibition and thus promote destruction of the invading bacterium²⁷.

Finally evidence is accumulating that atherosclerosis, the condition underlying most heart disease, is itself benefited by autophagy enhancement; this subject will be discussed in detail later in its own chapter.

10. The Protein Myth

One thing you discover when you try to formulate a low protein diet the difficulty of finding suitable foods. When the national Academy of Science established its Recommended Daily Allowances for protein, it started with the amount of protein and its breakdown products lost by the average adult male body per day²⁸. Since nitrogen is found in proteins but not fats or carbohydrates, its measure serves as a close measure of protein. Obligatory urinary nitrogen losses at about 37 mg/kg/day, fecal nitrogen losses at 12 mg/kg/day, perspiration, hair, fingernails, and sloughed skin nitrogen losses at 3 mg/kg/day and other losses at 2 mg/kg/day imply a protein loss of 0.34g/kg/day or 0.15g/lb/day. Any net recovery from the diet beyond that in the absence of growth is converted to carbohydrate (sugar) and burned for energy or then converted to fat. Of course energy is as legitimate a need as any other and there is nothing bad about getting it from protein, unless you have a condition like some kidney diseases for which a low protein diet is indicated.

Generally people eat until their energy needs, as measured in calories, are fulfilled. Some people do eat for other reasons but that is topic for a later discussion. A 170 lb adult male typically consumes 2700 to 3000 calories a day. His absolute minimum protein requirement then works out to be about 25 grams of protein per day (170 lbs X 0.15gram/lb/day).

Fat provides about 9 calories per gram consumed. Protein and carbohydrate provide 4 calories per gram. The 25 grams of protein consumed by our 170 lb male then represents (4 X 25)= 100 calories. So, if 100 calories out of 2700 calories consumed per day are protein then our 170 lb male has met his minimal protein requirement. 100 is 3.7% of 2700 so we can further say that a young adult male of any weight or caloric consumption gets his minimal protein requirement when the calories he gets from protein exceed 3.7% of his total caloric intake. Note that the elderly seem to have a somewhat higher protein requirement.

If he is less active, 2000 calories a day may be a more accurate number. In that case 100 is 4% of 2000 and we can say that a less active adult male of any weight or caloric consumption gets his minimal protein requirement when the calories he gets from protein exceed 4% of his total caloric intake (a nice round number).

All foods in the US now carry a nutrition label like the following:

Sample label for
Macaroni & Cheese

Nutrition Facts	
Serving Size 1 cup (228g)	
Servings Per Container 2	
Amount Per Serving	
Calories 250	Calories from Fat 110
% Daily Value*	
Total Fat 12g	18%
Saturated Fat 3g	18%
Trans Fat 3g	
Cholesterol 30mg	10%
Sodium 470mg	20%
Total Carbohydrate 31g	10%
Dietary Fiber 0g	0%
Sugars 5g	
Protein 5g	
Vitamin A	4%
Vitamin C	2%
Calcium	20%
Iron	4%
* Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.	
	Calories 2,000 2,500
Total Fat	Less than 65g 80g
Sat Fat	Less than 20g 25g
Cholesterol	Less than 300mg 300mg
Sodium	Less than 2,400mg 2,400mg
Total Carbohydrate	300g 375g
Dietary Fiber	25g 30g

The number of calories and the grams of protein per serving are always listed. If you multiply the grams of protein by 4 and divide by the number of calories, you will get the fraction of calories from protein. Multiply that fraction by 100 to get the percentage of calories represented by protein.

As becomes apparent, all staple foods have a protein percentage much greater than the 4% actually needed for protein synthesis by the body. Rice, for example, has the lowest protein content of the grains common in the Western diet yet is 8% in protein calories. A diet of just rice would provide over twice one's minimum protein needs. Even a diet of just cookies and diet cola (not recommended) could provide sufficient protein.

Of course this assumes that all the protein in the food is absorbed in the digestive process. Probably the less calorie-dense a food is the less true is this assumption. If you ate just broccoli, for example, even though the protein calorie percentage is high, the vast bulk you would have to consume to net

enough calories would likely prevent a large fraction of the protein from getting through to the bloodstream.

Note that human milk, the ideal complete food for humans for the period of time when they are growing the fastest, has only 6% of its calories represented by protein!

Increasing the 0.34 minimum as a safety margin, the World Health Organization recommends 0.45 grams/kg/day of protein. Doubling this the United States Department of Agriculture recommends 0.8 grams/kg/day for average healthy adults. For adults ranging from 100 to 200 lbs this is around 20 to 40 grams per day using the WHO number, 40 to 80 using the USDA number. By way of quick reference, a serving (6 oz) of meat or cheese is around 40 grams of protein, a similar serving of milk, beans or tofu around 10 grams, and a serving of grain or vegetable around 4 grams.

You can readily see that a typical Western industrial diet far exceeds these recommended minimums. Most protein ends up converted to carbohydrates and the nitrogen removed becomes a potentially toxic waste product that the body must then dispose of, principally as urea in the urine..

Why then does protein get such a positive press? The reasons are mostly historical. First children have elevated needs for protein to support growth and display symptoms of protein insufficiency during times of famine when adults do not. Second the high protein foods, meat and cheese, have been preferred by the rich and powerful (though this is as much for their fat content and energy density as for their protein) and have gained favor thereby. Third a significant portion of the population, especially in the past in Western nations, are or have been alcoholics and actually manage to consume their calories in alcohol to the exclusion of everything else. Finally government food policies have been given to departments of agriculture where meat and dairy interests are paramount rather than to departments of health.

The dangers of too little protein consumption are more severe than the dangers of too much and it is not surprising that advisories would be conservative on the up side.

Regardless, following the protein cycling diet does not reduce your overall protein consumption, just the timing of when it is consumed and not consumed.

11. Designing the Diet

With the goal of preventing or delaying neurodegenerative diseases by promotion of autophagy, we now must design a suitable diet plan. For this, certain questions must be answered: how much protein restriction is necessary and for how long and how often.

For the first question, how much protein restriction is necessary, the reasonable answer is that calories from protein as delivered to the bloodstream must be reduced to less than what the body normally needs for its own protein synthesis needs, namely 4% of total calories. When that level is achieved, the cells must then perform autophagy to obtain the amino acids they need to carry on.

Ideally most of the protein in your normal diet should be replaced by starches rather than by sugars or fats. Both proteins and starches are long polymers that take time to digest in a time-release manner so to speak. Sugars on the other hand digest very quickly and are more disruptive to your metabolism as a result. Likewise changing the balance of fat to non-fat can be disruptive as well. Realistically though, you will likely find it more practical to increase fat and sugar during periods of protein restriction. If these periods are of short duration, it should hardly matter. We will talk more specifically on this subject in later chapters in more detail.

For the second question, how long should a period of protein restriction last, we can consider the results of two areas of study. In free cell culture, starvation autophagy begins within two hours following transfer to a medium lacking in amino acids. And as will be discussed in the next chapter, there is a form of the Calorie Restriction diet where the dieter alternates days of fasting and eating. In both humans and mice this regime produces the benefits of the other forms of calorie restriction which are proposed to result from autophagy promotion. It is then reasonable to conclude that a single day of near total protein restriction is sufficient to initiate starvation autophagy.

The final question is how often should protein restriction be practiced. The answer to that is a little more involved.

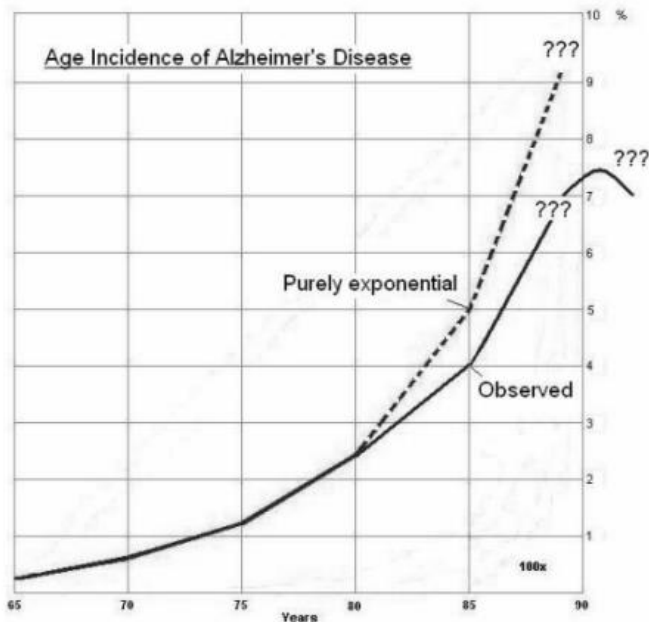
First we must consider what the purpose of promoting autophagy is. It is to clear the cell of degraded and aggregated proteins that are not being handled by the other recycling mechanisms of the cell. To prevent the accumulation we

must clear them at a least the same rate as they are produced. We do not really know the production rate of these aggregates but we can infer them from the known usual courses of the diseases we are trying to forestall.

We can calculate the rate of clearing by autophagy from certain known values. We know a 70,000g (154lb) male requires about 25g of protein per day to meet his own protein synthesis needs. Perhaps the body conserves a bit when protein is restricted so, to be conservative as well, we will say 20g of protein is the minimum. We know that cell contents account for about 2/3 of body weight*. We know that the body is about 17% protein and 17% fat by weight. $70,000\text{g} \times 17\% \times 2/3 = 8000\text{g}$ of cell protein. When autophagy is induced by protein starvation, at least 20g per day of protein must then be recycled. $20\text{g}/8000\text{g} = 0.25\%$ per day recycled. As long as that value exceeds the rate of accumulation of aggregates, a neurodegenerative disease produced by those aggregates can in theory be prevented.

Measured values for cytoplasm recycling by autophagy are actually much larger than 0.25% per event. When mouse cancer cells are deprived of amino acids for 2 hours, 2% of the cytoplasm is seen to be recycled³⁰. Of course we are concerned about the value in human neurons which could be much smaller. So we will stick with the 0.25% value. Even then it may be that some other tissue such as muscle is preferentially sacrificed to provide amino acids to the brain, and thus the real value could be less. 0.25% is only an educated guess.

Late-onset AD with 26.6 million sufferers worldwide in 2006 tops the list of diseases we would want to prevent. The numbers for AD are clouded by historical and cultural factors but, nevertheless, its real incidence rate seems to be rising over the decades. For the common non-genetic form, about 3 people per thousand will develop it in their 65th year. For every five to six years thereafter, the risk of acquiring the disease approximately doubles. From onset to death takes about 7 years on average. After age 90 the incidence may even decline.



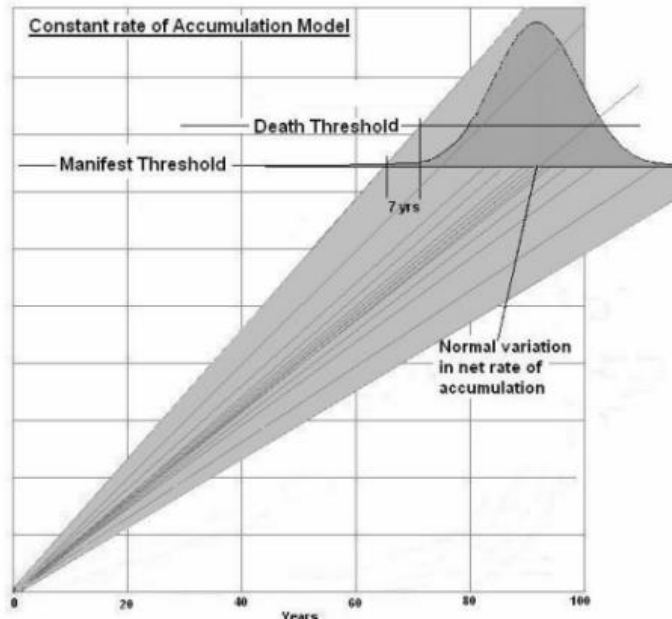
Lewy-body associated diseases including PD are next with about 8 million sufferers worldwide extrapolating from North American values.

The other recognized neurodegenerative diseases: prion diseases, frontotemporal dementia, Pick's disease, progressive supranuclear palsy, ALS, HD, and spinocerebellar ataxias altogether account for only around 800,000 cases worldwide by extrapolation from North American values. Compared to AD and PD, they may be of small concern. In many cases, however, the cause is genetic and people know they are at risk or even are certain of developing them should they live long enough.

First we shall look at AD. If the disease in fact results from an accumulation of aggregated proteins, what can we infer about the rate of the accumulation?

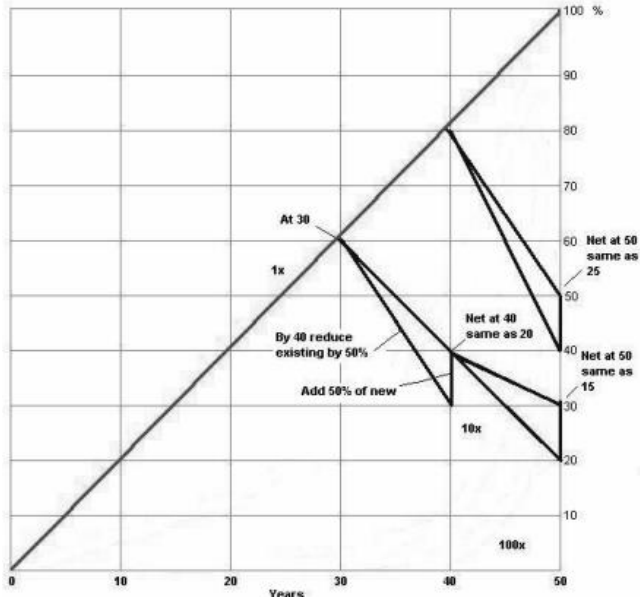
The simplest model assumes a steady rate accumulation over a life-time. When the accumulation reaches some threshold value, the disease manifests. When the accumulation reaches a second threshold, death occurs. Variation in

age of onset is then due to personal variation in rate of accumulation and most people do not live long enough to reach this threshold.



Applying this model to AD, we infer a worst case maximum increase of $1/65$ of the manifest threshold per year or $(1/65)/365 = 0.0042\%$. To delay the disease indefinitely, we must replace at least $1/65$ of our cytoplasm year or $(1/65)/365 = 0.0042\%$ per day. Since autophagy replaces 0.25% per day, the minimum number of days in the year where protein should be restricted is $(0.0042\%/0.25\%) * 365 \text{ days} = 6.2 \text{ days}$.

The following illustrates what might happen when more intensive protein cycling is used against an aggregating protein that reaches its manifest threshold at age 50.

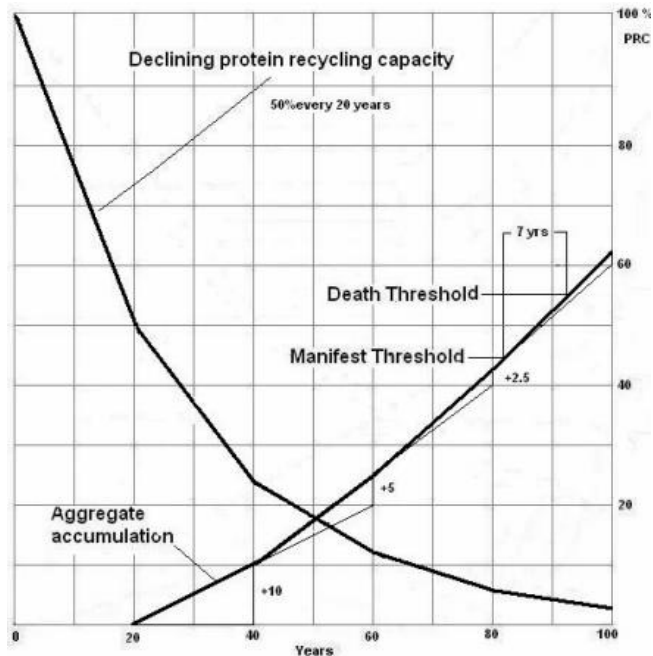


If at age 30 protein cycling is initiated at a 50% cytoplasmic replacement rate per decade, by age 40, the accumulation is knocked back to the same level as at age 20. We can break this down into two processes. In the decade after initiation the existing aggregate is cut down by half, the equivalent of 15 years of accumulation. At the same time, 10 years of new aggregate is added at half its normal rate resulting in 5 years of accumulation. 15 plus 5 is 20.

Since autophagy replaces 0.25% per day, one might think we could calculate the number of days per year of protein fasting required as $\text{days} = (50\%/\text{decade} / 0.25\%/\text{day}) = 200 \text{ days/decade} = 20 \text{ days/year}$. The error in such a calculation, of course, is that each autophagy event consumes not just old cytoplasm, but also some fraction of the new cytoplasm that replaced what was lost in previous autophagy episodes. I will give the correct calculations in a table below after discussion of some other models of aggregate accumulation that imply a much larger number than just 20 days a year of protein restriction.

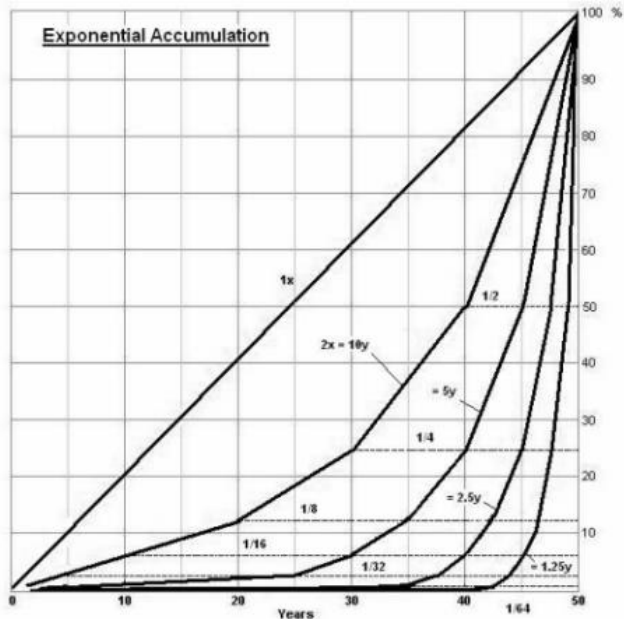
Next we will consider a slightly more complex model where the aggregate protein is again produced at a constant rate, but the cell employs a mechanism to recycle the aggregate. As long as the recycling rate exceed the production

rate, no accumulation occurs. However, in this model over time, the protein cycling mechanism of the cell declines and aggregate accumulation eventually begins. As the recycling capacity decays, the net rate of production minus recycling grows.



In this example we have the recycling capability declining by half every 20 years. As long as the rate of recycling is greater than the rate of accumulation of aggregate, no accumulation occurs. At the point where the rates are equal, accumulation begins and accelerates towards but never exceeds its constant rate of production.

In the next model we consider what happens when the rate of aggregate protein production is not constant but increasing with time. This is what would be expected with the protein mis-folding theory of degenerative diseases discussed in previous chapters where the accumulation of aggregate protein itself causes its production rate to increase. This is a true exponential increase.



The 1x line represents the aggregate accumulating at a constant rate. For the 10y line, the rate doubles every decade. For the 5y line, the accumulation rate doubles every 5 years. Likewise the 2.5y and the 1.25y lines double every 2.5 and 1.25 years respectively.

This model overcomes one of the problems with the other models, the relatively small difference between the level of aggregate at which the disease manifests and the level at which the disease proves lethal.

When a new drug is developed, two dose levels are determined: the therapeutic dose and the LD50 dose. The therapeutic dose is the level where the benefits of the drug manifest. The LD50 dose is the level where 50% of the test subjects die. Usually there is more than a ten-fold difference between the two values. neurodegenerative diseases generally manifest about a decade before death. In this chart, if 100% is the death threshold at age 50 then 80% is the manifest level at age 40 with the 1x constant increase model. Rarely would the therapeutic dose of a drug be as large as 80% of the LD50. If the aggregate

follows similar dynamics as a drug, it would seem doubtful that the constant production 1x model really applies.

With the 10y doubling, the difference between the onset and lethal levels is a more reasonable 2 to 1 for an onset at age 40 in the chart above. Likewise with the 2.5y doubling, the difference is a quite reasonable 10+ to 1. With the 1.25y line, on the other hand, it seems like onset would be more likely around 45 than 40.

The problem with doubling is that there first has to be something to double. We cannot start at zero. In the self-catalyzing protein mis-folding model, there is an initial spontaneous mis-folding or mis-foldings that forms the nucleus to catalyze further future mis-foldings. The question then arises as to whether the number of protein molecules that must mis-fold on their own without 'direction' from other mis-folded proteins of the same species is a reasonable number.

A typical nerve cell in the brain has about a 30 micrometer diameter. Such a cell might have around a thousand separate species of proteins. Calculating roughly, each species on average would have about a billion (10^9) protein molecules of its type. If a single protein molecule mis-folded at age zero and was capable of causing one other of its brethren to mis-fold similarly every 2.5 years, in 75 years, nearly 100% of the billion molecules of that type could be mis-folded. A similar result happens with a doubling every 2 years in 60 years or every 1 year in 30 years. Such is the power of exponential growth.

So it seems that the exponential model of aggregate accumulation passes the test and well approximates the actual dynamics of the neurodegenerative diseases. Unfortunately, this model implies a much higher accumulation rate than the previously discussed models, as much as a doubling every 2.5 years. A successful response would have to diminish aggregate at nearly the same rate. The following table shows the rates to be expected from various protein cycling regimes:

Estimated Annual Cytoplasm % Replacement with Various Protein Cycling Regimes:

Years	1/week	2/week	3/week	3.5/wk	4/week	3/month	5/month	7/month
1	12	23	32	37	41	09	14	19
2	23	41	54	60	65	16	26	34
2.5	28	48	62	68	73	20	0.31	41
5	48	73	86	90	93	36	53	65
10	73	93	98	99	99	59	78	88

Table 4

From the table we can see that 3 days a week of protein restriction would be sufficient to counteract an exponential accumulation that doubles every 2.5 years. This then is my most recommended regime: three 24 hour periods each week where very little protein is consumed.

Of course it is not necessary to match or exceed the rate of aggregate accumulation since a lower rate, though not preventing disease development, can delay its onset past the expected date of death from other natural causes.

It is not necessary to align the periods with day boundaries. One could say restrict protein from Saturday evening through Sunday afternoon, Monday evening through Tuesday afternoon, and Wednesday evening through Thursday afternoon every week.

Nor is it necessary to stick tightly to the schedule as long as, over time, the necessary numbers are achieved. If, for example, a social eating event came up on a scheduled no-protein period, one could just shift the period a few hours to accommodate the event.

Since the protein cycling diet is a life-long commitment, it needs to be as unobtrusive as possible. The remainder of the book will deal with ways to achieve this goal without turning your life completely upside down.

Note that after neurological disease symptoms appear, it may be too late to prevent further disease progression since the doubling rate may conceivably already exceed the maximum halving rate obtainable with protein cycling. If you are at particularly high risk of a neurodegenerative disease, you should commence protein cycling as soon as practical if you believe and intend to use it.

If you already have a neurological disease, you should be under the care of a physician and should clear with him or her before undertaking protein cycling, ADCR or any other diet.

12. The Alternate Day Calorie Restriction Diet

As I mentioned earlier, protein cycling was inspired as a less stressful way of achieving the benefits seen with the alternate-day calorie-restriction (ADCR) diet.

The ADCR diet has been around a few years and its practitioners have reported benefits for a broad range of conditions including:

- Obesity
- insulin resistance
- asthma³¹
- seasonal allergies
- viral diseases
- Lyme disease
- recurrent bacterial tonsillitis
- chronic sinusitis
- periodontal disease
- rheumatoid arthritis
- osteoarthritis
- menopause

In this diet calories from protein, carbohydrate and fat are restricted every other day as opposed to just the calories from protein in the protein cycling diet. It has been shown not to change the overall metabolism of the organism⁸⁷ and must therefore act through some other mechanism most likely autophagy. It should therefore be as effective as protein cycling in inducing autophagy and even more effective for weight reduction. Some of the other benefits to conditions listed above may not be seen with protein cycling; the studies have yet to be done.

For these reasons, especially for initial weight reduction, you might want to try this diet before starting a lifelong protein cycling regime. Fortunately there are ways to make this diet less stressful than it otherwise appears.

First it is not necessary to cease all eating. Many foods are so low in calories for their bulk that they effectively deliver zero net calories after digestion. Most raw or pickled vegetables and many cooked vegetables such as spinach fall into the category and can be freely eaten on CR fast days. Sugar free gum can occupy the mouth. Soluble fiber drinks can occupy the stomach and provide a sensation of fullness. Guar gum or psyllium work well for this purpose.

Nor do all calories have to be eliminated on fast days. In studies benefits have been seen with a mere 50% calorie reduction on fast days. I would think up to 300 calories a day would be allowed for milk in coffee or tea or other 'necessary' indulgences.

Nor does every other day have to be a fast day nor does a fast day have to begin at midnight. The week has an uneven number of days. Fasting every Monday, Wednesday and Friday might better suit a weekly schedule since you are likely to feel weaker and sleep more when fasting. The weekends are then freed for social or recreational eating and high energy activities.

One of the arguments advanced against the ADCR diet is that, since it resembles starvation, it would produce a condition sometimes associated with starvation, rhabdomyolysis, that occurs when muscle cells are damaged and release the protein myoglobin into the bloodstream where it gums up the kidneys. In fact, where it is seen in association with starvation, the cause is lack of vitamins or electrolytes (sodium, potassium, calcium, magnesium, chloride etc.). Or alcohol poisoning is also present and it the real cause. For the ADCR diet and for protein cycling, nutrients other than calories or protein are kept normal and this argument should not apply. The muscle 'wasting' from autophagy is internal to the cell. The cell does not die and does not release its contents into the bloodstream.

For perhaps most people, the ADCR diet is not so difficult as it first appears though one major concern for many is maintaining adequate blood sugar levels. Most people at some point in their lives have experienced the dizziness and distress from low blood sugar as a result of a delayed or missing meal, and that is what comes to mind when they envision dieting of any form. This is a particular problem for some people more than others.

I have myself followed the ADCR diet while monitoring my blood sugar levels. For me they have dropped some but have remained in the normal range on fast days. Home blood glucose testing kit are now freely available (in the USA at least) for about a dollar per test at the time this was written. You can then monitor your own blood sugar levels to see if your dieting is creating problems. (Tip: if you numb your finger with an ice cube, the necessary pricking can be painless though you then have to wait for the finger to warm before the blood flows.)

If you are unable to maintain adequate blood sugar levels on the ADCR diet, you can use protein cycling instead. You must then watch your over-all calories and accept a lower rate of weight reduction if that is your goal.

Even if your intention is to use the ADCR diet indefinitely, you may want to ease into it with some preliminary rounds of protein cycling. Since the protein cycling diet is less demanding, you might want to start with the protein cycling diet to get used to the pattern and then switch to the ADCR diet to lose weight or to get its 'proven' benefits.

13. Lysine and Methionine

The dietary requirement for protein is more precisely a requirement for the amino acids found in protein that the adult body cannot synthesize on its own. These so called essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. Tyrosine can be made from phenylalanine and vice versa. Likewise cysteine from methionine.

A protein source must provide these essential amino acids in the same proportions as in the proteins being synthesized in order to be 100% utilized. If say lysine were in only half its required proportion then a maximum of only 50% of the dietary protein could end up as body protein. Protein synthesis allows no substitutions. The rest would have to be converted to carbohydrate by removing the nitrogens and burned for energy.

Normally this matters little since the standard western industrial diet provides many times the amount of amino acids required for the body's protein synthesis needs. Lysine in rice protein, for example, is present in only 64% of the proportion needed for human protein synthesis. We then say its availability is 64%. Yet if you met your calorie requirements with rice alone, you would still get 256% of your lysine needs.

The goal of protein cycling, however, is to minimize protein availability during restriction, not to maximize it. We see from the food table that 100g of rice provides 130 calories of which 8% come from protein. 8% may be a little too high to trigger the autophagy we desire. For a meal where rice was the only protein source, however, we reduce the 8% to $(8\% \times 64\%) = 5\%$ since lysine limits availability to 64% in normal rice. Add a 100 calorie 0g protein pat of butter and the effective percentage drops to below 4%, well below the level where autophagy must occur.

As for other low-protein calorie dense foods, isoleucine is limiting in white potatoes at 60% and lysine is limiting in wheat at 45%. Modern corn has often been genetically enhanced to produce more lysine and tryptophan so exploiting the limiting amino acids in corn can no longer be done reliably. The same may soon apply to wheat as well. And sometimes lysine is added to flour or bread to 'enrich' it.

Lysine has another property we can exploit. When proteins are heated, especially in the presence of sugars, some of the lysines in the protein chemically react and become permanently unavailable. When bread is toasted,

it is this process that is thought to produce the characteristic browning and aroma.

From the food table we see that unenriched white bread has a protein calorie percentage of 11%. Since lysine is limiting in wheat at 45%, the effective percentage is $11\% \times 45\% = 5\%$. Slice it thin and toast it dark and the percentage may perhaps drop to 4%. Add a 100 calorie 0g protein pat of butter and the effective percentage drops to well below the magic 5%.

In some studies, the benefits of protein restriction to longevity have been traced to reduced total intake of methionine¹¹ (or tryptophan in a few studies). It is hypothesized that methionine is particularly susceptible to oxidation and the production of ROS that damage cells⁸⁹. Though protein cycling would not necessarily reduce total methionine intake, this issue does cloud interpretation of intermittent fasting studies on which protein cycling theory leans.

14. Anti-Oxidants

In chemistry, oxidation is any chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent, often but not necessarily oxygen. Oxidation reactions can produce so-called free radicals, the reactive oxygen species ROS mentioned previously (also some reactive nitrogen species). Bearing an unpaired electron, these radicals can readily attack and destroy other molecules in the cell. Anti-oxidants are substances that remove free radicals. They do this by being oxidized themselves. In this sense, anything that a free radical can oxidize can be called an anti-oxidant. Note that this does not mean that a given anti-oxidant is beneficial because it is an anti-oxidant. Likely over half of biological compounds can be considered as anti-oxidants but only in the same sense as books and antique furniture can be considered fuel for a fireplace; their true role in the cell lies elsewhere. Controlled free radical production is necessary to many cellular enzymatic reactions and an over-zealous anti-oxidant could be poisonous. Further some anti-oxidants when oxidized then become a pro-oxidants in some situations; ascorbic acid (vitamin C) is a case in point.

Both inside and outside the cell, anti-oxidant activity is largely mediated through enzymes specific to that function. Anti-oxidation is a tightly controlled process in the cell and the idea that swamping it with small-molecule enzyme-independent anti-oxidants from the diet would be beneficial seems far-fetched.

Higher primates have unusually high levels of anti-oxidant activity compared to other mammals. Concurrent with the loss of the ability to synthesize ascorbic acid they have lost the ability to degrade uric acid. Uric acid is thus present in high levels in human serum where it acts as a highly-effective anti-oxidant. Indeed humans could be considered saturated in anti-oxidant potential compare to say mice and dietary supplementation beyond overt deficiency levels likely has little effect on health outcomes and explains why promising mouse studies on anti-oxidants invariably fail when performed on humans. The point of this discussion is to argue that measures to prevent ROS damage in the first place, most popularly currently by anti-oxidant supplementation, are not likely to be effective whereas measures to enhance clean-up of the damage afterwards such as provided by autophagy are more promising. In fact many of the substances touted as anti-oxidants more likely produce their benefits by enhancing autophagy; resveratrol, for instance. One

issue that clouds all the studies is that ROS provokes a general response in the cell including anti-oxidant production and autophagy. In fact a lot of substances described as anti-oxidants are really just ROS mimics that provoke this response or even pro-oxidants that generate some ROS themselves that then provokes it (exercise for example). In the same way as a vaccine provokes an immune response, some level of ROS stress may be needed to provoke the cell to a proper level of anti-oxidant activity and extinguishing it with exotic antioxidants could be counter-productive.

15. Chemical Autophagy Promoters

Autophagy is a complex and vital cell process and just about any substance one tests turns out to have an effect on it. Whether or not that effect is significant in the real world is another question. A number of substances have been found that promote autophagy and clear the protein aggregates associated with neurodegenerative diseases³². Most are either prescription drugs or are unsuited to human medical use. A few, however, are freely available as supplements.

Curcumin

Curcumin is a chemical found in the spice turmeric used throughout Asia as a food additive and a traditional herbal medicine as well. In Japan, turmeric is fermented to diminish its bitterness and sold as a tea powder to cure hangovers. Cultures that consume curcumin regularly (as turmeric in yellow curries) have about a fourth the age-adjusted incidence of AD as those that do not³³. There are research results that curcumin can break up the plaques associated with that disease³⁴.

Curcumin has recently attracted the attention of cancer researchers where it has been shown to kill cancer cells by promoting autophagy^{35,36}. Cancer cells are fragile broken cells. Autophagy is often a cell process they cannot pull off and one explanation is that they end up killing themselves in the effort.

There are, however, some cancer types that can perform autophagy successfully. If such a cancer were being treated with a method that starved cells, inducing autophagy could then end up rescuing the cells and diminishing the effectiveness of the treatment.

Curcumin induces autophagy by the same pathway as calorie restriction, via the signal proteins mTor (mammalian target of rapamycin) and insulin³⁷. Autophagy induced by protein restriction uses a different mechanism that does not involve these proteins. Curcumin may not, therefore, be additive to the protein restriction effect though it should be additive to the calorie restriction effect.

Piperine

The principal argument against the effectiveness of curcumin as an autophagy enhancer is that very little is normally seen in studies to get past the digestion and reach the blood stream. Piperine, the active ingredient in black pepper, is usually added to curcumin in small amounts to enhance absorption by

as much as 2000%³⁸ (with 20 mg). The amount usually added in commercial capsules, about 3mg, is only about 1/6 the average American dietary consumption³⁹. The usual explanation for piperine's effects is that it inhibits the cytochrome systems in the intestine and liver cells that normally degrade the wide array of small chemicals (including curcumin and resveratrol) that plants produce. The observed increase in absorption seems to me far too large for the observed inhibition of the cytochromes and I suspect that it really acts by stimulating some form of endocytosis.

Nutrients generally move from the gut to the bloodstream by selective transport across the cell membranes of the cells lining the small intestine. They are then deposited in the hepatic portal vein and sent directly to the liver. If they make it past the liver they then enter the general circulation. If they can then make it past the blood-brain barrier, they can finally reach the nerve cells.

It is unlikely that the liver would let either curcumin or resveratrol pass intact. There is however a back-door to the general circulation through the lymphatic system. Fats are normally digested down to fatty acids and selectively transported into the cells that line the intestine. Short-chain fatty acids then proceed to the hepatic portal vein. Long-chain fatty acids, however, are transported across the cell and, after some mysterious processing, deposited in the lacteals, the capillaries of the lymphatic system, as so-called chylomicrons. The chylomicrons then move through the lymphatic system and deposit in the general circulation in the subclavian vein far beyond the reach of the ever-vigilant liver. Fat soluble substances such as curcumin and resveratrol can then perhaps hitch a ride in the fat and reach the general circulation.

In the lower reaches of the small intestine there are so-called M cells in the lining of the intestine that engulf particles as large as whole bacteria in a form of endocytosis called phagocytosis. Again fat globules and the substances dissolved in them could be engulfed as well and end up in the lymphatic system. Piperine may stimulate endocytosis and this is possibly why it enhances absorption.

There is another form of endocytosis called pinocytosis that is practiced by all cells to take in certain materials from the bloodstream. Though the amount of extracellular fluid enveloped is too small to contain something as large as a bacterium, a fat globule could conceivably enter a cell by this route. This process has yet to be demonstrated by the cells that line the intestine but

remains a possibility. If it exists, it could allow viruses to enter the body and this may be sufficient reason to disallow it to cells in contact with the outside world.

In fact there are organisms that exploit M cell phagocytosis to enter the body. The M cells pass what they envelop to phagocytes that normally digest them with their lysosomes. These invaders, however, may produce a substance that prevents the lysosomes from merging with the vesicles enclosing them and are thus saved from destruction. Or (as in the case of prion disease protein) the invader can survive the digestive process of the lysosome.

Bloodstream Half-Lives

The kidneys filter blood at a constant rate (GFR) that, apart from actual dehydration, is independent of water consumption. In the kidney, blood cells are mechanically filtered from the stream and returned to the general circulation. Water and solutes are then retrieved from the stream as needed and what is in excess flows to the bladder.

When a substance enters the blood stream, it is immediately subject to removal by the kidneys. The kidneys divert 20% to 25% of the blood flow from the heart and normally filter from 50 to 150 milliliters of blood per minute. For a typical adult blood volume of 5000 milliliters, this represents 1-3 percent per minute. So in 20 to 60 minutes, about half the blood is filtered. For an inert substance that spends no time attached to blood vessel walls or other blood components and that is not reabsorbed by the kidneys, the blood half-life would then be 20 to 60 minutes. (This is near to curcumin's half-life value.) In that time, its concentration would drop by half from its original concentration. In the next 20 to 60 minutes it would drop to one-fourth of its initial concentration, and so on.

A substance that spends some time adhering to vessel walls or blood cells or diffuses into and out of cells and intercellular spaces would have a longer half-life. Likewise for a substance that the kidneys reabsorb from the urine stream.

Conversely, substances that are actively removed from the bloodstream would have shorter half-lives. The liver does this for some substances, depositing them in the bile. The kidneys do some of it as well, actively depositing some wastes in the urine. Phagocytes in the spleen actively remove even solids from the bloodstream and perhaps deposit the remains in the hepatic portal vein that goes to the liver. There liver phagocytes may then

possibly deposit some of it in the bile. There may even be some active (energy expended) and passive transport of wastes from the linings of the intestines.

In a sense, substances may also be removed from the bloodstream by being converted into something else, usually by the liver. Resveratrol, for instance, is converted to resveratrol-glucuronide by the liver. In consequence, the half-life of resveratrol is only minutes but the half-life of the glucuronide derivative is about 9 hours. In the tissue, however, resveratrol-glucuronide can be converted back to resveratrol, and you can see that the half-life alone does not fully describe resveratrol's bioavailability⁴¹.

And, of course, a substance can be removed from the bloodstream because it is being consumed in the tissues.

Resveratrol

Resveratrol is a chemical found in grape skins and other plant sources that likewise seems to promote autophagy and produces results in cancer research similar to curcumin.

Since both curcumin and resveratrol seemingly promote autophagy and are safe, inexpensive and readily available without prescription, it is suggested that they be used in periods of protein restriction to enhance its effects.

At this point in time, their association with autophagy is circumstantial and their usage cannot be quantified so they cannot reliably substitute for protein or calorie restriction in inducing autophagy. They still may help by perhaps lowering the autophagy threshold, and they appear beneficial apart from any autophagy-inducing effects.

Spermidine

Spermidine is a poly-amine found in grapefruit and seminal fluid that promotes autophagy by a different pathway than resveratrol⁸⁰. It has been found to reduce the amount of aging in yeast, flies, worms, and human immune cells by inducing autophagy⁸¹. Currently it is not available as a supplement and one would presumably eat grapefruit or whatever to obtain it. As grapefruit disrupts the normal metabolism of many drugs such as statins, its chronic use may be contraindicated for some. Mature cheeses, fermented soybeans, fermented tea, Japanese Sake, domestic mushrooms, potatoes and fresh bread are also sources of spermidine. Note that grapefruit seed extract contains no spermidine.

Trehalose

Trehalose is a disaccharide, a complex sugar composed of two simple sugars bonded to each other. Sucrose and lactose are other common examples of disaccharides. In sucrose, a molecule of glucose is bound to a molecule of fructose. In lactose, a molecule of glucose is bound to a molecule of galactose. In trehalose, a molecule of glucose is bound to another molecule of glucose. It differs from maltose, another disaccharide composed of two glucoses, only in the orientation of the two glucoses in the disaccharide.

It is common in insects and crustaceans like crab and lobster and in some mushrooms. Similar as for lactose, the human body has a specific intestinal enzyme to split it into its simple sugar constituents. And, likewise, some people lack the enzyme and cannot digest it.

Since trehalose is usually digested down to glucose, it is somewhat of a mystery how any of it get into the bloodstream intact. Trehalose is known to interact with proteins and prevent their mis-folding. To get to the center of a protein, it must be somewhat hydrophobic and perhaps it travels with fat and is thus protected from degradation.

Trehalose has long been known to help with protein folding and has found use in free cell cultures for its protective effects. Recently it has been demonstrated to induce autophagy by a means independent of calorie restriction's mechanism. This autophagy induction has been shown to clear protein aggregates in models of HD, PD⁴² and oculopharyngeal muscular dystrophy (OPMD)⁴³.

Currently, trehalose can be purchased over the internet. If used in place of sucrose (at twice the amount since it is only half as sweet) in breakfast coffee or tea, it might help induce the autophagy that should ensue from the overnight fast when a low protein breakfast is consumed.

Lipids and Lecithin

Since curcumin, resveratrol and possibly trehalose likely travel with fat, you should take fat in some form when taking them, whether in food or in capsule. Lecithin also aids fat absorption and could be taken as well. (Mayonnaise is oil emulsified with egg lecithin and could handily serve this purpose.) As for timing, both curcumin and resveratrol peak about one hour after ingestion⁴⁴. They should probably be taken (in capsule form or in the

foodstuff that contain them) at midpoint in the protein free part of protein cycling or the calorie free part of ADCR so that their autophagy stimulation is in sync with the autophagy stimulation from the protein or calorie restriction. It may be, however, that they are more effective earlier or later in the restriction period if at all. We just do not know at this time.

Because so much is not known about absorption and timing issues for curcumin and resveratrol, it would be premature to depend solely on them to induce autophagy and not use protein or calorie restriction for that purpose. However, there is evidence for both that they may help with cancer and cardiovascular diseases as well, and, as we have seen, those are statistically far more life-threatening than the neurodegenerative diseases.

Lithium

The element lithium is also a known autophagy inducer and is available over the counter as lithium orotate in low dose form (5mg/capsule). Two capsules per day, the suggested dose, or 10 mg/day is somewhat less than the 30 mg/day dosage in the studies where it has shown benefits to neurodegenerative diseases and it might not have the same effect.

Lithium is also used at much higher doses (90-180 mg/day) as a mood stabilizer. It is then a prescription drug since the therapeutic level is close to the lethal level.

There is substantial confusion with lithium dosage because it is usable only as a salt. That means the lithium atom enters solution by giving up an electron. It must therefore be paired with something that can receive the electron. Just as sodium is paired with chloride in table salt, lithium is paired with another entity, usually carbonate or orotate. Because lithium is such a light atom, it is only a small portion of the total mass of the pair. 10 mg of lithium carbonate, for example, represents only 1 mg of lithium. The numbers I have given are for the mass of the lithium only. If you use lithium salts, you need to watch out for this distinction.

Cycling

Regardless of how autophagy is induced, it needs to be done cyclically so that the cytoplasm consumed by the autophagy can be replaced. For this the inducer needs to be out of the system by time the autophagy induction time ends and the recovery period begins. Curcumin levels drop by half every hour

whereas resveratrol drops by half every 9 hours. Using these numbers, resveratrol should probably be taken early in the protein restriction period and curcumin late. Lithium, on the other hand, drops by half in about 32 hours with much variation⁴⁵. This is too long to be useful for alternate day protein cycling, and I would not recommend its use for this purpose (though it may still be useful to lower the trigger threshold of another autophagy inducer). In most therapeutic uses of lithium, the level is always held high. From the point of autophagy induction, this is like always inhaling without exhaling. Or like pulling the trigger of a gun and holding it. The trigger must be released and pulled again to fire another shot. This absence of cycling may in fact be responsible for much of the contradictory data seen in research with chemical autophagy inducers. In protein cycling, the high protein portion of the diet is as important as the low and we use the term 'cycling' just to emphasize that point.

What often happens with a substance that induces autophagy is that a starvation condition is created where some essential substance is inadequately supplied by the body and must then be salvaged from the cell to permit continued synthesis. Lithium, for instance, creates a shortage of the lipid inositol required for membrane synthesis⁴⁶. The cell then initiates autophagy to recover inositol from existing surplus membranes.

The cell also begins to produce more of the proteins involved in inositol synthesis in response to the shortage. This so-called 'up regulated' inositol synthesis machinery then compensates for the shortage created by lithium and further autophagy does not occur. This is a general response to any shortage. Enzyme protein production is regulated by negative feedback mechanisms. The end product of the enzyme pathway feeds back a signal to the enzyme-production factory that inhibits further production. A balance between end product and the enzymes that produce it is then achieved. Autophagy happens because the need for nutrients cannot wait for the relatively slow process of 'up regulating' the synthesis machinery. Cycling then allows the 'up regulation' to be reversed by the same feedback mechanism (down regulated) so that the autophagy inducer can work again.

When autophagy is induced by protein or calorie restriction, however, there is a true shortage that cannot be corrected by simply up regulating synthesis machinery. Of course eventually the restriction must end to prevent starvation, but, for the entire restriction period, autophagy will be happening

since, in order to function, human cells must constantly make new proteins regardless of conditions.

Exercise and Alcohol

Exercise has recently been shown to initiate autophagy in response to the stress arising from the consequent production of ROS (reactive oxygen species)⁸³. Alcohol consumption likewise induces autophagy⁸⁴ presumably by the same mechanism. Apparently autophagy induction is so common that there seems to be little need to go out of one's way for it. All you need is some stress on the cells. So is everything bad you do to yourself then good? Indeed the accumulation of autophagosomes is a hallmark of neurodegenerative diseases. Taking a substance to accumulate even more hardly seems beneficial. What needs to be promoted is the completion of autophagy rather than its initiation, a topic discussed in more detail in the next chapter.

16. Incomplete Autophagy

Macroautophagy is a complex cell procedure involving many independent steps. Before it happens at all, the cell must recognize that a need to perform it exists. A cellular signaling system must therefore be activated. Such signaling systems have been extensively characterized and many of their component proteins and the genes for those proteins have been identified but many holes remain.

One well characterized signal to which the cell responds is a high level of insulin indicating that supplies of glucose from the bloodstream are currently adequate. The insulin binds to the outside of the cell on a special protein complex imbedded in the cell membrane. The binding of insulin on the outside causes the complex to then bind phosphate to a particular soluble protein on the inside. The phosphorylated protein then carries the signal to certain other proteins in the signaling network causing autophagy for the purpose of energy production to be suppressed. Conversely, the absence of phosphate on the protein causes autophagy for the purpose of energy production to be promoted.

This fits the general model for how information is transmitted throughout the cell. Immobile proteins enzymatically modify mobile proteins (or some other soluble substance). Often the immobile proteins are imbedded in membranes and modify soluble substances on one side in response to a soluble substance on the other. The modified mobile substance, be it protein or otherwise, diffuses or is actively transported to other locations in the cell where it modifies or otherwise influences other specific proteins.

More than one modified substance may be necessary to cause the responding protein to propagate the signal. And more than one responding protein may react to a single modified substance. Or a single responding protein may react to more than one modified substance. In this way, multiple conditions may be necessary to initiate an event, or a single condition may initiate multiple events, or a single event may be initiated by multiple conditions. In system dynamics terms, this provides the 'and' and 'or' logic functions used to define feedback systems.

In the case of the autophagy signal generated by low insulin, it alone may be insufficient to initiate autophagy if the cell currently has a surplus of energy substrates. The cell has another signaling system that reports on low levels of

glucose within the cell and both signals together may be needed to actually initiate autophagy.

The cell also needs supplies of essential amino acids and lipids to perform its vital functions. Signal systems must exist for them as well and must initiate autophagy even when the glucose-responding signal is absent. In fact, amino acid deficiency is signaled by the accumulation of uncharged transfer RNAs (tRNAs), that is tRNAs without their specific amino acids attached. Normally tRNAs are recharged by specific enzymes after they are discharged during protein synthesis by the ribosomes. If an amino acid species is unavailable then its tRNAs remain uncharged. Uncharged tRNAs bind to an enzyme that then signals the cell to initiate or complete autophagy to free up some of the needed amino acids.

Also autophagy is used by the cell for purposes other than supplying nutrients. In particular, autophagy is seen to be induced when reactive oxygen species (ROS) or mis-folded proteins accumulate. ROS usually appear when mitochondria break down and it makes sense that the cell would then initiate autophagy to clear up the debris. The same can be said for the accumulation of mis-folded proteins. The cell needs to isolate these noxious substances from the rest of the cell. Wrapping them up in an autophagosome does the trick nicely.

Macroautophagy is often selective. Mechanisms exist to drag degraded mitochondria or other organelles or even aggregates to the mouth of an enveloping autophagosome initiated for just that purpose.

As first mentioned, autophagy is a complex multi-step process whose details have been skipped-over in earlier discussion. Normally an autophagosome 'matures' by first merging with special vesicles produced by the ER. Two types of such vesicles have been identified, early and late. At least two things result from this maturation: the pH of the autophagosome is lowered and the autophagosome is now recognized by the transport system of the cell which then carries it from the region of the cell nucleus to the periphery where the lysosomes abound. There it merges with the lysosomes whose enzymes digest the inner vesicle of the autophagosome and its contents. The end products of digestion including fatty acids and amino acids exit the autophagosome and are reused for new synthesis or burned for energy.

What happens when the cell autophages for clearing debris but has no current need for its end products? Perhaps it can return the excess to the bloodstream. More likely it blocks completion at one or more of the many steps

in the autophagy process. The cell's immediate need was to isolate the noxious debris. It can wait for when it needs the digestion products before finishing. But what if that need never comes? Then autophagy is incomplete and autophagosomes accumulate in the cell perhaps to the point of disruption of normal cell processes by their sheer bulk alone.

In fact, extensive accumulation of autophagosomes is often observed in biopsies of nerve tissues from Huntington's, Alzheimer's, ALS, and Parkinson's patients. This is puzzling. Why would the cell have only half a mechanism to handle mis-folded proteins? Why would it not have simply evolved a mechanism to force a nutritional deficit to unblock the maturation process when autophagosomes accumulate?

Perhaps the reason is that there was never any selection pressure for it because there were always periods of nutritional restriction when the blockage would be lifted. That period was the overnight fast endured by all tropical mammals including humans. It may then be the effective extinguishing of the overnight fast by many cosmopolitan humans that causes the diseases associated with protein aggregates.

With this view, protein or calorie cycling is not just a trick to avoid diseases but rather a natural practice whose absence causes disease. Eating after sunset could then be said to cause (or at least to encourage) AD, HD, ALS, and PD. Likewise the eating of a high-protein breakfast.

Incomplete autophagy may account for studies where starvation is not seen to increase autophagy in the CNS. The explanation given is that the CNS is a privileged system that gets its nutrients regardless of outside events. Even though no new autophagosomes are seen in these studies, perhaps pre-existing ones created to handle mis-folded proteins, are then being matured³⁰.

Or the apparent lack of autophagy may just be a problem of methodology. Using a novel approach to detect, enumerate and characterize autophagosomes in vivo, researchers found extensive autophagy initiation in mouse neuronal tissue after as little as 24 hours of starvation⁸².

A failure of autophagy to complete after successful initiation has recently been implicated in the pathology of Paget's disease of the bone and frontotemporal dementia⁴⁷.

In view of these considerations, we may now restate the purpose of protein cycling – to clear autophagosomes initiated by earlier cell stresses

17. Fiber

The digestion of food involves several distinct steps. First it is chewed and enzymes in the saliva work on starches to free some short glucose polymers.

Next it enters the strongly-acid stomach where the acid kills any bacteria and denatures any protein not specifically designed to survive it. The lining of the stomach releases acid peptidases that work on proteins to chop them up into shorter polypeptides. Some small fat-soluble molecules such as alcohol, caffeine and aspirin can enter the bloodstream directly from the stomach but the fat, carbohydrate and protein fragments are still too large to be absorbed.

After 3 or 4 hours in the stomach, the food mixture, now called chyme, is released through the pyloric valve into a one foot long portion of the small intestine called the duodenum. Here it is mixed with bile produced by the liver and bicarbonate from the pancreas and duodenum lining that together neutralize the acid from the stomach. The bile further emulsifies the fat into microscopic droplets suspended in the watery flow. The lining of the duodenum and the pancreas release a wide range of specific enzymes to digest fat, protein, starch and sugar polymers to the simple molecules that can then be absorbed by the lining of all the segments of the small intestine. Aerobic bacteria normally found in the intestines may also aid somewhat in the digestion process.

The digested chyme then passes into the next section of the small intestine called the jejunum. This section is about 6 feet long and is specialized for absorption. Most of the small molecules from fat digestion (fatty acids and glycerol and the bile salts), carbohydrate digestion (simple sugars) and protein digestion (amino acids) enter the bloodstream here and are transported to the liver.

The remainder of the small intestine is the ileum which is characterized by areas of lymph tissue that become progressively more common down its length. As described in the previous chapter, so-called M cells in these areas ingest large molecules and solid particles including whole bacteria by a process called phagocytosis. It is currently unclear if this is significant nutritionally.

From ingestion to final absorption takes about 6 hours and this likely accounts for the normal daytime 6 hour periods between meals.

What is not absorbed passes into the large intestine, the colon, where bacteria are mixed in and water is removed. Anaerobic bacterial action then results in further digestion and absorption by the walls of the large intestine,

accounting for some vitamins and as much as a 100 calories per day. Finally the solid remains, the feces, are eliminated by defecation.

The term fiber can be applied to the material in the chyme that normally enters the colon. It consists largely of the cellulose and other highly-branched carbohydrates of fruits and vegetables. The grams of fiber in a serving are listed on the standard nutrition labels now required in the USA. We can see on these labels that 25 to 30 grams of fiber per day is recommended.

We see that for the digestion process to complete the body must invest its own proteins in the form of enzymes and mucus, usually with the expectation of a lucrative positive return. For a protein-free meal, the body might hold back on protein digesting enzymes but would still be obliged to provide fat and carbohydrate digesting enzymes as well as lubricating mucus. For a calorie free meal such as one would eat on the ADCR diet, the body would at least supply the mucus proteins required to lubricate the passage of food.

Though some supplied protein is recovered, a portion of total protein is always lost, held inside the bacteria that form about a third of the weight of feces. Normally this lost protein is of little significance. Studies have shown that, speaking roundly, even for a conventional high fiber diet (45 g fiber/day + 100g protein/day), only about 10% (10 grams) of the protein is not recovered⁴⁸. With 7g fiber/day + 100g protein/day, only about 7% is not recovered.

Protein is also lost from the shedding of hair and skin. The cells on the outer layer of our skin dry up and flake off at a rate estimated at 20 mg/minute⁴⁹ or $(20 \times 60 \times 24 / 1000) = 28.8$ grams of cells per day. If around half that is protein (largely keratin), then then you might think we lose about 14 g protein/day in this way alone. Actually there is still water remaining and the actual value is more like 3 g protein/day. If we add the losses from all sources, we lose about 25 g protein/day even on a total fast. If our protein input was chronically less than 25 g/day, we would eventually starve to death even with plentiful calories.

For the protein cycling diet, however, this seemingly unavoidable 25 gram per day protein loss gives us some wiggle room. As we have seen, meals totally lacking in protein are nearly impossible to devise. But any day with 25 or less grams of total protein is effectively protein free or even protein negative and certainly sufficient to require the cells to commence autophagy. 25 grams of protein represents about 100 calories or a bit less than 5% of the standard 2000 calorie diet. Under 5% is then the target we want to hit to guarantee autophagy induction.

On a low fiber diet (7g/day), however, that 25 gram margin, is reduced to maybe 22 grams. Though this may be insignificant small change, a high fiber diet does work a bit better than a low fiber diet. A no fiber diet would be even worse, so eating no calorie foods (salads, spinach, pickles, etc.) would be better than skipping meals entirely when following the ADCR diet.

High fiber also has been seen to reduce the incidence of colon cancer and, as we can see in the mortality table, colon cancer deaths alone exceed those from all neurodegenerative diseases below age 75.

Additionally fiber makes you feel full even when protein or calories are lacking. For all these reasons, sufficient fiber should be maintained when changing your diet for protein cycling or ADCR.

Fiber can be added with fruits and vegetables but, for those who dislike them, fiber can be supplemented from brans, guar gum, psyllium (as in Metamucil®), or any number of sources. Some are water soluble, some are not. Guar gum and psyllium are soluble, well-tolerated, taste-neutral, inexpensive, and readily available. They can be added to any beverage to make it filling yet still palatable. This is especially useful when on the ADCR diet.

18. Exercise while Dieting

Lack of sufficient exercise underlies many of the diseases of aging, especially diabetes and cardiovascular diseases. It is particularly important that we maintain a minimal level of exercise when in the protein or calorie restriction phase of protein cycling or ADCR. We feel weaker in a period of restriction and may, consciously or unconsciously, ease up. Human muscles are very adaptable and, if no demands are made on them, they shrink. When they shrink, the amino acids in their proteins become available to other cells and this could counter-act the intended autophagy-inducing affect of protein or calorie restriction.

Exercise should address three areas: strength, endurance and stretching. Strengthening muscles (pull-ups, push-ups, weight lifting, etc.) informs the large muscles to maintain or increase their mass. Aerobic endurance exercise (walking, running, swimming, cycling, etc.), where the heart rate is noticeably increased, maintains the mass of the heart and the small muscles involved with breathing. Finally stretching avoids cramps and other discomforts that might otherwise dissuade us from exercising at all.

Exercise also warms you up. One way the body responds to reduced calorie intake is reduced calorie output. Most of the calories we consume are used merely to keep the body at a constant 37°C. Simply by constricting blood flow to the skin, for instance, the body can conserve heat and reduce calorie output. Or the body can tell your brain that you are feeling a bit chilled and should put on a sweater. Or it may simply cause you to hold your arms in more tightly. However it happens, the net result is that reduced calories in leads to reduced calories out rather than to weight reduction. If weight reduction is your goal, then you may need to consciously override these unconscious decisions. This may be as simple as dressing lightly and using fewer bedclothes and cultivating an appreciation of how then feeling cool is helping you to lose weight. Generally, body awareness is good in any diet plan whether you want to lose weight, gain weight or just stay as you are.

Recent evidence shows that exercise itself is an autophagy inducer at least in muscle cells and that the benefits of exercise depend on its induction^{[83](#)}.

19. Neurodegenerative Diseases

Here are some details on the diseases we are trying to avoid and how they may fit the model of diseases that autophagy might benefit:

Huntington's Disease

Huntington's Disease (HD) is a rare neurodegenerative disease affecting at most around 7 people in 100,000 that produces a mental and physical decline starting most often in mid-life and ending in death. Woody Guthrie is its most famous victim. It is characterized by condensates of a protein named for the disease – huntingtin.

In many ways, HD is perhaps the best available research target for investigating neurodegenerative diseases generally. Its cause is entirely genetic and, in genetics parlance, is an autosomal dominant. This means that the disease results from the presence of a defective gene product rather than from the absence of a functional gene product as characterizes recessive or sex-linked genetic diseases. This makes tracing cause and effect more straightforward than otherwise.

Every cell has the defect and so no mechanism to account for cell-to-cell transmission is required. (That does not mean there is in reality no transmission.)

The gene mutation that results in HD is known and gene testing for it is routinely available. This means that a population can be identified who will eventually develop symptoms of the disease long before it manifests. These people, because it is untreatable and so devastating and because they likely have already seen the devastation in their relatives, are often willing and eager volunteers for scientific studies.

Serving as guinea pigs may be the only medical attention they can get for their condition. Since they do not yet have disease symptoms, physicians and insurers are generally unwilling to get involved. Providing an alternative to this population that does not require professional medical involvement was indeed the inspiration for this book.

Specifically the disease is caused by an defect in a region of a specific protein, huntingtin, where a chain of the amino acid glutamine that is normally around 16 to 24 glutamines in length is expanded to a length exceeding 35. The expansion in the protein is from an expansion in the gene that encodes the

protein: a normal 16 glutamine chain is encoded in the DNA by 16 repeats of the nucleotide triplet corresponding to glutamine, CAG. In HD, the CAG repeats are expanded to greater than 35 and so the disease is characterized as a 'CAG expansion disease', as are a number of other even rarer diseases. This expansion from the normal is transmitted through the generations in families that carry the disease.

Even though the disease is an autosomal dominant and generally proves eventually fatal, the gene has not been weeded out of the human genome. There are several explanations for this. Usually the disease manifests in the post-reproductive life of its sufferers after the gene has already been passed on. Also people with HD tend to have more children than average apparently because of some mental affect of HD that manifests earlier than the physical affects. This must compensate for the otherwise negative selection that normally extinguishes autosomal dominant genetic diseases.

It is unlikely that HD routinely arises from spontaneous mutation as do many other autosomal dominant diseases. It has been around for centuries at least and, though found in all populations, is far more prevalent in those of Western European descent. It would be evenly distributed if it were from a common frequent mutation event.

Occasionally HD will appear where there is no family history of the disease. Often this is from ignorance and denial of the true causes of decline and death within a family. Another reason is that the length of the CAG expansion tends occasionally to increase from generation to generation, sometimes dramatically. Thus a 36 CAG repeat gene that would normally not produce the disease can be expanded to a length that does. For some reason, this expansion, when it does occur, is more common when inherited from the father rather than the mother.

The 'happy' consequence of all this to researchers is that the HD gene comes in a whole range of variants differing in the length of the CAG expansion. The larger the expansion, the earlier, on average, the disease manifests though the character and progression of the disease is mostly unchanged. Animal models of the disease can thus be created with different expansions yet with some confidence that they mimic some real instance of the human disease.

Indeed a wide range of animal (and plant) models have been developed for HD including yeast, fruit flies, human and other mammalian cell lines, mice, and even sheep with any number of CAG repeats or portion of the huntingtin

protein. In some the protein can be conditionally produced depending on say the presence or absence of a particular drug or hormone.

As mentioned, there are other CAG expansion diseases besides HD, though they are all much rarer. They currently include eight forms of spinocerebellar ataxias and a disease abbreviated as DRPLA. In each, the particular protein species with the CAG expansion is different and otherwise unrelated to the protein species of the others. Regardless, the diseases are all similar in that afflicted neurons progressively decline until a particular nerve tissue or type fails.

Scientists can even create new CAG expansion diseases; one team added a jelly-fish fluorescence protein gene with a CAG repeat to an animal model and observed consequent neurodegeneration⁵⁰.

In all cases it is the CAG repeat protein with the expanded poly-glutamine sequence that forms aggregates. Clearing the aggregates by inducing autophagy could be effective on all forms.

CJD and the Prion Diseases

Creutzfeldt-Jakob disease (CJD) is the most common human instance of a class of neurodegenerative diseases called transmissible spongiform encephalopathies (TSEs). The class includes a disease of sheep, scrapie, and of cattle, bovine spongiform encephalopathy (BSE), which can be transmitted to humans and has so disrupted the international trade in beef of late.

It is extremely rare and nothing we would change our diet to avoid (apart from avoiding suspect beef). It is of interest here because it is transmissible and may be the model of how non-genetic neurodegenerative diseases like AD, ALS and PD can spread from one cell to another.

Unlike all other known diseases, the infectious agent of TSEs is a protein and not a nucleic acid, DNA or RNA. This protein, the prion, is a mis-folded version of a protein found in the infected host. In ways as we discussed in the chapter on protein folding, the mis-folded protein interacts with the normal and causes it to mis-fold and aggregate as well, disrupting and eventually killing the cell. What we have yet to discuss is how this mis-folding cascade within one cell could transmit to other cells.

The normal protein whose mis-folded form is the prion is named PrP. It is a common protein found in or on the outer surface of most (if not all) cells and may serve in a trans-membrane transport or signaling role. When mis-folded, it

forms aggregates with itself that may eventually condense to form inclusions or plaques just like the other neurodegenerative diseases. And also like the others, there are inherited genetic forms that account for some fraction of the cases (10-15% for CJD).

Somehow the mis-folded PrP proteins from the doomed cell must make contact with the same proteins in neighboring cells. The likely channel is through a process discussed earlier, endocytosis . It is a way cells take in many nutrients and the only way they can renew the cell membrane. When a cell dies, its neighbors use endocytosis to dispose of the body⁵¹. Specialized motile cells called macrophages also participate. In so doing they all bring the dead neighbor's proteins into vesicles within their cytoplasm. Normally these vesicles are merged with lysosomes exactly as in autophagy and the contents thoroughly digested for re-cycling. The mis-folded PrP protein, however, is resistant to the enzymes of the lysosome by virtue of its aggregation. When the lysosome itself decays or is destroyed as must eventually happen, the mis-folded PrP is released into the heart of its next victim.

This pattern accounts for the 'spongiform' (sponge-like) character of these diseases. A large hole appears in the afflicted tissue as the prion spreads from cell to neighboring cell, killing as it goes. The infected motile macrophages may travel to other areas of the tissue to start new holes when they die. Or the prion released from the burst dead cell may travel on its own to remote sites. Either way a slew of holes now develops in the afflicted tissue and it begins to take on the appearance of a sponge.

ALS and other Motor Neuron Diseases

Amyotrophic Lateral Sclerosis (ALS) is the most common human instance of a class of neurodegenerative diseases called motor neuron diseases (MND). Its most famous victim is Lou Gehrig and is about as common (or rare) as Woody Guthrie's HD. It is often characterized by condensates of neurofilament proteins and mutations in genes for a protein called superoxide dismutase (SOD). For unsurprising reasons, the motor neurons, by far the longest cells in the body, are least able to handle neurofilament malfunction and are the first cell type to die.

It is a progressive disease and, though it often first appears on only one side of the body, it eventually appears on the other as well.

More than for AD or PD, ALS is felt by many to arise from some specific, potentially identifiable cause such as exposure to pesticides and other toxins or

even mechanical injury. Clusters of cases have been observed among unrelated people leading to this view, but the presence of inheritable forms, known (5-10% of cases) and unknown, currently clouds the issue.

If it is truly not genetic, then there must be some way for the injured cell to transmit its injury to other cells or the disease would not be progressive. Perhaps the endocytosis mechanism proposed for the prion diseases applies here as well.

It should be noted that cell-to-cell transmission does not require that the cells be of the same type or that they all suffer the consequences of 'infection' equally. The motor neuron could simply be the cell type least able to manage the aggregate and the type for which it first proves lethal.

The benefits of autophagy to a mouse model of ALS have recently been directly demonstrated⁵². An autophagy promoter, lithium, increased survivability whereas an autophagy inhibitor, 3-MA, decreased it. Autophagy was directly observed in the cells and cited as the reason for the benefit.

Alzheimer's Disease

Unlike ALS that can strike people of any age, Alzheimer's Disease (AD) shows a strong preference for the aged and its study is complicated by all the other diseases that commonly accompany old age. Nevertheless, as we see in the mortality charts, it is the most common of the class of diseases called dementias and of all neurodegenerative diseases generally.

This is the disease we most want to avoid. The mean life expectancy following diagnosis is approximately seven years⁵³, marked by dependency and decline ending in death (if some other disease does not get there first). Fortunately it fits the pattern of diseases that might be prevented by pre-symptomatic autophagy induction by protein cycling or ADCR: it is progressive, there is an associated cellular protein condensate, and some evidence suggests that autophagy benefits the condition.

Again it is characterized by condensates of a protein called 'tau' and fragments of a protein called 'amyloid precursor protein'. Unlike the other diseases discussed here, condensates appear outside the cell as well as inside. There is some evidence that lysosomes may exocytose when they have fully digested their contents and thereby dump any indigestible outside the cell. This might explain how the aggregate proteins in AD appear as plaques outside the

cell as well as within⁵⁴. It may also explain how aggregate proteins could move to other cells to seed new mis-folding cascades.

Curcumin, a known autophagy inducer, has shown some benefit⁵⁵ as has resveratrol⁵⁶.

And, like ALS, lithium, another autophagy inducer shows benefits as well⁵⁷ - including condensate clearance.

Parkinson's Disease and other Lewy Body Diseases

Parkinson's Disease (PD) is the second most common neurodegenerative disease after AD. Inherited forms are very rare. Though progressive in its symptoms, it is rarely terminal of itself.

The disease appears as a particular tissue in the brain, the substantia nigra, deteriorates. The substantia nigra normally produces the substance, dopamine, necessary to the cells that direct muscle movement. Any condition that destroys this tissue will produce parkinsonism, the paralysis characteristic of the disease. Parkinson's Disease, however, is a specific progressive condition for which parkinsonism is only one aspect. Among the other aspects is dementia which may occur without parkinsonism. One aspect common to all forms of the disease is the presence of condensates called Lewy bodies so, in the case of dementia in the absence of parkinsonism, the disease may instead be labeled as dementia with Lewy bodies (DLB). If the Lewy bodies are in the neuron support cells called glia cells instead of in the neurons themselves, the disease is labeled as multiple system atrophy (MSA)

Regardless these diseases also fit the pattern of those that might be prevented by presymptomatic autophagy induction by protein cycling or ADCR: it is progressive, there is an associated cellular protein condensate, and some evidence suggests that autophagy benefits the condition.

It is characterized by condensates (the Lewy bodies) of a protein of unknown function called 'alpha-synuclein'. Interestingly fragments of this protein appear in some AD plaques as well.

And like the other neurodegenerative diseases, the autophagy promoters rapamycin⁵⁸, curcumin⁵⁹, and lithium⁶⁰ clear the aggregate and/or inhibit disease progression. Also some genetic forms of PD have been traced to a lack of a protein needed to autophagosome damaged mitochondria⁶¹.

There is substantial evidence that PD can sometimes result from head injuries⁶² even though the disease manifests long after the trauma and then gets

progressively worse. The protein mis-folding model might explain this rather puzzling observation. Perhaps the injury temporarily disrupts the blood-brain-barrier and allows circulating mis-folded alpha-synuclein fragments, harmless elsewhere, into the brain where they then seed a mis-folding cascade.

Other Degenerative Diseases

All the diseases discussed here are classed as ‘proteopathies’⁶³ as a specific protein species is seen to misbehave. There are other tissues besides neurons with progressive degenerative diseases characterized by cellular inclusions, most notably muscle. The list is long, including some not uncommon diseases like amyloidosis, but most are rare and I will not go into them. Nevertheless, where protein aggregates are involved, autophagy as induced by protein cycling or ADCR might be preventative for them as well.

Speaking of amyloidosis, there is evidence that type 2 diabetes (adult onset) may be caused by amyloid plaque formation in pancreatic cells⁶⁴. A protein called amylin forms the aggregate. Perhaps even diabetes may be prevented or delayed by protein cycling. (Or is it just that everything looks like a nail to a man with a hammer?)

There are certainly progressive degenerative diseases that do not involve aggregates. The auto-immune diseases like multiple sclerosis, rheumatoid arthritis, diabetes, etc. come to mind. The affect of protein cycling on their development is unknown but for them I have no plausible scientific rationale to believe promoting autophagy would be positive, neutral or negative.

There is, however some evidence that protein cycling might benefit these diseases (and others such as scleroderma, inflammatory bowel disease, eczema and psoriasis) by a mechanism not yet tied directly to autophagy. The drug halofuginone, derived from hydrangeas, is effective against autoimmune inflammation. A recent study⁶⁵ showed that it works by stimulating the cell’s amino acid starvation response, and, of course, protein cycling is amino acid starvation.

20. Cancer

A cancer cell is a damaged cell where the damage allows the cell to replicate without constraint. There are as many types of cancer as there are ways to damage a cell in this way. The damage must be to the genetic machinery of the cell else it would not transmit to all daughter cells. Likely all fatal cancers trace back to a single cell among the 50 to 75 trillion cells of the adult human body that has acquired the ability to replicate without constraint, to evade the body's cancer defenses, to convince the body to feed it and/or to spread throughout the body.

This genetic damage is usually from an external source such as tobacco for lung cancer, UV rays for skin cancer, or viruses for cervical cancer. These common cancers often strike at an early age and the incidence varies greatly between regions. Other cancers are more characteristic of old age and are said to be senescent.

Senescence

Cancer and cardiovascular disease are the quintessential senescent diseases in that their general incidence rises exponentially as an organism approaches the maximum lifespan characteristic of its species. A 20-year old dog will be as cancer riddled as a 90-year old human despite their similarities at the cellular level.

The question then arises as to what mechanism accounts for the exponential rise and the differences among species? Depending on definitions, it has been estimated that around 5000 cells per day out of 50 to 75 trillion total cells of the body become cancerous. Normally these cells are destroyed by the body's cancer fighting mechanism, the immune system. Cancer rates therefore depend on two opposing factors: the rate of mutagenesis producing cancer cells versus the efficiency of the immune system in killing them.

Mutagens

A mutagen is anything that causes a mutation: an alteration in the DNA of the cell that materially and permanently changes the cell. X-radiation is an example that directly damages DNA. The chemicals associated with tobacco use are the most prominent direct mutagens. Other mutagens are produced by the body itself in reaction to what may be termed indirect mutagens; alcohol,

obesity and inactivity, all known causes of cancer, probably work this way by causing an increase in ROS (reactive oxygen species) that then do the actual damage.

One can minimize but not entirely eliminate exposure to external mutagens. Further there are internal mutagens such as genetic predispositions independent of behavior. Some genes, for example, detach from their original position in the chromosomes and reinsert into some other position. Other genes make copies of themselves that then insert into somewhat random positions on the chromosomes. This jumping gene process, though likely beneficial to the evolution of the species, can create cancers to the detriment of the individual. It is thought that something similar to this jumping-gene mechanism is used by the body to create the large random assortment of antibodies produced by the immune system.

Antibodies

Antibodies are proteins that bind specifically to other substances, their antigens, for the purpose of signaling their presence. An antibody producing cell is genetically altered to make just one species of antibody. The body generates hundreds of thousands if not millions of species of such cells by a random process early in development. Then those cells whose antibodies are currently binding their antigens are culled and eliminated. After the culling period, the remaining cells wait for the remaining life of the organism for their antigens to appear. If and when they do, the particular cell proliferates producing more antibodies that now signal other cells to destroy the antigen and/or whatever is displaying the antigen.

All cells are required to display pieces of their internal proteins on their outside membranes. Those that display antigens for existing antibodies are destroyed by the immune system as are those who fail to display anything. Only those proteins present at the time of the antibody cull are allowed (or those by chance not recognized by any of the body's remaining antibody species – presumably a rare event perhaps more common in old age). An invading virus, for instance, is thus recognized by the “foreign” proteins it encodes and its host cell is killed to limit the infection.

Cancer cells are usually killed by the same process. The genetic damage that makes a cell cancerous in the first place often causes the cell to make proteins not present during the cull. These may be fetal proteins only required

for early development or others normally not ever produced at all. Or a mutation may alter a protein sufficient to make it appear foreign. Either way the cell is seen as producing illegal substances and is killed. Very few cells with major genetic damage make it this far (a mere 5000 a day) to be eligible for an external attack. Most die from internal processes many of which are likely designed just to prevent cancers from forming. These internal detection systems command an orderly death when triggered.

Apoptosis

About 50-70 billion cells normally die every day in the human body. Usually when a cell knows its time is up, it packages itself up into bite-size pieces and hangs eat-me signs on its outer membranes. Its neighbors and passing macrophages then consume the dying cell. This orderly process is called programmed cell death or apoptosis and the cell maintains a system of enzymes just for this purpose, the caspases. If a cell dies without apoptosis, a so-called necrotic death, its contents spill out and provoke an inflammatory response, a less desirable outcome from the point of view of the entire organism. Sometimes a cell dies from excessive autophagy and that is then called autophagic cell death. This dual role of autophagy, preserving the cell or killing the cell, creates many problems of interpretation especially in cancer studies where the goal of treatment is to selectively kill human cells. It is a current controversy whether autophagic cell death is programmed or accidental. It is certainly messier than apoptosis and one would wonder why the body would sometimes select it when a better mechanism is available. More likely autophagic cell death reflects the cells heroic but futile rescue efforts in response to a fatal stress.

In some cancers, the apoptosis mechanism is broken and the cell fails to respond to internal or external signals that apoptosis should initiate and complete. The damage can occur at any point in the complex apoptosis system and cancers will behave differently depending on where the damage has occurred. Some, for example, will not respond to external requests to kindly die such as provided by immune cells when they detect that the cancer cell is producing unusual proteins. Others may simply be unable to complete the apoptosis program regardless of who initiates it.

Autophagy

As autophagy is likely involved in all cell processes including apoptosis and antigen presentation, drugs or other treatments that affect autophagy might be expected to affect cancer growth as well. Indeed this is seen: autophagy enhancers promote some cancers and retard others, and the converse is seen for autophagy blockers.

A cancer cell with a broken autophagy system may thereby avoid apoptosis but then not get the benefits of autophagy, namely the ability to survive a period of starvation and stress. Many conventional cancer treatments are thought to work in just this way, by stressing all cells to the point where the weaker cancer cells die. Some autophagy enhancers might then be expected to override the breakage and allow some cancer types to better survive the treatment, making things worse. For other cancer types, overriding the breakage may then allow apoptosis to complete, making things better. Other cancers have intact autophagy systems and would show no affect. Cancer is complicated...

The question then arises concerning protein cycling, an autophagy enhancer; does it promote or retard cancer generally? From an earlier chapter it was seen that cancer underlies far more deaths than neurodegenerative diseases and this is not a trivial concern. Many cancers though have known environmental causes (smoking, obesity, sun exposure, alcohol, etc.) and are mostly preventable. To subscribe to protein cycling while not dealing with these known cancer causes is disproportionate to say the least. Certainly if one is currently under treatment for cancer, that is perhaps not the time to practice speculative diet measures. If otherwise one might look to the data.

Though protein cycling has not been studied in this context, intermittent fasting including ADCR has and the results have been encouraging^{[86,104](#)}. At least in mice and rats, alternate day fasting (ADF) in the few studies available showed lower values of cancer markers or longer survivability compared to the controls. Though it may be premature to practice protein cycling for the sole purpose of diminishing cancers, one can be reasonably confident from studies so far that it would not make cancer incidence worse.

21. Cardio-Vascular Diseases

Phagocytes

Phagocytes are the class of cells that perform phagocytosis, an invagination of the outer cell membrane that envelops external solid material (>0.75 μm by definition) in much the same way as autophagy envelops internal material. The liposomes so formed, phagosomes, are often processed in the same way as autophagosomes, namely by fusion with lysosomes. It is thought that most cells (excepting perhaps extremely specialized cells such as nerves, muscle and red blood cells) can at least phagocytose their dead neighbors, and so the term, professional phagocyte is applied to those cell types for which phagocytosis is their main function. These cell types comprise a dizzying array of named forms differing in their purpose, location, mobility and maturation, but, for this topic, only the macrophage, its precursor the monocyte, and its senescent form the foam cell need be considered. Monocytes are white blood cells that leave the blood stream and enter tissue when invited where they transform into macrophages (big eaters). If they then overeat on oxidized fats, they can transform into foam cells.

Atherosclerosis

Atherosclerosis is a specific form of arteriosclerosis (lit. hardening of the arteries) in which foam cells play a central role. It begins when cholesterol deposits between the cells of the inner lining of an artery and the layer of smooth muscle around them forming a so-called plaque. Oxidation of this fat causes the epithelial cells lining the artery to signal its presence and thus draw in monocytes from the circulating blood to clear it up⁹⁶. The monocytes then transform into macrophages and start eating. Human cells, though capable of producing cholesterol, are incapable of breaking it down. Instead excess cholesterol is transported by a particular protein in the blood (as HDL) to the liver where the cholesterol is deposited in the bile as bile salts. By this process, the plaque is cleared up, the macrophages eventually die or emigrate⁹⁰ and no trace of the cholesterol deposit remains.

At least that is what is supposed to happen. Instead the fat seems to enter the macrophages at a greater rate than it can be processed and exported. The fat droplets accumulate in the constipated macrophages which are then designated as foam cells⁹⁵. These foam cells are unable to extricate themselves

from the plaque and so they expand and weaken it as they accumulate and die. Rupture of the weakened plaque can then release solid matter that then blocks the artery causing a heart attack if in the heart, a stroke if in the head or thrombosis generally anywhere in the body.

It is now known that the ingested fat droplets are normally processed in the macrophage by autophagy⁹² and that this process is the rate limiting step in cholesterol export. Perhaps this accumulation of fat droplets arises from a failure of the macrophages to initiate and complete autophagy to clear them out. Perhaps the macrophages are depending on the usual fasting episodes of the organism to complete the process, and the high incidence of cardiovascular diseases, like neurodegenerative diseases, may arise from the extinguishing of routine fasting in industrial societies. If so then protein cycling should work to clear atherosclerotic plaque and prevent most heart disease, statistically a far more significant outcome based on mortality rates than preventing neurodegenerative diseases.

Is there any evidence to support this hypothesis? There is some argument in the current literature as to whether autophagy is helpful or harmful in atherosclerosis⁹⁴. If autophagy is indeed helpful then things that promote autophagy such as ADCR and rapamycin should show some positives:

- Intermittent feeding shows benefits in cardio markers in all cases in a review of many studies^{86,108}.
- Rapamycin (as everolimus) eluting stents placed in atherosclerotic arteries have been shown to reduce the plaque through autophagy enhancement^{99,100}.
- Smooth muscle cells, another component of plaque, are seen to be protected from cell death by cholesterol overload by rapamycin. The opposite occurs with the autophagy inhibitor, 3-methyl adenine⁹³.

Other studies have shown that autophagy promotion enhances the ability of heart cells to recover from a brief period of oxygen starvation as happens in the cardiac artery blockage of a typical heart attack. A similar protective benefit is seen in brain tissue and stroke⁹⁷.

Lipofuscin

The heart muscle itself can be the site of origin of cardiovascular disease. Heart muscle cells, like most human cells, are differentiated to perform a specific task and, as a result, have lost the ability to divide and multiply. The

heart, like most organs and muscles, keeps a population of undifferentiated stem cells to replace any cells that die, though in the heart's case, the few if any stem cells present appear inadequate to handle even a moderate die-off. In consequence heart muscle cells are like neurons; their life span matches the organism's life span and are not renewed and rejuvenated sufficiently by cell division. Cellular junk that the cell cannot dump can accumulate over the decades to the point of interfering with normal operations. Lipofuscin is the name given to the most prominent junk in human cells. It is what remains in the lysosome after all that can be digested has been digested. It consists mostly of cross-linked oxidized unsaturated fatty-acids and proteins entrapping metals, sugars, etc notably aluminum and iron. One would think the cell would just dump it by exocytosis to be picked up by macrophages who would then migrate to the liver or spleen to dump it into the bile. Some studies suggest that something like this indeed happens¹⁰⁵. Others suggest otherwise, that the lipofuscin just accumulates in the cell¹⁰⁶. Regardless, its accumulation is a hallmark of old age and may ultimately cause heart failure. Calorie restriction diets have been shown to reduce lipofuscin¹⁰⁷ and enhanced autophagy may be the mechanism, yet another way in which protein cycling could be heart protective.

In summary, protein cycling has the potential to diminish cardiovascular disease in three ways: by promoting cholesterol export from atherosclerotic plaque, by enhancing heart muscle recovery from temporary oxygen starvation, and by reducing lipofuscin accumulation in heart cells.

22. Chronic Infections

Infections are diseases caused by invading organisms be they viruses, bacteria, protozoa (nucleated single-cell organisms – protists), or higher organisms such as worms or lice. Usually the course of an infection is short as the body mounts its defenses and eliminates the invader. Some invaders, however, can survive what the body throws at it and produce a so-called chronic or latent infection: chronic if disease symptoms persist, latent if they recur later. This usually involves the invader finding a refuge within the body where the body's immune system cannot reach and from which counter-attacks can be mounted.

Some viruses such as those that cause chicken pox and other herpes conditions find refuge in the nucleus of the cell. They incorporate themselves into the DNA and wait for suitable conditions to again reproduce. This is how the chicken pox herpes virus can cause the disease condition known as shingles decades after its initial infection.

Other organisms find refuge in the body spaces outside the cell: tape worms for instance in the intestine or lice on hair follicles.

Some can directly invade and survive within the cytoplasm of the cell. Many, however, find refuge within the cell's own vesicles. They enter either by their own mechanisms or by being endocytosed by phagocytes. Normally the vesicle they then inhabit would merge with lysosomes and the invader would be thereby destroyed. These invaders, however, prevent this 'maturation' process by producing substances that block or counter-act it. They are then safe and secure often within the very cell whose job is to destroy all invaders, the phagocyte

Protein cycling may be a therapeutic approach against all diseases of this class. Promoting autophagy repeatedly may cause the blockage produced by the invader to be overridden, allowing the invader's vesicle to 'mature' to lysis. Or the autophagosome induced by protein cycling may itself engulf the invader's vesicle. Either way, the invader is destroyed. Induced autophagy may or may not then be adequate in and of itself to entirely eliminate the invader from the body.

These organisms that hide in vesicles produce some of the most significant diseases that plague mankind including malaria, schistosomiasis, tuberculosis and even ulcers and may underlie diseases yet unknown and

consequences of those diseases such as cancer, nephritis or arthritis. Suppression of such organisms may even account for the benefits documented for autophagy -promoting drugs and practices such as calorie restriction and ADCR. We shall now examine some of these diseases in detail from the perspective of autophagy promotion.

Malaria

Malaria is caused by a protozoan called plasmodium that, despite having a nucleus, a mitochondrion and a plastid, is small enough to invade red blood cells and take up residence there. Proliferation in red blood cells produces acute malaria that resolves as the spleen destroys the infected cells. Similar organisms cause schistosomiasis and trypanosomiasis, all of which are very significant chronic diseases in the tropics.

Two species of plasmodium, *P. vivax* and *P. ovale*, can produce chronic or latent malaria by hiding inside of liver cells. Interestingly chloroquine, the traditional drug of choice against malaria, is an autophagy inhibitor. Red blood cells have no nuclei or mitochondria and do not perform autophagy. A plasmodium in a red blood cell would be untouched by autophagy promoters, though that would not be true for the plasmodia in liver cells. Perhaps the plasmodium in the red blood cell needs its own autophagy to survive nutrient restriction and its inhibition blocks its proliferation.

Hepatitis B

Chimpanzees can be infected with the Hepatitis B virus where up to 75% of liver cells contain visible virus particles that disappear as the chimpanzee fights off the disease. Cell destruction, however, is not seen in this process as would be expected if the immune system was clearing the particles⁶⁶. Presumably then it is autophagy doing the clearing. Hepatitis B is sometimes chronic in humans perhaps because autophagy is inadequate in those cases.

Dengue Fever

The Dengue virus initially enters the cell by inducing endocytosis at the cell surface. The resulting single-membraned endosome containing the virus particle then fuses with the double-membraned autophagosome. The virus then recruits the cells machinery to produce substances that prevent lysosomes

from merging with the autophagosome and destroying it and the virus it contains⁶⁷.

Tuberculosis

Tuberculosis is caused by a number of species of mycobacteria when the organism takes up residence, most often in vesicles in the macrophages of the lung. Usually the body can eliminate the infection, but, in many cases, it becomes chronic. Other immune cells cluster around the infected macrophages to produce the 'tubercles', the granules that give the disease its name. It has been found that autophagy helps eliminate the organism⁶⁸. Leprosy is caused by a similar organism.

Lyme Disease and other tick-borne diseases

Lyme disease and its relatives are caused by infection with a species of corkscrew shaped spirochete bacterium called *Borrelia*. The organism assumes a globular form, the so-called cyst state, that can survive inside human cells and create a chronic disease often misdiagnosed as ALS, MS or another neurological disease.

Crohn's Disease, Colitis and IBS

Though historically thought to be genetic conditions, recent evidence suggests that the common gut bacterium *Escherichia coli* may be the cause when it takes up residence in vesicles inside intestinal macrophages⁶⁹. Interestingly, a number of unrelated bacterial species share this capability by sharing a ring of DNA called a plasmid⁷⁰. This plasmid is separate from the main DNA ring of the bacterium and encodes a number of proteins required for invasion of human cells. In this way, the plasmid could be considered as a virus of the bacterium. Indeed many of the proteins it encodes are similar to the proteins of true bacterial viruses. Further the plasmid can sometimes be transmitted to 'uninfected' bacteria even across species. This bacterial 'virus' may then be true infectious agent responsible for these and perhaps many other diseases that vary only on the particular bacterial 'host' species involved.

Ulcers

Ulcers are usually caused by a bacterium *Helicobacter pylori* that has figured out how to survive within the hostile acid environment of the stomach.

Recent evidence shows that ulcers result when the bacterium takes up residence in vesicles inside the cells lining the stomach⁸⁵.

23. Statins and the Blood-Brain Barrier

Statins are drugs that inhibit the body's production of cholesterol. They include simvastatin (marketed as Zocor), atorvastatin (marketed as Lipitor) and lovastatin (marketed as Mevacor) among others. Their effectiveness against cardiovascular diseases and relative safety have made them by far the most widely-prescribed class of drugs ever.

Statin use is becoming about as common as aspirin use. A majority of people over 50 in the United States now have statin prescriptions. In most countries, including even the medically cautious UK, statins are now available over the counter without prescription. And, as their patents have expired, their costs continue to plummet and they will soon sell for pennies a pill. Since statin use is so widespread and growing rapidly, we must consider its interactions with protein cycling.

All commercial statins are given names in the form prefix + 'vastatin'. Hence simvastatin, atorvastatin, lovastatin, cerivastatin, fluvastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin etc. They all work by direct inhibition of a specific enzyme in the cholesterol-synthesis pathway. They differ primarily in their degree of oil solubility. The more oil-soluble (lipophilic) is the statin, the more easily it can cross the blood-brain-barrier and directly affect neurons and their support tissues.

The blood-brain-barrier prevents cholesterol and other fats in the bloodstream from reaching the neurons. In consequence the neurons or their support tissues (glia, astrocytes) must synthesize the cholesterol required for neuron structures. If this synthesis is inhibited by statin use, the neurons might be impaired thereby in some vital function. This is in fact the argument advanced by some against statin use. It may be behind the rare memory-loss side-effects reported in statin use.

Besides cholesterol, the metabolic pathway inhibited by statins is also used to make the chemical Co-enzyme Q10 (CoQ10). CoQ10 is one of the few entirely-hydrophobic molecules of the cell. As such it resides entirely within the lipid phase in the interior of cell membranes where it serves a vital role as an electron transporter. It is especially important in the mitochondrion. When mitochondria fail, they release their acid interiors into the cytoplasm leading to death and dissolution of the entire cell. It is speculated by some that the depletion of mitochondrial CoQ10 by statins may thus be behind the cell death

phenomenon rhabdomyolysis (discussed in a previous chapter) seen in a small percentage of statin users.

Since protein cycling or ADCR may stress cells already made fragile by statin use, you may choose to supplement CoQ10 when using statins. In pill form, CoQ10 supplements are expensive so I suggest you use the powder form instead. It should be added to the fat component of your meals at about 50 mg/day and should cost no more than 8 cents per day.

Certainly statin use should not be stopped just because you are protein cycling. The evidence of its benefits to cardiovascular profiles is just too strong. Unsurprisingly, cardiovascular diseases aggravate dementia and parkinsonism, the very things protein cycling seeks to avoid. There is even a remarkable statistical study where the incidence of dementia and parkinsonism was seen to be reduced by half with simvastatin and much less with atorvastatin or lovastatin. Simvastatin, unlike atorvastatin, can cross the blood-brain-barrier. This suggests that lipophilic statins like simvastatin could prevent dementia and parkinsonism by direct action on the brain rather than just indirectly by cardiovascular benefits⁷². If the patents on simvastatin had not run out, you would probably be hearing a lot more about these results.

In fact cholesterol depletion by lipophilic statins is a known inducer of autophagy⁷³ and this may be the mechanism by which it reduces dementia and parkinsonism, clearing the mis-folded protein aggregates associated with these conditions. In the near future statins may be prescribed for AD and PD even where cholesterol levels are normal.

Alternate day statin dosing has been shown to be as nearly effective as daily dosing⁷⁴. Perhaps statins could be cycled concurrently with protein or calorie cycling to synergize each other's benefits.

It is not too surprising that blocking cholesterol synthesis seems to promote autophagy. When cell membranes are recycled, the cholesterol they contain is made available for new membrane synthesis in the same way as amino acids are made available for new protein synthesis. Perhaps cycling statins could be as effective as cycling proteins in promoting autophagy. We may never know since statins are coming off their patents and there is little incentive to do the necessary studies. Further statins have some problems in crossing the blood-brain barrier and would likely promote autophagy less in the CNS than in the rest of the body, the exact opposite of what is desired to restrain the development of neurodegenerative diseases.

BHC

The ideal autophagy inducer would freely cross the blood-brain barrier, or better yet, act just on the barrier itself. There may in fact be such a drug, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BHC), a leucine analogue. It selectively inhibits a protein complex, LAT1, that is required for the essential aromatic and branch-chained amino acids to cross the barrier⁷⁵. When this enzyme is inhibited, essential amino acids, especially aromatics (phenylalanine, tyrosine, tryptophan) are no longer available to the cells of the CNS and autophagy must ensue. This drug, or one that behaved the same, in a cycling regime would induce continual rounds of autophagy in exactly the same way as protein cycling but without the hassle of a restrictive diet and without affecting uninvolved parts of the body. The vast financial potential of such a drug should justify the studies necessary to engineer it into a pharmaceutical product.

24. Low Protein Foods

The food table presented in an earlier chapter guides us in planning our low protein meals. It should be readily apparent that meat, cheese, eggs, beans and milk are off the table so to speak. We instead concentrate on the calorie-dense low-protein items.

One goal of the protein cycling diet is to minimize the disruption to our normal eating patterns. Ideally just the protein portion of the diet is replaced by starch. To achieve this, we look for starchy substitutes for the high protein items in our diet. Of course the low calorie-dense items, fruits and vegetables, can be consumed in any quantity desired. Their bulk alone can make a low calorie meal satisfying. Watermelon works well for this purpose.

Starch is a polymer of glucose just as protein is a polymer of amino acids and they thus share some physical properties. Starch can be purchased in many forms, as cornstarch, tapioca, potato starch, manioc flour, sweet manioc starch, sour manioc starch, or arrowroot. They have minor differences in chain lengths and degree of branching, but all behave similarly and can be used to make substitutes for meat, cheese, eggs, bread and milk.

Or the starch can be in the food itself. A meal of just rice or potatoes and vegetables can be quite satisfying when autophaging. After all, you are the entrée! Remember that if you autophage 20g of your own protein per day you also get 180 calories from the 20 grams of fat that accompany it and you will find that your hunger is easily satisfied. My experience has been that, if I feel hungry, anything I put in my stomach including water stops the sensation.

Finally if you have not already, you should discover cassava root (yuca, yucca) the third most common human calorie source⁷⁶. Boiled it produces a very tasty vegetable similar to potato but with zero protein.

Now for some recipes. Since the goal of protein cycling is to be unobtrusive on your normal routines, so should be the recipes. For that reason they are presented in their most basic forms to be elaborated to your tastes.

Meat - You can thicken the fat or drippings from meat with starch to make a gravy. Eat over thin-sliced toast with mushrooms and onions. Save the meat for the next day and consider meat substitutes that have the mouth feel of meat without the protein. Commercial gravies are available, some with protein and some with none. Check the label.

Microwaved frozen spinach flavored with any kind of vinegar provides a calorie and protein free dish with the warmth and chew of meat suitable even for ADCR. Other frozen green vegetables work too.

You can use the gravy from meat instead of vinegar with microwaved frozen spinach as well. It then has calories and can no longer be used for ADCR but is still suitable for protein cycling.

Black olives likewise can provide something of the texture of meat with the calories but none of the protein. Chopped olives can make a satisfying sandwich spread.

A microwaved slice of eggplant can fill a sandwich and give something of the shape and texture of a lunch meat.

Cheese - Cheese is very protein dense. A protein-free imitation of sliced cheese can be made by mixing cheese flavor powder (available in many flavors and forms on the Internet if not locally) with starch, a little oil and just enough water to make a fluid batter. Pour thin on a griddle and apply medium high heat. It will immediately set up and form a dry opaque or translucent top depending on the amount of oil. When the edges begin to curl up, flip it over. The revealed side may be somewhat gummy. Fold it so that the gummy side is on the inside and you have something with the shape, texture and taste of a slice of American cheese (yum!).

Eggs - Egg yolk and egg white are both very high in protein. If egg is being used as a binder in a recipe, try starch instead and save the eggs for the next day.

Bread - Manioc flour is made from cassava (yuca) and is available in the Latin foods section of supermarkets or in tropical markets generally. Quick breads can be made from it that are virtually free of protein.

A flat bread called 'casabe' can be made as follows: Make a paste with a quarter cup of flour, a pinch of salt and about a half cup of water and immediately spread it to an 8 inch circle on a frying pan or griddle over a medium flame. When the edges brown, flip it and cook the other side for the same amount of time as the first side. The result will be a cassava tortilla that can be used like any other flat bread. If it turns out gummy, you have made it too thick, added too much water, not cooked it long enough or have waited too

long between mixing and cooking. If you add other ingredients, the gelling of the starches may be disrupted and less water may then be needed.

A quick and dirty way to make casabe is to spread the dry flour in a fry pan and then spray with a water mist and heat. For my breakfast, I have a mix of equal parts oat bran and manioc flour with some salt and pepper. I wet a fry pan with a water sprayer and dust on a tablespoon of the mixture. I then spray it until it is thoroughly wet and then heat it until it is dry. The entire outer edge will curl up slightly as heat is applied. When this happens, the casabe releases from the pan and I flip it and turn off the heat. I then spread the casabe with apple butter and eat with tea. Tasty yet zero protein.

A leavened bread can be made with manioc flour by adding baking powder to the above mixture and baking instead of frying.

Wheat bread can be used if it is thin sliced and toasted to destroy its lysine as described in an earlier chapter. Dilute its protein calories further with butter and other no-protein spreads.

Milk - Milk is a mixture of cream (fat), lactose(sugar) and protein with significant amounts of calcium, magnesium, phosphorous, citrate, potassium, etc. It is often fortified with vitamin D.

A simple substitute is cream (not half and half) for coffee or tea. To get closer to milk, dilute with water and add starch, about 2 tbs per quart.

Rice milk is a protein free milk substitute now commonly available in supermarket; likewise coconut oil as a cream substitute.

Lacking protein, all these milk substitutes last longer in the refrigerator before souring or otherwise degrading.

Beans - Beans and peas are fairly high in protein and cannot be eaten in the protein restriction phase of protein cycling. If you need a substitute and have considerable imagination, you can substitute spheres of cassava starch called tapioca pearls. They are a staple of tropical cuisine and are available in a wide range of sizes and colors. When soaked or boiled, they absorb whatever fluid they are in and expand into somewhat transparent spheres. You may be familiar with them if you have ever had bubble tea.

All meals - The target of all your meals in the protein restriction phase of protein cycling should be 5% of calories from protein. Protein digestion is not

100% efficient and a meal that is nominally 6% or 7% likely would be sufficient. Unfortunately, short of biopsy, there is no direct test to know if autophagy is occurring in the neurons and we are left with subjective measures. From my experience, if autophagy is happening, you will know it. I have gone for a week with virtually no protein. The sensations I got of calmness and tightness in the gut are the same sensations I get from a single day of calorie restriction. You might try a multiple day extreme no-protein fast yourself to determine your own sensations when autophagy is sure to be happening. After, if you feel those same sensations with a one day fast, you can have some confidence that your low protein meal is sufficiently low in protein.

We use a one day minimum for protein fasting in the protein cycling diet since that is what is used in the ADCR diet. There is positive data for that diet that a 24 hour fasting period is sufficient. However it has not been ruled out that some value less than 24 hours might work as well. 12 hours is obviously too little as that is the normal overnight interval but 18 hours might be enough, especially if you are on a low to moderate protein diet. If so, you could achieve protein cycling simply by skipping evening snacks and eating a low protein breakfast. Or you could extend a 24 hour protein fast to 32 hours in the same way.

Experiment.

In protein cycling, the protein restriction phase is always followed by a non-restriction phase so that the cell proteins (and fats) lost by autophagy are replaced. Since Western industrial diets are already so high in protein, usually no special efforts are required for non-restricting meals though your overall protein consumption should at least meet the WHO recommendations of 140 to 280 grams per week for adults ranging from 100 to 200 lbs.

25. Supplements

Normally, our natural diet provides everything we need, calories, vitamins, minerals, amino acids and fatty acids. The body maintains some reserve of each and can endure some period when one or more elements are lacking with no apparent stress. To some degree, our appetites even select from among available foodstuffs for what is currently needed.

Though no stress may be apparent from temporary malnutrition, there may in fact be some. In a worst-case scenario, if that nutritional stress results in misfolding a protein, the result may be the initiation of a misfolding cascade that only shows up decades later as a neurodegenerative disease.

When we overrule our appetites and impose an artificial diet, we run some slight risk of a nutritional deficit, not in the bulk items, calories and amino acids, but in the micronutrient vitamins, minerals and essential fatty acids. Taking supplements during a diet is a simple and cheap way to guarantee against such a deficit.

Also as discussed earlier, there are items in industrial diets that are near the border-line of insufficiency that need some attention. If you are at particular risk of a neurological disease, you might be more concerned about sustaining optimal levels of micronutrients rather than merely meeting minimal levels.

My recommendation, from an insurance perspective only, is to take an inexpensive full-range multi-vitamin and mineral supplement while on the protein cycling diet. I would also supplement Vitamins C, D and E separately for anti-oxidant support. Also I would add omega-3 fatty acids to the list. If you are at particular risk of a neurological disease, you might add other anti-oxidants such as melatonin, lipoic acid, taurine, etc.

26. Suggested Diet Plans

Protein cycling is designed to get the benefits of ADCR with much less of the pain. Though not as intense as many other diets, unlike them, it is a life-long commitment if your intention is to delay the onset of neurodegenerative diseases. Because it is a life-long commitment, it can be dropped whenever circumstances warrant and picked up again later with no real consequence. Such circumstances might include infection, pregnancy, holidays, weekends or social events.

The diet can be practiced at any level. It could be as little as restricting evening and breakfast proteins or as much as restricting all protein every other day as well. The level you choose would depend on your age and susceptibility to the disease or diseases you wish to avoid. Or perhaps your real interest is in ADCR and protein cycling is just a way to prepare you for that.

Though not intended for weight loss, in my experience if overweight, you will lose a few pounds permanently at the start. If you wish to lose more, you can simply cut back a bit on calories when in the protein restriction phase while your mind is on your diet. Of course you must then not over-compensate when in the non-restriction phase. Since it is a life-long diet, there is no rush to reduce. If you are in a rush, however, you could combine protein cycling with ADCR to more rapidly lose weight. Once you are accustomed to the protein cycling pattern, ADCR seems much easier as well. Remember that the diseases associated with overweight are far more common a threat than the diseases associated with aggregates.

**Years for 50% Cytoplasmic Replacement
with Various Protein Cycling Regimes:**

1/week	2/week	3/week	3.5/week	4/week	3/month	5/month	7/month
5.3	2.65	1.75	1.5	1.35	7.7	4.6	3.3

Table 5

The intention of protein cycling is to force your cells to replace their cytoplasm at a rate greater than or equal to the rate of accumulation of aggregating proteins. Since the rate of aggregation likely increases with age, the level of protein cycling needed to counteract likewise rises with age. With that in mind, I somewhat arbitrarily suggest the following protein cycling regimes:

No protein cycling

This regime is suggested for those younger than 20, pregnant or lactating women, those with active diseases or demonstrably weak immune systems, and those trying to add muscle mass to meet some sports objective. You need your protein now and have the rest of your life to worry about senility and your old age.

18 Hour Daily Protein Cycling

This regime is suggested for those older than 20 yet younger than 30 or anyone for whom a whole day of protein restriction is unbearable.

Protein is restricted from the end of the evening meal until lunch the next day (18 hours). Breakfast can include wheat-based products such as toast, muffins, donuts, danish or bagels provided that the wheat is the only substantial source of protein (the lysine deficiency trick) and its calories are heavily diluted with calories from non-protein sources (butter, sugar, juice, fruit, etc.). Overall protein calories should not account for more than about 15% of your daily calories. You then hope that 18 hours is sufficient to induce autophagy with the assurance that you have decades before you might need to take stronger steps.

If autophagy occurs, it would be in the last 6 hours before lunch. Conservatively the body would then autophagy to satisfy $6/24$ or $\frac{1}{4}$ of its daily amino acid needs. If practiced 7 times a week, it would add up to the equivalent of 7 days per month. From table 4, we see that that computes to an 88% cytoplasmic replacement rate per decade.

From table 5 we see that it recycles half the cytoplasm every 3.3 years. If the presumed aggregate is doubling exponentially at a value greater than 3.3 years, the aggregate should eventually be eliminated. If it is doubling at a value less than 3.3 years, it will continue to accumulate.

One Day per Week Protein Cycling

This regime is suggested for those older than 30 yet younger than 40 or for those experimenting with protein cycling. Calories from protein is restricted towards 5% of total calories for a 24 hour period once every week. From table 4 we see that this computes to a 73% cytoplasmic replacement rate per decade,

We can bump that up to about a 95% rate by combining it with the 18 hour daily regime.

Curcumin and resveratrol can be taken at restriction midpoint hopefully to enhance the autophagy induction. Either or both can be taken in capsules as supplements or in the foodstuffs that contain them.

From table 5 we see that it recycles half the cytoplasm every 5.3 years. If the presumed aggregate is doubling exponentially at a value greater than 5.3 years, the aggregate should eventually be eliminated. If it is doubling at a value less than 5.3 years, it will continue to accumulate.

Two Days per Week Protein Cycling

This regime is suggested for those older than 40 yet younger than 50 or for those who feel that 24 hours of protein restriction is insufficient to induce autophagy. Calories from protein is restricted towards 5% of total calories for two 24 hour periods every week. From table 4 we see that this computes to a 93% cytoplasmic replacement rate per decade.

The two 24 hour periods can be contiguous with no non-restriction period between them if you feel that a single 24 hour period is insufficient. This may indeed be the case for those with very high protein diets where 24 hours may not be enough time to clear the gut. Otherwise the two periods are separated by the usual non-restriction periods.

Again curcumin and resveratrol can be taken at restriction midpoint

From table 5 we see that it recycles half the cytoplasm every 2.65 years. If the presumed aggregate is doubling exponentially at a value greater than 2.65 years, the aggregate should eventually be eliminated. If it is doubling at a value less than 2.65 years, it will continue to accumulate.

Three Days per Week Protein Cycling

This regime is suggested for those older than 50 or who face a high likelihood of soon developing a neurodegenerative disease. Calories from protein is restricted towards 5% of total calories for three 24 hour periods every week. From table 4 we see that this computes to a 98% cytoplasmic replacement rate per decade or an 86% rate every 5 years. 50% replacement occurs in 1.75 years meaning that it can keep aggregates indefinitely at their current level if they are otherwise doubling every 1.75 years.

This regime is particularly calendar friendly as the restriction days can be Monday, Wednesday, Friday leaving the weekends free for excess and frivolity.

And again curcumin and resveratrol can be taken at restriction midpoint.

This, in combination with no protein breakfasts, is the regimen I have followed for almost a year with minimal disruption to my usual habits.

Four Days per Week Protein Cycling

This regime is suggested for those older than 50 who feel that 24 hours of protein restriction is insufficient to induce autophagy or those who face an imminent likelihood of developing a neurodegenerative disease and wish to maximize their response. Calories from protein is restricted towards 5% of total calories for four 24 hour periods every week. From table 4 we see that this computes to a 99% cytoplasmic replacement rate per decade or an 93% rate every 5 years. 50% replacement occurs in 1.35 years meaning that it can keep aggregates indefinitely at their current level if they are otherwise doubling exponentially every 1.35 years.

With only seven days in a week, this regime requires that some of the restriction periods if not all be contiguous. The pattern could say be Sunday, Wednesday, Saturday alternating a 48 hour restriction period with a 24 hour restriction period or say Monday, Tuesday, Thursday, Friday with two 48 hour restriction periods.

Since the intent is maximization, a protein restricted breakfast should also be employed to add 6 hours to the restriction period.

Whatever Days per Week Protein Cycling

Since you understand the scientific rationale behind protein cycling, you can design your own protein cycling schedule to suit you own lifestyle.

Other Diets

There a many, many diet plans in the world, traditional and synthetic and quite a few likely promote autophagy. Among them are Macrobiotics, vegetarian diets and a number of diets of cultural or religious origin. Generally they explain themselves in metaphorical terms like 'toxins', 'purity', 'balance', 'yin', 'yang', 'sacrifice' etc, rather than giving a scientific rationale. Regardless, if they do in fact promote autophagy by restricting protein or calories in quantity or timing, then they should work as well as any I have to offer. If you are on such a diet,

then you need only continue to achieve the same benefits as from protein cycling.

The term 'protein cycling' is also applied to other diets that promote body building purposes⁷⁷. Perhaps they can be tweaked to promote autophagy as well.

27. Summary

- Neurodegenerative diseases like AD and PD are characterized by protein aggregates and condensates.
- The aggregates, or at least the proteins that eventually form the aggregates, may be responsible for the disease.
- The aggregate proteins may accumulate because the usual protein recycling systems of the cell cannot handle them.
- The aggregate proteins may induce newly made proteins of the same type to mis-fold as well and accelerate the disease progression.
- Non-selective autophagy as occurs during periods of restriction may recycle the aggregate proteins.
- Non-selective autophagy may be triggered by amino acid restriction alone.
- Alternate day amino acid restriction may be sufficient to counter-act and reverse the accumulation at any point and indefinitely delay progression of neurodegenerative diseases.
- Nutrients other than protein must remain adequate in the diet including vitamins, minerals and essential fatty acids and should be guaranteed by taking supplements.
- Other foodstuffs such as curcumin and resveratrol may enhance the autophagy inducing effects of protein cycling.
- Heart disease, diabetes and cancer are much greater risks than all neurodegenerative diseases combined and the risk increases as overweight increases so weight control is a first priority.
- Protein cycling may reduce heart disease, diabetes and cancer by enhancing autophagy.
- Protein cycling can be used to reduce weight as well though the alternate day calorie restriction diet works faster while still promoting autophagy.

A website for discussing this book has been opened at:

<http://proteincycling.blogspot.com>