INTRODUCING

THE SECRET LIFE OF MITOCHONDRIA

YOUR CELLS’ MICROSCOPIC POWERHOUSES FOR LONGEVITY, APPEARANCE AND PERFORMANCE

JOSEPH L. EVANS, PH. D.
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ABOUT THE AUTHOR

Joseph L. Evans, Ph.D., is Founder and President of P & N Development Ventures (Redwood City, CA), a consulting firm to the pharmaceutical, nutraceutical, and venture capital industries. Dr. Evans is a pharmaceutical scientist with over twenty years experience in the research, development, and commercialization of pharmaceutical and natural product therapeutics for insulin resistance, type 2 diabetes, and obesity. Dr. Evans received his Ph.D. in Biochemistry from Drexel University (Philadelphia, PA), and received post-doctoral training in molecular biology, biochemistry, cell biology, and physiology at Dartmouth Medical School (Hanover, NH) and the University of Copenhagen (Copenhagen, Denmark).

Dr. Evans’ previous corporate experience includes positions of increasing responsibility in drug discovery and development at Syntex, Merck, Novartis (formerly Sandoz), Shaman, SUGEN, and Telik. Dr. Evans is Founder and President of JERIKA Research Foundation (Redwood City, CA), a privately-held nutraceutical company that identifies and commercializes novel and proprietary nutritional supplement products for unmet and underserved markets. Dr. Evans is also the Co-founder and President of Leptogen (Davis, CA), a biopharmaceutical company focused on the development of small molecule pharmaceuticals for the treatment of obesity and type 2 diabetes.

Dr. Evans has also served as Vice President, Research & Development and Chief Scientific Officer of Medical Research Institute (San Francisco, CA), where he provided scientific leadership/expertise in product development, manufacturing, regulatory affairs, technology evaluation, and clinical research for a variety of highly successful products including NO2™, CE2™, and AgeLess™ (controlled release lipoic acid). Dr. Evans has extensive expertise in the areas of natural product pharmacology, including antioxidants and nitric oxide biology, especially the regulation of nitric oxide production by natural compounds, including arginine and polyphenolic compounds. Dr. Evans has played a major role in the development, introduction, and commercialization of a first-in-class controlled release α-lipoic acid for individuals with type 2 diabetes, and a first-in-class nitric oxide generator product for the sports nutrition industry. Medical Research Institute (MRI), was acquired by Natrol (NASDAQ: NTOL) in June, 2007.
FORWARD:

THE SECRET LIFE OF MITOCHONDRIA

The authors have written an interesting, informative, and timely book about mitochondria. It was once thought that the levels of these intracellular organelles, which produce ATP, were under constitutive control and not subject to regulation. It is now known that the quantity of mitochondria in cells, such as muscle, change in response to diet, exercise, and pharmaceutical intervention. The current work summarizes our recent concepts concerning mitochondrial function and regulation. At the level of transcription, the regulation of mitochondrial biogenesis is both complex and only partly understood. A major point is that many laboratories studying mitochondria believe the pre-eminent positive regulator of mitochondrial biogenesis is the transcriptional co-activator, PGC-1α. Mitochondrial biogenesis is mediated by exercise, changes in the cellular levels of nitric oxide, and activation and/or increased content of the proteins, AMPK, and SIRT1. To increase mitochondria, both exercise and these other three factors require the involvement PGC-1α. In fact, nutritional or pharmaceutical interventions that increase either PGC-1α activity or its content may serve as exercise mimics. This book presents, in a highly readable fashion, a unique, timely, and interesting description of mitochondrial biogenesis and the major pathways that have been shown to increase mitochondrial biogenesis. I believe that the data presented is scientifically accurate and provocative, and trust it will have the same impact on you.

Sincerely,

IRA D. GOLDFINE, MD
Professor of Medicine
University of California, San Francisco
Life is too short to worry about things that we cannot control, so we are repeatedly advised to focus only on those important things which we can control. When it comes to our health, well being, and longevity, we are all at the mercy of our genetics, for better or worse. But, clearly, there are controllable variables. Despite the fact that we are not all CEOs of a company, we are all CEOs of our bodies. That’s right, of all the important things over which we have total control, none are more important than our bodies. Now, there is no shortage of reading, listening, and viewing materials extolling the benefits of various approaches that will make us look and feel younger and live longer. Some of them even mention regular exercise as part of the process! (If you have not figured this one out yet, exercise is very good for you, in a wide variety of areas.) However, none of the approaches really addresses a fundamental, root cause of increased body fat and reduced lean muscle mass, low energy levels and inefficient metabolism, increased low-grade inflammation, inadequate performance, accelerated aging, and for some, unfortunately, premature death. While these aspects of our general health might seem vastly different and impossibly related to a single cause, you will learn after reading this book, that there is a single causative factor—one I am sure many of you have not even heard of before reading the title of this book. But, I assure you, of all the important components of the cells in our bodies, none are more important than mitochondria. By analogy, think of a world without any source of energy, not even solar. That’s right, not much of a world, even by prehistoric standards—might as well as well be living on the moon! But wait, even living would be impossible without an energy source. This book will not lead you on a journey to become energy independent, as I assure life without energy is impossible. However, this book will show you how to take control and increase your energy reserves and make them more efficient—the far reaching benefits will impact every aspect of your general health and well being. We hope you enjoy the read.
CHAPTER 1

THE MITOCHONDRION:
EVOLUTION, STRUCTURE,
AND FUNCTION
CHAPTER 1

INTRODUCTION

The most fundamental and essential need of our bodies, indeed all living things, in order to survive and reproduce is energy. Energy is stored in food, but it is not biologically useful until it is converted into a form that our cells and tissues can use. No matter what type of food is consumed as our primary source of energy, and we all know that some sources are better than others, this potential energy must first be absorbed into our bloodstream, and then into our cells, the smallest functional units of the tissues. The food we ingest is analogous to the crude oil that is drilled from oil wells. While it is absolutely necessary in the overall production of energy, it must first be processed and refined to be of any use as an energy source.

Once in our cells, useable energy is produced through a sequential series of biochemical reactions. The most important energy molecule that our cells make is called ATP (adenosine 5’- triphosphate). This is a high-energy molecule that is necessary to drive the biochemical reactions that permit our cells and tissues to function normally. ATP is analogous to the gasoline that fuels our cars. Without it, our metabolism grinds to a halt, and our cells rapidly die.

Our primary objective in achieving and maintaining an attractive physique, low body fat, and overall healthy body should be to maintain an adequate supply of ATP. The more ATP we have available, the more efficient our cellular metabolism will be, and consequently the aging process slows. However, just like the gasoline tanks in our cars which require all too frequent refueling ATP must also be re-synthesized and replenished to resupply our body's continual demands for energy. The healthier we are (read: less body fat), the better positioned we are to provide our body with all of our required ATP. Thus, the rich get richer, metabolically speaking. In contrast, the more excess body fat we carry around, the more difficult it becomes to meet our daily energy needs. This situation leads to a downward spiral, in that the less energy (i.e. ATP) we have available to replenish our energy stores leads to an energy deficit, which we are poorly positioned to resolve. Clearly, the failure of many individuals to stick to their exercise program or even start one, because they are too fatigued or become too exhausted before seeing results, reflects this downward spiral.

Ok, so we have now discussed the critical need for us all to have an ample supply of ATP to enhance our physical appearance, maintain our general health now and in the future,
improve the quality of our life, and slow down the aging process. But is this possible? How is it accomplished, and what are our best options for increasing ATP? Is it too late, if we are already overweight? Is it too late, if we are already over 40? Will it hurt? Is there a pill?

Before we go on to answer these and many more questions, we need to introduce to you the mitochondrion (plural, mitochondria), the featured performer in our story since this ‘Mighty Mo’ is center stage for our ATP production (Figure 1.1).1, 2

While the cell’s nucleus and DNA have long held ‘Rock Star’ status among the media, general public, and even many health care professionals for many decades, mitochondria can best be viewed as the Rodney Dangerfield of cellular organelles, i.e. they don’t get
no respect! For example, a recent search of the biomedical literature (using PubMed) revealed over ten times as many scientific references related to nucleus and DNA compared to references on mitochondria.

Mitochondria are analogous to the oil refineries; the basic purpose of a refinery is to separate and transform various hydrocarbon groups from crude oil so that they may be used, combined, or further treated to create the thousands of products made from petroleum, including gasoline. In an analogous fashion, mitochondria are the specialized little factories within each of our cells in which carbohydrates and fat are converted into ATP. Not surprisingly, they are often referred to as the cellular ‘power plants’ due to their primary function of producing energy in the form of ATP. In our day-to-day life, whether we are feeling good or feeling down, mitochondria really do go unnoticed, even when we cannot understand why we are so tired, or crave a nap at 2 PM. Despite their fundamental importance in determining so many aspects of our general well being, mitochondria still fall below the radar of most individuals. We hope to change that situation forever through the information in this book.

Mitochondria Overview

Mitochondria are one of many types of cellular organelles which, in cell biology speak, are specialized functional sub-units within cells that are partitioned off from the cellular matrix by their own lipid membrane. Mitochondria are found in all of our cells, but are especially abundant in metabolically active cells, such as brain, skeletal muscle, heart, liver, and kidney. These cells can contain thousands of mitochondria, which can comprise about 40% of the cytoplasm. The female oocyte (egg cell) will pass on more than 100,000 mitochondria to the next generation; sperm cells typically possess less than 100 mitochondria. In contrast, blood cells and skin cells (epithelial cells) have few if any mitochondria. According to Professor Enzo Nisoli, a pioneer in this field, and colleagues, adults possess approximately 10 million billion mitochondria, which corresponds to approximately 10% of our body weight! That is some serious energy generating capacity!

As discussed above, the primary function of mitochondria is to produce ATP. However, mitochondria have other important functions including (but not limited to) the storage
and release of calcium, an important signaling molecule within the cell, and the control
of cell death. Since a discussion of these additional functions of the mitochondria is
beyond the scope of this book, the reader is referred to two excellent references for more
information.2, 4
Mitochondria have many structural and functional features in common with bacteria
and other ‘simpler’ organisms (prokaryotes). This has led to the widely held belief that
mitochondria originated from an endosymbiotic (one organism living within another)
relationship between bacteria and more complex cell types.5 This idea was strengthened
significantly after the detection of DNA in mitochondria, which shows remarkable similarity
to bacterial genomes.

STRUCTURE

Each mitochondrion consists of four main compartments, including the outer membrane,
the inner membrane, the inter-membranous space, and the matrix. The outer membrane
encloses the entire organelle and is relatively porous, allowing the passage of small
molecules. The outer membrane contains the enzymes that transport fat into the matrix,
where it is metabolized and converted into ATP. The inner membrane is characterized
by a series of complex folds and tubules called cristae, which contain a variety of the
enzymes, including those responsible for making ATP. Thus, the more cristae, the greater
the capacity for producing ATP. Cells that have a greater energy demand, such as skeletal
muscle cells, have more cristae than, for example, epithelial (skin) cells. The matrix is
all the space enclosed by the inner membrane. This is the location in the mitochondrion
where ATP is actually made, through the action of the ATP synthase enzyme. In addition,
the matrix contains many important enzymes including those responsible for the oxidation
of pyruvate (formed from the metabolism of carbohydrates) and fat. The matrix also
contains the mitochondrial DNA, which directs the production of some but not all of the
mitochondrial proteins.
CHAPTER 1

FUNCTION

The most important function of mitochondria is the production of energy in the form of ATP. The coordinated and highly regulated group of biochemical reactions involved in ATP production is collectively known as the citric acid cycle (also called the tricarboxylic acid cycle or Krebs cycle) along with the electron transport chain. As you might have surmised, making ATP is a very complicated, but essential process. As a testament to the complexity of this area of research, two Nobel Prizes in Chemistry have been awarded: the first in 1978 to Professor Peter Mitchell for his work on describing the proton gradient and its role in driving ATP production, and the second in 1997 to Professors Paul Boyer and John Walker for their elucidation of the enzymatic mechanism underlying the synthesis of ATP by ATP synthase.

Due to the essential role played by the electron transport chain in producing ATP, the early development of pharmacological agents that targeted these complexes had a significant impact on the ability of cells to produce energy (ATP) and were generally regarded as toxins. That is, if you block electron transport, you block the formation of the proton gradient, and therefore the supply of energy to produce ATP. This class of agents is also referred to as uncouplers (also called metabolic uncouplers or uncoupling agents) since they uncouple the process of electron transport from ATP synthesis. One of the earliest weight loss drugs was the uncoupling agent DNP (2,4-dinitrophenol). This agent was used from 1933 until it was removed from the market by the passage of the US Food, Drug, and Cosmetic Act in 1938 due to several fatalities, a number of cases of cataracts, and other reported toxic effects. The pesticide rotenone inhibits the transfer of electrons at complex 1, and carbon monoxide (one of the emissions from your car’s exhaust pipe) inhibits the transfer of electrons to oxygen by competing for the critical binding site. The antibiotic oligomycin inhibits ATP synthase by blocking the pore through which the protons pass.

However, not all uncoupling agents are toxic; we all have mitochondrial proteins, called uncoupling proteins, which are proton channels that allow the passage of protons and thus do not drive ATP production; instead an elevation in body temperature (heat) is produced. Metformin, the most widely used oral medication for type 2 diabetes, is a mild uncoupling agent. Despite this activity, or perhaps because of it, metformin has a beneficial effect on blood glucose levels, and its use is also associated with mild weight loss in many individuals. Recent evidence indicates that metformin activates the enzyme AMPK
(5’AMP-activated protein kinase),\textsuperscript{11-14} which is activated when the levels of ATP decrease and the level of AMP (adenosine monophosphate) increases. AMPK acts as our body’s fuel gage, and plays a very important role in temporarily shutting down our body’s use of ATP, while simultaneously increasing our body’s ability to generate ATP, including burning fat, and stimulating the uptake of glucose from our blood. In addition, very recent research has shown that AMPK plays an essential role in mitochondrial biogenesis.\textsuperscript{15, 16} That’s right! When you uncouple, you increase AMPK, which quickly switches your metabolism from energy consuming to energy conservation and more… it increases fat burning, and directs your body to make more mitochondria! Sort of like a Toyota Prius, only much better. But wait, it gets even better… guess what exercise does---- it also stimulates AMPK!\textsuperscript{17} So, if you are thinking straight, you are thinking that agents that stimulate AMPK have the potential to lower blood glucose, conserve your ATP, so that you have enough energy to generate even more, burn fat, and stimulate your cells to make mitochondria.\textsuperscript{18-20} We will discuss this concept in a lot more detail later in the book.

Although oxygen plays a critical role as the final acceptor of the transferred electrons resulting in the formation of water (i.e. H\textsubscript{2}O), intermediates called reactive oxygen species (ROS) or free radicals can and do form during this process, and are potentially harmful if they are produced in excess.\textsuperscript{21} These ROS can react with and damage DNA, proteins, and lipids, leading to structural and functional abnormalities in these macromolecules. And since mitochondria are a prime source of ROS, their DNA, proteins, and lipids are inviting targets for attack, as they are in such close proximity. Think of mitochondria as being at ‘Ground Zero’. This can be very bad! The formation of ROS increases as we age, as we gain weight, and in virtually all disease states.

The cell has several antioxidant defense systems to counteract the potentially harmful effects of ROS, including the antioxidant enzymes, superoxide dismutase, peroxidase, and catalase, along with the antioxidants vitamin C and E.\textsuperscript{21} However, when the overproduction of ROS exceeds the capability to ability to remove them, oxidative stress ensues, as if frequently encountered with obesity, aging, and many diseases states.\textsuperscript{22, 23}

Now you are probably already asking “Is there anything we can do to reduce or prevent the excess formation of mitochondrial ROS?” Well, yes there is, but that will come later in the book. For now, the point to grasp is: we all need energy, and lots of it. We produce virtually all of our useable energy in the mitochondria in the form of ATP. The best way to
increase your energy, reduce your body fat and improve your physique and to maintain a healthy body is to exercise. Exercise is the most proven method to improve the function of your existing mitochondria, to stimulate the production of additional mitochondria (called mitochondrial biogenesis), which in turn provides even more ATP for your cells. However, as we discuss this in more detail, we will see that very recent research from some of the best laboratories in the world is providing us with a road map to identify novel and effective approaches to enhance or mimic the effects of exercise. That’s right, I said. There are strategies that can be exploited to increase the efficiency of your exercise time. Now, I do not mean to give the impression that you can get sixty minutes benefit for 5 minutes in the gym. If you expect that, keep on dreamin’. But what I am saying is that it will be possible to perhaps get sixty minutes benefit for 30 minutes in the gym. Now, I hope that got your attention. Let’s read on to see learn about how aging and disease affect the efficiency of your mitochondria, and vice versa.
CHAPTER 2

THE ROLE OF MITOCHONDRIA IN AGING AND DISEASE
Obviously, having well-functioning mitochondria is important for optimum energy production. But do you really have to worry about the condition of your mitochondria? Can the function or efficiency of your mitochondria really decline? And, if so, does this really lead to health problems, or is this just a problem for top-level athletes, who need to maximize their energy production for optimum athletic performance? We’ll answer these questions and more in this chapter, where you’ll come to understand just how vital your mitochondria are for your own well-being and vitality. In addition, your partner will be eternally grateful for taking the time to read on! And after we take you through some of the most recent discoveries made by scientists studying energetics and health, you will understand why the focus on mitochondrial function represents a new paradigm in the way aging and disease will be viewed and treated in the years to come.

As we discussed in Chapter 1, mitochondria have their own set of DNA. This fact was known for over 25 years before it was first discovered that defects or mutations in mitochondrial DNA played a role in several very serious human diseases. The conditions first linked to mitochondrial DNA mutations were rare, inherited disorders that were passed down exclusively from the mother, since we get our mitochondria from the egg, and not from the sperm. One example of a severe condition that can develop as a result of mitochondrial dysfunction is a form of blindness that develops in young adults, called Leber’s hereditary optic neuropathy (LHON), the first disease linked to mitochondrial mutations. Other inherited mutations in mitochondrial DNA lead to relatively rare diseases producing, muscle myopathy (a progressive muscle weakness), cardio myopathy (an abnormal enlargement and deterioration of the heart muscle, movement problems, and dementia. Even a substantial number of diabetes cases that develop in younger individuals are linked to mitochondrial mutations.

What these diseases have in common is one or more defects in mitochondrial DNA that result in fewer or abnormal proteins being produced that are critical for proper mitochondrial function. Accordingly, mitochondrial function in the affected tissue, be it skeletal or heart muscle, the brain, or the optic nerve, and often throughout the entire body, is dramatically impaired. While these diseases are not common, they are instructive about the consequences of impaired mitochondrial function. Interestingly, if you put the symptoms together from different diseases that have been shown to originate from mitochondrial dysfunction, for example loss of hearing and vision, loss of muscle
strength and coordination, as well as dementia, you start to build a profile of what we normally think of as simple aging.

The ways in which these genetic diseases are different come from the fact that distinct organs are differentially effected by the mutations, and that the symptoms can develop at different stages of life, sometimes not until a relatively old age. One reason why an inherited mutation might not develop into a symptomatic disease until late in life is that mitochondrial DNA damage and impaired mitochondrial function form what is known as a vicious cycle, which is fueled by the generation of ROS that we discussed in Chapter 1. In this vicious cycle, reductions in functioning mitochondrial proteins caused by mutations in the mitochondrial DNA lead to an increased production of ROS and free radicals. These ROS and free radicals over time can induce more mutations in the DNA, further impairing the ability to produce healthy, fully functional mitochondria. LHON, for example, typically results in vision loss in young adulthood, but the age of onset can be in middle age or later. Recently, scientists identified carriers of the most common mitochondrial mutation (affecting 1 in 6000 people) and studied them at a young age while they were still symptom-free. Individuals with this mutation typically develop brain degeneration and stroke-like symptoms, as well as diabetes and deafness, and degeneration of the heart and skeletal muscle, which were common problems among the older relatives in the families of these still healthy subjects. What the researchers did find was that even before these symptoms developed the ability of their hearts to generate ATP was already significantly impaired.

Together, the studies of diseases derived from a mitochondrial mutation(s) give us a glimpse of the effects over time when starting with impaired mitochondrial function. Together, the evidence suggests that once mitochondrial function is reduced, there is an ever-increasing cycle of damage, as ROS and free radicals can produce damage to proteins and DNA in the mitochondria. The DNA damage leads to production of defective proteins and the replication of mutated DNA to produce new, defective mitochondria. The transmission of biochemical reaction to DNA damage therefore makes the problems more permanent and more difficult to treat. In the next section, we’ll show you the evidence that the aging process is intimately tied to the accumulation of oxidative damage to cells, and in particular to mitochondrial DNA. We’ll also show you how accumulated defects in mitochondrial function can produce many of the most common health problems in our society.
CHAPTER 2

AGING

Long before scientists began looking toward the mitochondria as a critical player in multiple diseases, its central role in the process of aging was already well established. Professor Denham Harman first proposed the free radical theory of aging in 1956, although at the time he found little support for his stance that accumulated cellular damage resulting from ROS was responsible for the symptoms associated with aging. By 1972, when he published his theory that mitochondria, by determining free radical production, functioned as a “biological clock” determining the ultimate life span of an organism, the contributions of mitochondria to aging were more widely accepted. However, it would be many more years before the interrelationships between mitochondrial function, uncoupling, ROS, mutations in mitochondrial DNA and mitochondrial biogenesis would be understood sufficiently to allow for a more full understanding of the role of mitochondria in aging.

Eventually, the contributions of mitochondrial DNA mutations to human aging were observed directly. Initially, these genetic changes were seen within mitochondria in tissues from individuals in their 70s. This resulted in the mistaken impression that mitochondrial dysfunction was a problem only in senescence -- the deterioration that occurs near the end of the life span -- and was irreversible. However, it was later observed that mitochondrial dysfunction actually was occurring decades earlier than widespread DNA damage had been observed. The cellular damage that results from this reduced mitochondrial function contributes to pre-senescence aging and generates the mitochondrial DNA mutations seen in the aged.

But let’s not forget that aging is a very complex issue involving all the body’s organ systems. You could legitimately wonder how alterations in mitochondrial function could produce so many symptoms that you’ve probably previously attributed to the body just wearing out. To see how mitochondrial dysfunction can produce many of the health problems associated with growing old, let’s first look at the way that proper brain function is absolutely dependent on efficient mitochondrial activity.
CHAPTER 2

MITOCHONDRIAL DYSFUNCTION AND NEURODEGENERATION

Since you’re used to thinking of your muscles as doing all the heavy lifting as you go through your day, you’ll probably be surprised to learn that your brain is your most metabolically active tissue. Thinking all those brilliant thoughts you have takes a lot of energy, as does processing all those sights and sounds around you. All the neural impulses involved in these mental processes require ATP production and involve mitochondrial respiration. That’s why, at rest, your brain is responsible for 20% of your total energy expenditure even though it accounts for about 2% of your body weight.32 As we’ve discussed previously, any time the activity of the electron transport chain is high, there is a high risk of ROS production and oxidative damage.

Unfortunately, the anti-oxidant defense system in your neurons is not as strong as other tissues, and the brain is rich in structures particularly susceptible to oxidative damage.33 The end result is that the brain is highly susceptible to mitochondrial dysfunction, and neural and cognitive problems are common in the syndromes of inherited mitochondrial DNA mutations.26 Beyond that, mitochondrial dysfunction contributes to several other degenerative brain diseases that do not result from inherited mutations.26 These progressive and incurable diseases point out the importance of preserving mitochondrial function and preventing oxidative damage in the brain throughout your life.

ALZHEIMER’S DISEASE

As mentioned earlier, aging is associated with reduced mitochondrial function and mutations in mitochondria. The brain is no exception, as almost half of the mitochondrial DNA in the brains of elderly subjects have severe mutations.34 In Alzheimer’s disease, the damage goes a step further, with formation of amyloid plaque throughout the brain and significant neuron death.35 However, there is an ever growing body of evidence that mitochondrial dysfunction is among the earliest events that produce the lethal cascade of neural damage.

In patients with Alzheimer’s disease studied before the development of significant cognitive impairment or visible damage to the brain, energetic imaging techniques have revealed an increase in the amount of oxygen consumed relative to the amount of glucose
being metabolized.\textsuperscript{36} This inefficient use of oxygen shows that mitochondrial dysfunction in the brain is one of the first observable symptoms of this disease. The brains of patients with Alzheimer’s disease show reduced activity of the mitochondrial electron transport chain (specifically complex 4) and display significant oxidative damage.\textsuperscript{36} Since amyloid plaque accumulation can result in significant ROS generation,\textsuperscript{37} some of the mitochondrial damage certainly develops later in the disease. Still, despite lingering questions over the initial cause of mitochondrial dysfunction in patients with Alzheimer’s disease, the contribution of mitochondrial energetics and oxidative damage in the progression of the disease is undeniable.

**PARKINSON’S DISEASE**

Parkinson’s disease is a progressive neurodegenerative disease that causes disintegration of the afflicted patient’s motor skills, speech, and other physical functions. It is most commonly associated with a characteristic tremor. A small fraction of the Parkinson’s disease cases are attributable to inherited mitochondrial mutations.\textsuperscript{35} The primary evidence that this disease progresses as a result of oxidative damage comes from several sources. As with Alzheimer’s disease, the mitochondrial electron transport chain function is reduced in Parkinson’s disease, although at a different site, primarily complex 1.\textsuperscript{37} Some very clever scientists took cells that did not contain mitochondria and injected mitochondria from either Parkinson’s disease patients or normal volunteers, they saw that the cells with the Parkinson’s disease mitochondria were significantly less efficient at generating ATP, and generated higher levels of ROS.\textsuperscript{38} In addition, a synthetic neurotoxin targets the electron transport chain and produces Parkinson’s disease-like symptoms.\textsuperscript{37} The origin of mitochondrial dysfunction in Parkinson’s disease is not clear, although environmental toxins have been hypothesized to contribute. The presence of multiple markers of oxidative stress and accompanying damage to proteins, lipids and DNA in mitochondria, in addition to a depletion of the intrinsic anti-oxidant system in the neurons of patients with Parkinson’s disease, provide compelling evidence that impairments in mitochondria contribute to the progression of this disease.\textsuperscript{35}
OTHER NEURODEGENERATIVE DISEASES

Mitochondrial dysfunction, as well as cellular oxidative damage and mitochondrial DNA mutations have been tied to the development and/or progression of several other neurological diseases such as Huntington’s disease, Amyotrophic Lateral Sclerosis (also known as ALS or Lou Gehrig’s disease), and Multiple Sclerosis. You might wonder, then, whether neglecting your mitochondria will put you at risk for developing any or all of these conditions. The truth is that, aside from Alzheimer’s disease, these are relatively rare conditions that likely require something more than moderate mitochondrial inefficiencies to develop. The point we’re making here is that the brain is extremely dependent on oxidative phosphorylation, and dangerously sensitive to oxidative stressors. Because of that fact, the fate of you and your brain is exquisitely tied to your mitochondria, and you will need them to function as efficiently as possible for as long as possible if you are to avoid cognitive decline as you age.

MITOCHONDRIAL DYSFUNCTION AND CARDIOVASCULAR RISK

So how important is having robust mitochondrial function for good cardiovascular health? Well, it is well known that being sedentary can both decrease the aerobic capacity of muscle and the body as a whole and can increase the risk for heart disease, but that doesn’t answer the question of whether poor aerobic capacity can actually cause poor cardiovascular health. This question was important enough to a group of scientists to study this question in a novel way. These investigators noticed that, just like in humans, in a group of rats, there was a wide range in their ability to perform aerobic exercise. They then selected the rats that could run for the longest time on a treadmill and bred them with each other. They did the same selective breeding between rats with the worst endurance during treadmill running tests. For 11 generations, they kept testing offspring from the high endurance group and breeding the most proficient runners, as well as breeding the worst runners from the low endurance group. At the end of the 11 generations of selective breeding, they predictably had a strain of rodent couch potatoes, as well as a strain of rodent ‘Olympic’ champions. The high-endurance rats could run three and a half times longer at the same speed as could the low endurance group.

Not surprisingly, given what we know about the role of mitochondria in producing energy...
for aerobic activity, the content of mitochondrial proteins were significantly higher in muscle from the high endurance group. Also, the proteins responsible for stimulating the production of new mitochondria (which we’ll discuss in the next chapter) were also generated in higher amounts in the high endurance group. The important findings, however, were that these rats with a low aerobic capacity displayed many of the characteristics that cause doctors to worry about the risk of heart disease in their patients. These rats had elevated blood pressure, serum triglycerides, and other aspects of the cardiovascular risk profile termed “the metabolic syndrome”. The hearts of these low endurance rats showed signs of increased cellular stress and greater susceptibility to arrhythmias.

Let me spell out why these findings are so important. Doctors and scientists have believed for the longest time that improved aerobic capacity and improved cardiovascular health were two separate benefits of performing regular exercise. Most doctors, if asked, would still support this idea. But these studies show that the important component is the high capacity for aerobic energy production, due to large amounts of highly functioning mitochondria that is important for cardiovascular health. If, like the rats in this study, you are born with it, you have an advantage without ever stepping on the treadmill. If you’re like the rest of us and don’t have Olympic marathoners for parents, you are going to have to work a bit for your health. But, if you take advantage of some methods to improve your mitochondrial function that don’t involve exercise, as you’ll read about later in the book, you could get benefits similar to someone who might exercise twice as hard.

Other scientists have taken a different approach to reducing the ability of mice to produce mitochondria by genetically blocking the production of proteins that stimulate mitochondrial biogenesis. They have found that doing so leads to multiple defects in the function of the heart. In these mice, the energy reserves are so minimal that the hearts just can not respond to increased demands and are unable to produce stronger contractions. These studies showed that hearts with deficiencies in mitochondrial function were predisposed to failure when stressed.
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MITOCHONDRIAL DYSFUNCTION AND DIABETES/OBESITY

The risk of heart disease is also increased by being overweight, and by the presence of diabetes.\textsuperscript{44} In the pre-diabetic state, blood sugar levels are still kept somewhat under control, although insulin, the hormone the body uses to trigger an uptake of glucose into muscle and repress a release of glucose by the liver, is much less effective at producing these effects.\textsuperscript{45} This condition raises the risk of heart disease as well, although the symptoms of pre-diabetes are typically too subtle to be easily detected. Because type 2 diabetes, as well as the resistance to insulin that predisposes an individual to this disease, are much more common in the overweight and obese, it was assumed that the accumulation of large amounts of fat throughout the body somehow affected the cellular response to insulin in muscle, liver, and other tissues. Recently however, diabetes experts have come to realize that it is an accumulation of fat stores directly in the muscle and liver cells that is causing the breakdown of their normal function.\textsuperscript{46} While it is easy to understand how these cellular fat stores are elevated in an obese person, it was less clear why the intracellular fat would accumulate in normal weight people, although when it does insulin resistance is the result.

Several studies have now examined the mitochondrial function in these individuals, and have found that a reduced mitochondrial content and impaired oxidative phosphorylation are at least part of the problem. These signs of mitochondrial insufficiency were seen together with an accumulation of fat in muscle from lean individuals who were at increased risk for diabetes due to a family history of the disease.\textsuperscript{47, 48} So even in the absence of over abundant fat stores throughout the body, an inability of muscle to burn off fat stores within the cell because of inadequate mitochondrial capacity caused an accumulation of lipids in the muscle cell and resulted in a disruption of normal cell metabolism.

And remember those rats that were bred for their low mitochondrial and aerobic capacity? When they were provided a diet high in fat, which is probably a lot more like your diet than is standard rat chow, they gained more weight, added more fat, and became more insulin resistant than did the rats with a high mitochondrial capacity.\textsuperscript{49} This shows you the importance of being able to burn off the fat you’re putting into your body by optimizing the performance of your mitochondria.
MITOCHONDRIAL DYSFUNCTION AND MYOPATHY

Just like cardiac muscle, skeletal muscle is highly sensitive to mitochondrial defects, since it also needs to produce large amounts of ATP via oxidative phosphorylation. This makes muscle a primary site of damage in individuals with inherited mitochondrial mutations. In these individuals, the accumulated mitochondrial damage leads to severe muscle weakness and exercise intolerance. Three-time (1986, 1989, 1990) Tour de France champion Greg LeMond eventually was forced to retire when the diagnosis of mitochondrial myopathy explained his progressive loss of exercise capacity. It has been speculated that his problems arose from environmental toxins, and were not inherited. However, mitochondrial dysfunction arising from different causes can also significantly impair muscle function.

Muscles from aged individuals show decreased mitochondrial content and lower amounts of energy produced per amount of mitochondria. The evidence suggests that the lower efficiency in muscle mitochondria from the elderly is a result of ROS damage to the inner mitochondrial membrane causing an inefficient uncoupling of the electron transport chain. That means that much of the weakness and vitality loss that we attribute to just getting older is really a result of mitochondrial dysfunction related to cumulative oxidative stress.

Fortunately, studies have shown that the decreased efficiency and impaired energy production capacity of muscle mitochondria that develops with aging can be at least partially reversed by exercise. This shows that mitochondrial dysfunction in the aged is not entirely due to irreversible mutations in mitochondrial DNA, as some have hypothesized. Instead the evidence suggests that not only can the production of new mitochondria be triggered by regular physical activity, but the efficiency of existing mitochondria can be improved, perhaps by reversing oxidative damage. The ways in which exercise can rev up energy production and reverse the aging process obviously deserve a closer look.
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EXERCISE AND MITOCHONDRIAL BIOGENESIS
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Proper mitochondrial function is obviously crucial for many aspects of health. Fortunately, you can take steps to increase the energy production capability of your cells, even if your mitochondrial capacity has declined due to age or weight gain. The amount of mitochondria in your cells is not fixed, and while the process whereby new mitochondria are synthesized is complex, there are a few key sites that are responsible for regulating of mitochondrial biogenesis that we have only recently come to understand. This process is best understood by looking at the best known and most effective way to produce new mitochondria... exercise! 15, 53-58

It’s no secret that aerobic exercise is good for you. 59-61 Many have heard from their doctors to get at least 30 minutes of vigorous activity each day. However, the exact way in which regular physical activity improves health remains a great mystery to most individuals, doctors included. You might think that the key aspect of going for a jog or using the elliptical trainer at the gym is burning a few extra calories each day. However, one of the primary benefits of that activity is in the long-term cellular adaptations that result from the work your muscles perform. 53, 62

While exercise is the best way to avoid the continual accumulation of fat stores that is becoming more and more common across the globe, it is clear that the benefits of regular physical activity go beyond maintaining a svelte waistline. The risk for developing chronic diseases such as diabetes, cardiovascular disease, neurological degeneration and even cancer is higher for sedentary individuals than their active counterparts whether or not they are overweight 63. So, clearly, exercise is positively affecting some organs other than your fat cells.

Try to workout alongside an elite athlete and you’ll quickly realize that there is a huge difference in the performance capacity of their body, especially their cardiovascular system and their muscles. What is less obvious is that there is also a dramatic difference in the way the muscles look on a microscopic level. To some extent, you can see the signs of their high endurance capacity just by looking at the composition of their individual muscle cells (called fibers). The biggest difference you’ll notice is that an endurance athletes muscle is jam packed full of mitochondria. 15, 53 This is not a new observation, nor is it limited to individuals participating in regimented exhaustive workouts. In fact, the ability of mitochondrial production to increase in proportion to the amount of physical activity was first hypothesized when it was noticed that the breast muscles of pigeons contained...
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much more mitochondria than that of chickens, which, unlike the airborne pigeons, did not use these muscles to fly.

So, while there are many generalized benefits of regular exercise on the body as a whole, many of these occur as a result of changes that occur within the organ that is performing the majority of the work, skeletal muscle. Muscle has a tremendous ability to adapt both its structural and functional characteristics in response to the demands of repeated exercise bouts. The adaptations occur incrementally over time, eventually resulting in improvements in the ability of muscle to handle the mechanical and energetic requirements of repeated contractile activity.

We’ll need to explore just what happens within a muscle fiber when it is active in order to understand the cellular triggers that stimulate mitochondrial biogenesis. This understanding can help maximize the effects of exercise and perhaps generate some of these same responses in other cells and tissues. We’ll be talking specifically about skeletal muscle, the muscle type that produces movement of the body. Skeletal muscle is not the only type of muscle in the body, as cardiac muscle contracts to propel blood flow to the body’s organs, and smooth muscle contracts to regulate the diameter of blood vessels, the movement of food through the digestive tract, along with other bodily functions that occur involuntarily. Skeletal muscle attaches to bone on opposite sides of one or more joints, and by shortening the length of individual muscle cells, called fibers, muscle causes movement of body parts across the joints. So while a single movement may require the coordinated actions of multiple muscle fibers, the fibers stimulated to contract by the nervous system (recruited) may have very different structural and functional properties.

Because the primary function of muscle is to contract in order to produce movement, and this contraction requires energy, the primary components of a muscle cell can be divided into two principal categories, the contractile machinery (responsible for shortening the muscle fiber), and the metabolic machinery (responsible for generating ATP for the contraction). The functional profile of a muscle fiber, which can vary widely throughout the body, is a factor of the makeup of both the contractile and metabolic machinery.

The contractile properties (reflected by a fast- or slow-twitch phenotype) determine the types of activities for which the muscle fiber is best suited either quick movements that require a high force production, or prolonged use. These properties are largely pre-
programmed, and are a factor of the type of contractile proteins that the cell produces. A muscle fiber shortens when the contractile proteins actin and myosin bind each other and pull towards each other, bringing the opposite ends of the contractile units closer together. Cells that produce fast-contracting myosin (termed fast twitch fibers) will shorten more quickly, and are better suited for faster, more explosive activities. Cells that produce slow contracting myosin (slow-twitch fibers) are more suited for slower, long-lasting activities, where their greater efficiency at producing force will be advantageous.66

The metabolic properties of a fiber are matched to the contractile properties, such that fast twitch fibers are more capable of generating large quantities of ATP rapidly, for short periods of time, while the slow twitch fibers are adept at producing ATP for long periods of time while resisting fatigue. These metabolic characteristics show greater diversity across individual fibers than the contractile properties which fit neatly into two categories. This is because the metabolic machinery is much more adaptable to changing demands, and adjusts rapidly to increased and decreased use. These metabolic demands placed upon the fibers therefore can vary dramatically as a result of the contractile machinery, but also as a result of the amount of time they must spend working. The ways in which the energy production capabilities of muscle fibers are improved in response to increased use not only contribute to the fatigue resistance of the muscle, but contribute dramatically to overall health.

As the site of energy production in the cell, mitochondria are critical determinants of the function and endurance properties of muscle. The important metabolic machinery of fast twitch muscle fibers that are used only in short explosive movements are enzymes involved in anaerobic glycolysis, the rapid breakdown of glucose for ATP.65 However, this process, although ideal for generating ATP quickly, results in the incomplete breakdown of glucose and the accumulation of the by product lactic acid. Because of problems in handling lactic acid, anaerobic glycolysis proves to be an unfeasible option for prolonged activities. An improved energy production and endurance capacity of muscle requires an increase in the aerobic machinery of the cells, which, as discussed previously, depends on the mitochondrial content of individual muscle fibers. This increase in mitochondrial content is the hallmark response of muscle fibers to increased use.

Muscle has by far the highest energy production capacity of any tissue, in order to meet the demands of vigorous activity.65 However, when not contracting, muscles use very
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little energy. At rest, skeletal muscle accounts for only 16% of the entire resting energy expenditure of the body, although it might account for 40% of total body weight. In contrast, your brain, heart, liver and kidneys, which are hopefully active even when your muscles are not, burn energy at about 30 times the rate per pound as resting skeletal muscle.

Once activity starts, however, and muscles begin to contract, their rate of energy production can increase 200-fold. Thus, the cellular machinery of skeletal muscle must be equipped to respond to contractile activity with rapid increases in the delivery of fuel sources, and the capacity to rapidly generate ATP from these compounds. One such response is the very rapid increase in the uptake of glucose from the blood stream into muscle cells following the initiation of contractile activity. The effect of contractile activity is quite rapid, reaching a maximal increase in the rate of muscle glucose transport within minutes. These types of reactions require an ability of the cell to sense the increased demand for energy and respond. It may seem obvious, but each cell needs to have a system in place whereby it can sense the demands placed upon it and respond appropriately. In muscle, this means integrating changes in the energy state of the cell, with signals related to the actual work being performed. The exact cellular mechanisms responsible for activating the metabolic machinery of muscle in response to contractile activity have yet to be fully detailed. However, many of the same intracellular systems that are used to help the muscle cell respond to the acute need for fuel are also involved in triggering the long term response to repeated exercise bouts involving a gradual adaptation in the metabolic profile of muscle.

As mentioned earlier, the primary long term adaptation that muscle makes to repeated metabolic demands is an increase in mitochondria. Mitochondrial biogenesis is accomplished when one of these exercise-related signals activates the most important regulator of mitochondrial biogenesis, PGC-1α (peroxisome proliferator-activated receptor-gamma coactivator-1alpha). PGC-1α functions as a transcription factor, a protein that binds to your DNA in specific sites to regulate the synthesis of new proteins. PGC-1α is a master regulator of mitochondrial biogenesis, because nearly all of the genes coding for proteins that are required to make new mitochondria have binding sites on their DNA regulatory regions for PGC-1α. Using genetic manipulation, when mice were produced that made higher than normal amounts of PGC-1α in their skeletal muscle, the mice had significantly greater proportion of fatigue resistant slow twitch muscle fibers,
and had much greater mitochondrial content. These mice also showed much greater running ability, exercise endurance and improved recovery, highlighting the key role of PGC-1α in mitochondrial biogenesis and improved energy production. Simply stated, all roads to mitochondrial biogenesis travel through PGC-1α, and increased PGC-1α appears to improve exercise performance.

To understand how going from rest to activity can produce a disruption in the intracellular environment that will lead to an immediate compensation to increase energy production and also trigger long term adaptations via mitochondrial biogenesis, let’s look at the different ways in which contractile activity lead to the production of several “second messenger” signaling molecules. These signaling molecules pass on the message that the muscle is now active, and that very specific responses to the enormous metabolic and mechanical challenges are needed.

When contractile activity starts, the levels of calcium and several metabolic products increase. These serve as second messengers to activate enzyme pathways designed to both stimulate metabolism and increase the production of components of the contractile or metabolic machinery. The signals that are generated by low intensity aerobic activity differ in part from those generated by high intensity resistance activity. This explains why the long term adaptations to these different types of activities are different. Unlike the increase in mitochondrial content observed with regular aerobic exercise, resistance training, such as lifting weights, is associated with an increase in the contractile machinery. This increase in the production of contractile proteins results in increased muscle mass with very little enhancement of mitochondrial production.

With the slow rhythmic contractions of aerobic activities, there is consistent, modest increase in intracellular calcium within the muscle cell, which is responsible for inducing the contractile process. Calcium plays an important role in stimulating an immediate metabolic response to muscle contraction, and in initiating the process whereby the production of additional mitochondria will occur. Both of these processes, the immediate and long term responses are possible because there are specific enzymes within the cell that are activated by calcium. One of the calcium-activated enzymes, calcium/calmodulin-dependent protein kinase (CaMK) acts to stimulate the uptake of glucose into the cell to support the increased metabolic rate.
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More important for the long term adaptation, CaMK, along with the other calcium-activated enzyme, calcineurin, both increase the production of PGC-1α leading to mitochondrial biogenesis. Mice that produce higher levels of CaMK in their muscle also show increased PGC-1α, and a corresponding increase in total mitochondria. In fact, just raising the cellular levels of calcium in muscle cells stimulates the production of mitochondrial proteins.

By having these processes regulated by calcium-activated enzymes, the cell has an excellent measure for how much activity a muscle fiber performs on a regular basis. The more neural impulses that a muscle fiber receives telling it to contract, the longer calcium levels stay elevated in the cell, and the more time these calcium-sensitive enzymes will stay activated, stimulating mitochondrial biogenesis. This certainly helps explain why the mitochondrial content of muscle is directly proportional to the amount of use it gets.

One more way the muscle fiber has to sense the need for immediate and long term responses to an increased demand for energy is through an enzyme called 5’AMP-activated protein kinase (AMPK). AMPK has been called the “master switch” regulating cellular metabolism due to its ability to quickly activate or turn off metabolic pathways. AMPK can quickly sense the energetic state of the cell by being sensitive to falling ATP levels and changes in the ratio creatine to phosphocreatine.

At the start of exercise, phosphocreatine is used as an immediate source of energy to power the muscle contraction, and is converted to creatine, increasing the creatine to phosphocreatine ratio. As ATP is broken down to replace the phosphocreatine, the ATP levels begin to fall as well. This change in the fuel status of the muscle cell as the immediate energy sources are depleted activates AMPK. To immediately jump start the cell metabolism to replenish the ATP stores, AMPK can stimulate the uptake of glucose into the muscle cell for glycolysis, and activates the oxidation of triglycerides and fatty acids in muscle and liver cells. AMPK is such a sensitive indicator of the metabolic demands placed on the cell, it also contributes significantly to the long term adaptation of muscle to increase its energy producing capabilities. AMPK activation has been shown to increase PGC-1α production and mitochondrial biogenesis.

Recently, a new role for nitric oxide (NO) as an exercise second messenger has been presented. NO is produced from L-arginine and oxygen by the enzyme nitric oxide synthase.
(NOS). We will discuss NO in greater depth later, but for now it is just important to know that NO production increases dramatically in contracting skeletal muscle as nitric oxide synthase (NOS) activity is stimulated. The NO produced during exercise, like the other second messengers discussed has profound immediate effects and contributes to long term adaptations as well. In order to increase the delivery of oxygen and fuel sources required to maintain the high levels of energy production required, NO dramatically increases blood flow to contracting muscle by relaxing the smooth muscle that surrounds blood vessels allowing them to expand. NO also stimulates the uptake of glucose into muscle, although it is unlikely to contribute to this effect as much as some of the other second messengers. NO has recently been shown to be a potent stimulator of PGC-1α, providing one additional mechanism where the total amount of work a muscle fiber performs can be monitored and translated into an appropriate amount of mitochondria for the typical energy production needs.

Each of these cellular sensing systems allows the cell to respond to increasing energy demands by stimulating the production of additional mitochondria (Figure 3.1). So, the good news is that despite aging, obesity, and many disease states being associated with low amounts and poorly performing mitochondria, you can quickly jump start the production of new mitochondria to improve your endurance and health. The only catch is that this exercise effect is mostly specific for your skeletal muscle and your heart, which will be performing the real work during exercise, and in whose cells these signals will be generated. Fortunately, there is even more good news, as you can take advantage of new advances to activate these same second messenger pathways beyond what you would do in your normal exercise routine, and you can generate these signals for adaptation in cells throughout the body, not just in your muscles. To learn how, read on.
Figure 3.1. Critical factors involved in mitochondrial biogenesis in skeletal and cardiac muscle.
CHAPTER 4
CALORIC RESTRICTION, MITOCHONDRIAL BIOGENESIS, RESVERATROL, AMPK, AND NITRIC OXIDE (NO)
In 1935, nutritional scientist Professor Clive M. McCay (Cornell University) published his seminal paper showing that rats fed a diet with 30-40% fewer calories lived about 33% longer than rats fed *ad libitum* (i.e. rats who could eat as much as they wanted). Although Dr. McCay initiated the caloric restriction regimen shortly after these animals were weaned from their mothers, later research has shown that caloric restriction can be started in adult animals with the same impressive results. On their own merit, these results, albeit in rats, are quite amazing. However, since the initial report of the lifespan extending effect of caloric restriction in rats, this dietary manipulation has produced similar effects in a variety of other organisms including yeast, nematodes (roundworms), fruit flies, fish, spiders, other rodent models (mice and hamsters), and dogs. In fact, caloric restriction has evolved as the most reproducible approach to increase lifespan in experimental models. Beyond altering survival, caloric restriction in rodents has delayed the onset of most major diseases that are common as we age. These diseases include cancers of the breast, prostate, immune system, and the gastrointestinal track, along with cardiovascular and metabolic diseases, and neurodegenerative diseases.

Caloric restriction is currently being evaluated in non-human primates, and the results appear very promising. In the two major ongoing studies (one at the National Institute on Aging examining rhesus and squirrel monkeys, and the other at the University of Wisconsin-Madison evaluating rhesus monkeys), the caloric-restricted monkeys (~30% less calories than control animals on normal diet) are reported to be happy and healthy, with lower body weight, % body fat, blood pressure, glucose, insulin, and triglycerides. Thus, it appears that the monkeys are responding to the calorie restricted diet in much the same fashion as do the rodents. As you might well imagine, this topic is very exciting, and one of the hottest research areas in all of science.

There are skeptics out there who doubt the applicability of a caloric restriction regimen to humans and well they should, as very few of the people whom we know including all the eccentrics, would stick to such a regimen for very long. However, research in this area is already shedding light into the molecular mechanisms that may be responsible for increased lifespan along with all the other beneficial effects on health and survival. These discoveries are already providing us with an initial blueprint for identifying nutritional agents that could mimic the beneficial effects of caloric restriction. We will discuss this in more detail later on.
Early on, researchers addressed the question of whether it was caloric restriction per se, or a component of the diet (i.e. fat, protein, carbohydrate, vitamins, antioxidants) that was responsible for observed physiological and metabolic benefits. Well, from the work of many laboratories, it has been shown that neither restriction of fat, carbohydrate, or protein, nor supplementation alone with multivitamins or high-doses of the antioxidants vitamin C or E proved effective. Thus, the answer to the above question is that the benefits are due to caloric restriction and not from a dietary component.94

As we have discussed above, caloric restriction produces a multitude of biochemical and physiological changes resulting in increased lifespan and a delay in the onset of age-related disease.97 Over the years, scientists have considered, tested, and discounted two original ideas offered to explain how this occurs, including caloric restriction-mediated growth retardation and lowered body fat. These hypotheses no longer are considered viable. However, two other ideas have at least some supporting experimental evidence, including the caloric restriction-mediated slowing of cell division (consistent with the observed delay in cancer in rodents), and the caloric restriction-mediated reduction in blood glucose. Less blood glucose in the circulation would slow the formation and accumulation of glucose-modified proteins (sometimes referred to as advanced glycation end-products), deleterious molecules which are hazardous to your health!102, 103

The most widely accepted theory and the one with the most experimental support is that caloric restriction promotes healthy longevity by reducing damage to mitochondria from free radicals and other reactive oxygen species (ROS).95, 104, 105 The detrimental impact of reactive molecules on biological function was first brought to light over fifty years ago by Professor Denham Harman (University of Nebraska), who proposed a causative role of free radicals in the aging process.28, 106 The interest in free radicals continues to attract widespread experimental attention. Back in the 1980’s, scientists began to realize that it was mitochondria that were most vulnerable to attack and damage from free radicals.107 As we discussed in Chapter 1, this is due to the biochemical reactions occurring in the mitochondria during the synthesis of ATP, a complex process that utilizes lots of oxygen and can give rise to the generation of free radicals and other reactive oxygen species.

Free radicals are highly reactive molecular species, usually derived from oxygen but can also be nitrogen derived, that possess an unpaired electron.108 Molecules in this condition are absolute renegades, reeking havoc on any lipid, protein, or even DNA molecule in
close enough proximity to offer an electron target which the free radical will snatch to complete its pair. This is not good news for the molecule that was just attacked, as this oxidizing damage (as this loss of electron is termed) typically results in a reduction or loss of function. Although cells have developed ways to protect against and even repair this oxidative damage, these systems are limited and some absent in mitochondria, especially those protective mechanisms involving the mitochondrial DNA. Indeed, mitochondrial DNA exhibits more oxidative damage that nuclear DNA from the same tissue. The oxidative damage inflicted on the mitochondrial infrastructure results in a downward spiral, since mitochondrial ATP production becomes less efficient and generating even more free radicals and reactive species. Of course, the molecular damage is not limited to mitochondria, but will eventually involve other parts of the cell, and will accumulate over time. Experimental evidence clearly indicates that caloric restriction reduces the degree of age-associated free radical production along with its consequent damage.

How, in fact, caloric restriction exerts these effects is not know for certain, but it is likely that a reduction in energy sources, especially glucose and fat, will lead to an overall reduced demand on mitochondria in terms of oxygen consumption. Alternatively, caloric restriction might improve the efficiency of ATP production and oxygen utilization, such that fewer free radicals and reactive species are produced per molecule of oxygen. There is another potential mechanism that could be involved, and this is where our story starts getting really hot, so buckle up.

During a search for genes and proteins affected during caloric restriction, one candidate has moved to front and center stage. This gene is called SIRT1 (Silent Information Regulator of Transcription), and its protein is markedly increased by caloric restriction. When SIRT1 is overexpressed in mice, they live longer and live healthier. In addition, their metabolism is much more efficient (i.e. they make more ATP), and they become more resistant to disease. In fact, many of effects of caloric restriction are mimicked by SIRT1, leading it to be called the ‘Fountain of Youth Gene’. SIRT1 is a member of the Sirtuin family of genes. Sirtuins are a family of seven NAD-dependent enzymes, called histone deacetylases, and named SIRT1 through SIRT 7 in mammals. By enzymatically removing acetylase from proteins, including histones and non-histone target molecules including transcription factors, transcriptional co-activators, or transcriptional co-repressors, sirtuins play a very prominent role in regulating gene activity. Sirtuins are found, in various forms, in virtually all living organisms including bacteria, fungi, plants,
animals, and humans. Their fundamental role throughout evolution, in each of these organisms, seems to be the reduction of cellular stress and the promotion of cell survival. SIRT1 has received the most attention due to the critical mechanistic role(s) it plays in mediating the physiological effects of caloric restriction. Recent data even indicate that SIRT1 is increased in response to exercise. Other sirtuins especially SIRT3 and perhaps SIRT4 may also play a role in mediating the effects of caloric restriction. SIRT5-SIRT7 are the least characterized members of the sirtuins family.

OK, what it is it about SIRT1 that allows it to elicit such wide-ranging physiological effects. Work from Professor Pere Puigserver (Johns Hopkins University School of Medicine) has revealed that NAD (an activator of SIRT1) levels increase in liver cells during fasting (i.e. an experimental version of caloric restriction) resulting in increased SIRT1 activity. One of the target proteins of SIRT1 is PGC-1α. As we saw in Chapter 3, PGC-1α is a master regulator of mitochondrial biogenesis and energy metabolism. Indeed, when SIRT1 is overexpressed in cells, a physical interaction of SIRT1 and PGC-1α is observed, and more importantly SIRT1 deacetylates and activates PGC-1α. This activation of PGC-1α triggers a domino-like effect at the level of gene expression and results in the production of new mitochondria (that’s right, mitochondrial biogenesis), along with many other improvements in glucose and lipid metabolism. I think that you will have to agree that the team of SIRT1 and PGC-1α are rather impressive. But, it gets better, I assure you.

It has long been known that the French consume a much greater amount of saturated fats in their diet, yet suffer from a lower incidence of cardiovascular disease. This phenomenon has been termed ‘The French Paradox’. It has been proposed that the greater amount of red wine consumed by the French protects them against the development of cardiovascular disease. The intensive search for the actual molecule(s) that could be responsible for this almost miraculous activity led researchers to a compound identified as resveratrol. Resveratrol is a polyphenolic compound (a stilbenol) found in plants, especially red grapes and peanuts. Red wine, produced from red grapes, contains the highest amount, on a percentage basis, of resveratrol ranging from 0.1 – 14.3 mg per liter. The more resveratrol is investigated, the more its diverse health benefits pile up. In animals, resveratrol has been associated with anti-cancer activity, cardioprotective activity, antioxidant and glutathione-sparing activities, anti-inflammatory activity, anti-viral activity, and anti-neurodegenerative activity. But without a doubt, the activities
that affords resveratrol rock star status, is its ability to increase lifespan and delay age-related deterioration in a variety of experimental models. In experiments using yeast, nematodes (roundworms), fruit flies, and fish, resveratrol has been shown to increase mean lifespan by 18-70%, and maximum lifespan by 15-66%. While resveratrol has not yet been shown to increase lifespan in mammals, it has been consistently shown in mice to delay age-related diseases, improve metabolic efficiency, including a reduction in body weight and blood glucose, and largely mimic the biochemical and physiologic effects of caloric restriction, qualifying it, to a degree, as a caloric restriction mimetic.

What does resveratrol do at the mechanistic level that has led to its Rock Star status? The striking similarities of the benefits of caloric restriction and resveratrol led researchers to evaluate the effects of resveratrol on SIRT1 activation. These experiments have conclusively shown that resveratrol does, indeed, activate SIRT1 and its teammate, PGC-1α. While there might be (and are) other important molecular targets of resveratrol, none are more important than SIRT1 and PGC-1α. In light of the critical roles played by SIRT1 and PGC-1α in mitochondrial biogenesis, the obvious question is what does resveratrol do, in this regard? Well, just about everything that you would predict, and the then some! Treatment of mice with resveratrol significantly increased their aerobic capacity, judged by their increased running time and oxygen consumption in their muscles. Resveratrol induced the genes for oxidative phosphorylation (remember, this is one of the distal steps for the production of ATP in the mitochondria) and mitochondrial biogenesis, effects largely explained by the resveratrol-mediated decrease in PGC-1α acetylation and an increase in PGC-1α activity. This mechanism is consistent with resveratrol being a known activator of SIRT1, and by the lack of effect of resveratrol in cells lacking SIRT1. Importantly, resveratrol treatment protected mice against diet-induced-obesity and insulin resistance. If there was any doubt, these results obtained with resveratrol clearly implicate SIRT1 and PGC-1α as key regulators of energy and metabolic homeostasis. In fact, resveratrol and several related resveratrol analogs are now being developed as potential new therapies for type 2 diabetes.

Our story with resveratrol and mitochondrial biogenesis does not end here. As we saw in Chapter 3, there are other convergent pathways that can lead to increased mitochondrial biogenesis including activation of the fuel-sensor enzyme, AMPK, and also increased production of nitric oxide (NO). Now, as if resveratrol’s effects on activating SIRT1 and PGC-1α were not impressive enough, there is evidence that resveratrol increases AMPK.
activity,\textsuperscript{144-148} and increases NO production in a variety of experimental models including animals.\textsuperscript{149-152} So, of all the compounds studied to date, resveratrol scores the ‘hat trick’ when it comes to mitochondrial biogenesis, in that it activates 3 of the 4 major pathways (SIRT1, AMPK, NO) leading to activation of PGC-1$\alpha$. Resveratrol mimics many of the effects of both caloric restriction and exercise, providing many of the gains of each, without the concomitant pain! But, as you will read, resveratrol has some fast company in this regard. That is to say, there are other natural compounds that are known activators of additional pathways know to stimulate mitochondrial biogenesis. Let’s drill down a bit more on these additional pathways leading to mitochondrial biogenesis.

First, we will start with AMPK. AMPK can be regarded as the cellular fuel sensor, because it is activated by a drop in the energy status of the cell.\textsuperscript{11, 84, 85} If ATP is consumed faster than it can be re-synthesized, ATP levels fall, and AMP rises. This rise in AMP results in an increase in the AMP:ATP ratio, and triggers the activation of AMPK and leads to the phosphorylation of a large number of downstream targets. The overall effect of AMPK activation is to switch off energy-using pathways, and to switch on energy-generating pathways, thus helping to restore the energy balance within the cell. The conservation of AMPK throughout evolution emphasizes its importance: homologs of this enzyme have been indentified in all eukaryotic species, including plants.

AMPK plays a crucial role in lipid metabolism, including fatty acid oxidation in the liver and skeletal muscle. AMPK increases fatty acid oxidation, thereby providing a much more plentiful source of ATP production, compared to carbohydrate metabolism. AMPK has been shown to drive mitochondrial biogenesis.\textsuperscript{15} As we saw in Chapter 3, exercise is a potent stimulator of AMPK, PGC-1$\alpha$, and mitochondrial biogenesis. While the exercise studies are informative, they do not provide a direct link between AMPK activation and mitochondrial biogenesis. However, a direct link is provided by the use of pharmacological tools, such as $\beta$-guanadinopropionic acid ($\beta$-GPA, for short, and AICAR (ok, if you really want to know: 5$'$-aminoimidazole-4-carboxamide-1-$\beta$-D-ribofuranoside). These compounds reduce energy capacity, and consequently activate AMPK as a compensatory response. When mice are treated with either of these compounds, they show an increase in AMPK along with PGC-1$\alpha$, mitochondrial enzymes, and mitochondrial biogenesis.\textsuperscript{16} The mice also have improved exercise capacity. In contrast to their effects in normal mice, these compounds do not have any beneficial effects in mice which lack the AMPK genes and proteins.\textsuperscript{15} The results provide solid evidence that AMPK is necessary for mitochondrial biogenesis,
CHAPTER 4

and that both exercise and pharmacological activation of AMPK convey their signals to induce mitochondrial biogenesis through PGC-1\(\alpha\). The implication here is that it should be possible to identify agents that stimulate AMPK and induce mitochondrial biogenesis, i.e. identify agents that function as exercise mimetics.\(^{153, 154}\)

NO mediates an extensive array of regulatory tasks in the cell, many of which involve mitochondria.\(^{155-158}\) These heterogeneous research areas have been tied together with the finding that NO stimulates the mitochondrial biogenesis.\(^{3, 92, 93, 159}\) Originally identified as a vasodilator, NO regulates the flow of blood to tissues. This, in turn, controls the supply of oxygen and respiratory substrates to mitochondria, and the redistribution of heat generated by those mitochondria. More recently, NO has been found to directly regulate the binding and release of oxygen from hemoglobin, and in this way controls the supply of oxygen to mitochondria.

The ability to regulate the number of mitochondria in our cells is crucial for all types of physiological processes, including embryonic development, movement, fat metabolism, and aging. From what we have learned, the more mitochondria we have the more energy efficient we become and the less oxidative stress our cells and tissues encounter. The take home message here is that mitochondria are our friends, and we could all use more. In fact, we should all be doing everything we can to increase our supply. But how do cells control the number of mitochondria they contain?

The discovery of a master regulator of mitochondrial biogenesis, PGC-1\(\alpha\), has shed light on the underlying mechanisms. Overexpression of PGC-1\(\alpha\) in transgenic mice results in increased numbers of mitochondria in cardiac and skeletal muscle. PGC-1\(\alpha\) is a transcriptional co-activator that increases expression of nuclear respiratory factor–1 (NRF-1) and mitochondrial transcription factor A (mtTFA), which in turn promote the expression, respectively, of nuclear and mitochondrial genes that are required for mitochondrial biogenesis.\(^{76-78}\) PGC-1\(\alpha\) is known to be up-regulated under conditions that promote the synthesis of new mitochondria, for example, during prolonged exposure of rats to cold temperatures, and as we saw in the previous chapter, exercise. Cold exposure activates the rat’s brown adipose tissue through stimulation of \(\beta_3\)-adrenergic receptors, leading to an increase in cellular calcium ions along with the signaling molecule, cAMP. This results in enhanced PGC-1\(\alpha\) production and an increase in the number of mitochondria in brown fat, which generates body heat.\(^{92}\)
Figure 4.1. Critical factors involved in mitochondrial biogenesis in muscle and non-muscle cells. The CaMK pathway is not shown.
Professor Enzo Nisoli, a pioneer in this field, and his colleagues reported the missing link in this causal chain: NO generated by endothelial nitric oxide synthase (eNOS), which may have been activated by calcium ions and/or phosphorylation, increases cGMP levels. This, in turn, up-regulates production of PGC-1α and the biogenesis of mitochondria. These scientists found that overexpression of NO, or a cell-permeable cGMP analog, or eNOS dramatically increased the numbers of mitochondria in a range of cell lines and in differentiating brown fat adipocytes. Furthermore, mice lacking functional eNOS exhibited a decrease in mitochondrial enzymes and oxidative phosphorylation in the aerobic skeletal muscles, along with decreased numbers of mitochondria in a wide range of tissues, decreased energy metabolism, and increased weight gain.

The implication is that NO produced by eNOS is itself a master regulator of mitochondrial number, and thus potentially of aerobic exercise, heat production, and obesity. This surprising discovery has important implications for understanding energy metabolism and suggests therapeutic interventions for treating obesity. Although these studies do not provide evidence for the regulation of mitochondrial biogenesis by NO in humans, it would be very surprising if such an important mechanism for the physiological adaptation and survival would be restricted to rodents. If NO indeed regulates mitochondrial number in humans, it should be possible to stimulate production of mitochondria in muscle and other tissues with dietary interventions, in order to increase energy stores and athletic performance, reduce obesity, improve overall health, or even delay the aging process. The major pathways that have been shown to stimulate mitochondrial biogenesis in muscle and non-muscle cells are shown in Figure 4.1.
CHAPTER 5

EXERCISE MIMETICS,
MITOCHONDRIAL
BIOGENESIS, INCREASED
ENERGY, REDUCED
ADIPOSITY, IMPROVED
HEALTH, AND LONGEVITY
Wow! We have covered quite a bit of science since we began this journey. We have discussed some of the most cutting-edge and hotly pursued research areas of biomedical science over the last few years. Ordinarily, when you hear about new research discoveries, you will also hear the caveat that it will be another 10 to 20 years, at least, before any practical applications of the work can be realized. That is not the case with this information, I assure you. We have come to the point where we can begin to capitalize on this information now, not 20 years from now! There are nutritional interventions, available to us now, that will allow us stimulate the pathways that lead to mitochondrial biogenesis. That’s right, available to us now! There are dietary interventions that are directed towards the primary molecular targets required for mitochondrial biogenesis, including SIRT1, AMPK, and NO. As you now know, these same biochemical signaling pathways are activated by exercise. So, in a sense, we now have available a formula for a nutritional cocktail for an ‘exercise mimetic’. The cocktail can be used to activate PGC-1α from multiple directions, complementing exercise with the objective of achieving the wide array of beneficial metabolic changes.

Now don’t take this the wrong way: we are hardly suggesting that you give up exercise, swallow the exercise cocktail, and presto! New mitochondria magically appear. No way. This prototype cocktail should be regarded as more like an ‘exercise sensitizer’, rather than a pure exercise mimetic. By sensitizer, we mean that the cocktail will act in a complimentary fashion to exercise, priming the pump, so that you can get more bang for the buck, so to speak! That is to say, that you might be able to reap the benefits of 60 minutes of exercise in a lot less time. And who would complain at that prospect?

So, let’s get started. Our first component of the cocktail is, as you probably guessed, resveratrol and its close relative, methylated resveratrol (a compound also known as pterostilbene). The methylated resveratrol plays an important role in achieving the maximum benefit of resveratrol. Here’s how: for all the amazing things about resveratrol, it has very poor oral bioavailability. This means the vast majority (~75%) of the compound gets excreted in your urine and feces, and what does get absorbed gets converted by your liver into a variety of metabolites. Some of these metabolites might have resveratrol-like activity, but this is an open question. Furthermore, the plasma half-life of resveratrol is extremely rapid, less than 10 minutes. That means it does not stay around very long to be helpful!
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The intestinal absorption of methylated resveratrol is much greater than resveratrol (approximately 5-8 times), and methylated resveratrol possesses greater metabolic stability than resveratrol. The plasma half-life of methylated resveratrol is much longer compared to resveratrol, and is approximately 45 minutes. That is to say, more gets absorbed into your blood and tissues, and less gets metabolized into other compounds. Importantly, since the chemical structure of methylated resveratrol is closely related to resveratrol, it possesses the biological activities of resveratrol on the molecular targets of interest (i.e. SIRT1, AMPK, and NO).

The resveratrol component contains a third active ingredient called quercetin. Quercetin is a polyphenolic antioxidant (a flavanoid) found in fruits and vegetables. Adding quercetin to the cocktail is important for several reasons. As we have discussed above, resveratrol is metabolized in the liver to form conjugates, which are more easily excreted. Quercetin is an effective inhibitor of the enzymatic sulfation and glucuronidation reactions, and effectively extends the life of native resveratrol. In addition, quercetin is also able to activate SIRT1, albeit to a lesser degree than resveratrol.

Our second component of the cocktail is directed at AMPK. In addition to its critical role in stimulating mitochondrial biogenesis, AMPK is a validated molecular target for increasing energy expenditure, improving insulin sensitivity, and reducing appetite. That is right. I said appetite reduction! In animals, when AMPK in the brain is inhibited (in contrast to being activated in peripheral tissues), their appetite goes down, the animals are leaner, and they weigh a lot less. This makes sense when you think about it. Since AMPK is the primary biochemical sensor for our body’s fuel status, it is not surprising that it would be turned-off (inhibited) in response to a meal that has just delivered a plentiful resupply of fuel, thereby signaling our body to stop eating. While it is intriguing to consider that an activator of AMPK in the periphery could be not only an exercise mimetic, but also an attractive approach to treat obesity, type 2 diabetes, and the metabolic syndrome, it is also possible that such an activator could also result in increased food intake (i.e. if it activated AMPK in the brain). So, the ideal agent that regulated AMPK would activate AMPK in peripheral tissues and inhibit AMPK in the hypothalamus.

To date, only a single orally available agent exhibits this profile: α-lipoic acid (lipoic acid). Lipoic acid is a multi-functional antioxidant that has been used for many years as a treatment for diabetic neuropathy. In rodents, lipoic acid decreases hypothalamic
AMPK activity and causes profound weight loss by reducing food intake and increasing energy expenditure. Administration of lipoic acid to obese rodents increased AMPK activity and fatty acid oxidation in skeletal muscle; lipoic acid also increased insulin-stimulated glucose disposal both in whole body and in skeletal muscle. These results indicate that lipoic acid-induced improvement of insulin sensitivity is mediated by activation of AMPK and reduced triglyceride accumulation in skeletal muscle. Based on these results, lipoic acid has been proposed as an anti-obesity agent. In 3T3-L1 adipocytes, lipoic acid treatment promotes mitochondrial biogenesis. That's right, I said increased mitochondrial biogenesis. Just like resveratrol! In conclusion, these studies indicate that AMPK is a validated target for reducing appetite, increasing energy expenditure, improving insulin sensitivity, and last but not least, increasing mitochondrial biogenesis. Lipoic acid is the only oral agent that has been shown to increase AMPK in periphery and decrease AMPK in the hypothalamus.

Although lipoic acid has been used quite successfully for many years to treat diabetic neuropathy, its full range of benefits are limited by the abbreviated time that therapeutic plasma levels are maintained. This plasma profile is a function of the short half-life of LA, along with its extensive pre-systemic elimination, which is thought to be primarily hepatic. Human pharmacokinetic studies have found that LA possesses an extremely short plasma half-life of about 30 minutes after both oral and IV administration. Thus, following oral LA administration, a maximum plasma level is quickly reached, but falls just as quickly to a level insufficient to impact insulin sensitivity, glucose control, or AMPK activation. To overcome this limitation, a proprietary controlled release formulation of lipoic acid has been developed. The original version of this controlled release formulation has been evaluated for safety and efficacy in humans with type 2 diabetes. It was found to be safe and well tolerated, and it significantly reduced plasma fructosamine (an intermediate-term indicator of glucose control) by approximately 15%. This formulation has also been reported to reduce markers of oxidative stress in patients with type 1 diabetes. So, to summarize what we have discussed so far, our exercise mimetic cocktail contains the resveratrol/methylated resveratrol/quercetin component which targets SIRT1/AMPK/NO, and the controlled release lipoic acid component which targets AMPK. And now, for the final component of the cocktail.

Consistent with our theme of increasing mitochondrial biogenesis, we want to include a
component that increases NO production. The most effective way of achieving that goal nutritionally is by including L-arginine. L-arginine, a semi-essential (i.e. can be produced by liver under certain conditions) amino acid, is the primary chemical precursor (i.e. building block) for the biosynthesis of nitric oxide (NO), an endogenously produced cellular signaling molecule that increases blood flow in the vasculature, and a major regulator of mitochondrial biogenesis. Virtually all studies in animals report that acute and chronic administration of L-arginine improves blood flow and vascular health. In humans, there are a large number of published studies that report beneficial effects following oral L-arginine supplementation. These benefits are derived from the L-arginine-mediated increase in NO production.

The effectiveness of L-arginine can be increased by combining it with α-ketoglutaric acid (AKG). AKG plays a vital role as an intermediate in the Krebs cycle, and the eventual production of ATP. AKG offers a benefit by supporting protein synthesis, and by promoting healthy nitrogen balance. Supplementation with AKG results in a nitrogen-sparing effect, and a reduction in loss of lean body mass. Glutamine is the most abundant amino acid found in the human body, and one of the most important in the process of building muscle and gaining strength. Glutamine is formed from glutamic acid and AKG, which can serve to increase both glutamate and glutamine levels.

A proprietary, extended release formulation has been developed that combines L-arginine and α-ketoglutaric acid, along with a unique whey protein fraction processed using a patent-pending technology to yield both high and low molecular weight peptide fractions. There is convincing evidence from mechanistic studies that suggests that these unique peptide fractions are activators of the enzyme nitric oxide synthase (NOS), resulting increased production of NO production, and independent of arginine. These peptide fractions may also increase the transcription of NOS gene. The synergistic activity of the size-based fractions of this unique whey protein has been shown to increase NO production in human endothelial cells in vitro from 9.5 (~950%) to 12.7 (1250%) times compared to control. This proprietary formulation works by 1) elevating the plasma level of L-arginine, the substrate of the nitric oxide-generating enzymes (NO synthetases; NOS), and 2) by increasing the activity of NOS. This formulation drives the biosynthesis of NO in various tissues including the vascular endothelium and skeletal muscle, and increased NO production is one of the primary ways to increase mitochondrial biogenesis.
CHAPTER 5

So, there you have it: a cocktail that contains three remarkable nutritional components directed against the major biochemical approaches to increase mitochondrial biogenesis. The strategy has been summarized in Figure 5.1. As is shown in the figure, each of the nutritional interventions stimulates one or more of the same pathways activated by exercise, including SIRT1, NO, and AMPK. This means that the cocktail can (and should) be used in combination with an aerobic (i.e. cardiovascular) exercise program. As we see from the flow diagram, the pathways converge on PGC-1α. Activation of this transcriptional co-activator is critical for mitochondrial biogenesis. As a result of making more mitochondria and / or increased volume of existing mitochondria, a variety of additional physiological benefits are achieved. These include reduced production of ROS and decreased oxidative stress, stemming from more efficient energy production (lower oxygen usage per unite mitochondria). The new and improved (i.e. healthy) mitochondria lead to increased energy production and, quite likely, exercise performance.\textsuperscript{57, 79, 153, 154} The more energy we have, the longer we can exercise and this will lead to reduced body fat stores. The less body fat we carry around, the less inflammation, and the more exercise we are able to achieve. Instead of a downward spiral, this spiral is clearly pointing up. And we can see from Chapter 3 that risk of developing (and possible dying) from one of the chronic diseases is markedly reduced by our level of exercise.

We introduced this book by saying that we should focus only on those important things which we can control. As far as attaining and maintaining optimal health, there is nothing more important that we can do for ourselves as regular exercise, especially the aerobic type. That being said, we have now seen how we can influence the amount of what can be regarded as the most important parts of our cells, the mitochondria. The potential payback for us is huge, and will impact every aspect of our general health and well being. Now is the time to take control and increase our energy reserves!
Figure 5.1. Nutritional supplement approach to stimulate mitochondrial biogenesis.

- L-arginine
- α-ketoglutarate
- Whey protein fractions
- Resveratrol
- Quercetin
- Dimethylresveratrol
- Lipoic acid
- Biotin

EXERCISE

↑ NO

↑ SIRT1

↑ AMPK

↑ PGC1α

↑ Mitochondrial Biogenesis / Function
↓ ROS / Oxidative Stress
↑ Metabolic Function
↑ Energy Level
↑ Exercise Performance
↓ Body Fat / ↑ Lean Muscle Mass
↓ Age-Related Deterioration
↑ Increase Lifespan (?)
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Ref Type: Abstract
As we age, it becomes easier to gain weight and harder to maintain muscle. Thus, our waistlines tend to fatten, our muscles weaken, and the physical tasks that once seemed trivial now seem tiring. The deterioration of mitochondria, the microscopic power plants within every cell responsible for converting food into biochemical fuel, has been implicated as a major cause. The loss of mitochondrial efficiency and number over time deprives us of living a longer, healthier life. It may come as no surprise, therefore, that mitochondrial biogenesis, the natural process in which your cells build new mitochondria, has been suggested hold the key to delay the deteriorations in appearance and performance, and even aging.

*The Secret Life of Mitochondria* seeks to explain the role of mitochondria as a major component of the beneficial health effects of exercise, and to provide a simple, but scientifically sound, template for using nutrition to maximize your body's ability to produce mitochondria.

“...many laboratories studying mitochondria believe the preeminent positive regulator of mitochondrial biogenesis is the transcriptional co-activator, PGC-1α. In fact, nutritional or pharmaceutical interventions that increase either PGC-1α activity or its content may serve as exercise mimics.”

— Ira D. Goldfine, MD; Professor, University of California, San Francisco