Pick one:

- I want to die from heart disease
- I want to die from cancer
- I want to die in the arms of my beloved after great sex and a long life.

If you picked number 3, the odds are overwhelmingly stacked against you. And if you live to be 85 years old you have a 50% chance of having diagnosable Alzheimer’s. We are in the grip of a conspiracy spearheaded by food companies, pharmaceutical concerns, and a medical establishment determined to ensnare you from cradle to grave.

Can we do away with the bell curve and the probability condemns us to a life of disease and distress? After all, people in the Amazon or rural China do not die from cancer or heart disease. They die peacefully in bed.

Making your Healthspan Equal your Lifespan

In my late twenties I left a prestigious teaching position and my own research laboratory at San Francisco State University to explore the tributaries of the Amazon River. I’d had enough of academia and eager students wanting to read about indigenous cultures. I wanted to visit undiscovered villages and explore ancient civilizations firsthand. My travel expenses at that time were paid for by a pharmaceutical company hoping to find the bark or root that could become the next great cure for cancer or heart disease. After all, the jungle is nature’s pharmacy, filled with exotic plants whose powers have yet to be discovered, and I was one of only a few medical anthropologists exploring the Amazon.

During my travels I saw how Amazon peoples live in harmony with their environment, in the bounty of nature. Whereas the farmer needs to work an average of 8 hours every day to meet the caloric needs of his body, the Amazonian needs to work only 3 hours to get the same nutritional value from nature. This left them many hours of the day to do what all of us do when we go to the Rain-forest – be in awe of its beauty and commune with Nature. They had no masters making them build pyramids, castles, or fortifications. Instead, they became consummate explorers of consciousness. In the jungle there is little call to outer exploration. Every patch of jungle looks just like every other. There is no faint shore that calls you, no mountain pass to cross, no distant port to lure you. The shamans were consummate explorers of an inner landscape during their states of shamanic ecstasy.

I spent many months paddling to villages that had seldom seen a white man and wherever I went, I found no cancer or heart disease. The two greatest killers of Western civilization were not present in aboriginal societies. And to top it off, I met no one with dementia or Alzheimer’s. People died of old age, infections, and accidents. And many died peacefully, in their sleep. Clearly, the Indians knew something about health that we westerners didn’t know. What was their secret?

The shaman believes that when you enter shamanic ecstasy you create the conditions for health, and healing happens naturally. Illness is seen as an imbalance in a natural harmony that occurs when we’re disconnected from the invisible side of nature and have forgotten its existence. What’s more, when you are ill, it’s possible that you’re experiencing the consequences of imbalances that you personally had little role in creating. It could be because of the actions and deeds of your parents and grandparents, which can have an effect lasting for many generations, what we call today epigenetics.

Clearly, the Indians knew something about health that we westerners didn’t know. What was their secret?
Disappointing Pharma, Discovering Ayahuasca

My sponsor could not contain his disappointment that I hadn’t discovered the ingredient for a blockbuster bark that would make all of us rich and save some lives at the same time. Unbeknownst to them, or to me at the time, I did find the preventive cancer, as well as many of the ailments of civilization. I learned there was an ingredient to life-long health that could be found in the virgin rainforest, but would not fit in a backpack or be turned into pills. It was shamanic ecstasy. This was at the Upper Madre de Dios River, nearly three decades ago. One day I was invited by the shamans to participate in a ceremony with the legendary ‘vine of death.’ But first I had to take the earthly medicine, the cleansing herbs and week-long dietas or fasts to detoxify my body and repair my brain. Then I could begin to explore a reality that has lain hidden in plain sight all along.

Psychoactive brew & DMT

At the time, I had difficulty understanding why I had to ingest the foul tasting plants the shamans offered me, the barks and leaves that would make me purge out of both ends of my body and break out in a feverish sweat. Years later, in the laboratory at San Francisco State University, I learned that the dieta was rich in Omega 3’s that repaired the region in the brain responsible for learning, (in my case learning an entirely new world view) including how to live healthfully and stress free.

The plants were natural anti-inflammatories that turned on major detox pathways and switched off the death clock that ticks away inside every cell in our body. They upregulated more than 500 genes that create health, and switched off more than 200 genes that create disease. Thankfully, today you can find these ingredients that repair our broken brains and detox our body in your local health-food store. The dieta was the necessary step required to enter the state of consciousness – known as shamanic ecstasy - where all healing can happen. It was only after that evening ceremony on a pristine sandy beach at the edge of the river that I began to comprehend what the shamans were talking about. Ayahuasca is a psychoactive brew, whose main ingredient active is DMT, a compound nearly identical to the neurotransmitter serotonin. The fact that there are receptor sites in the brain for this molecule suggests that we produce it naturally. DMT is a found in all living things, from trees to horses to eagles. Everything that has DNA has DMT. There are two notable instances when we produce abundant DMT. During shamanic ecstasy, and at the moment of our death. The word ayahuasca means the “vine of death” in the Quechua language. Legends say that it brings you out of the ghost-like existence where you have been spending most of your time, into a life where you are truly alive. Back then, thirty years ago, I was not prepared for what I would experience. Who I was, anthropologist, white male, modern man, son, explorer, would all be forcefully stripped away from me. Everything and everyone I thought I had been sloughed off like crusty dead skin. And then, after no one was left to interpret or reason or catalogue or annotate the experience, absolute clarity enveloped me, and I realized that I was much greater than any of those roles; I was a part of all of creation. Insignificant, yet indispensable.

Spirit of Gaia

During my journey I met the spirit of Gaia, our living planet, who showed me that what we call the ‘real’ world is the hallucination. The goddess revealed to me that everything that I believed to be reality was a distortion of the very truth I sought. I am not the first, nor will I be the last, to meet the spirit of Mother Nature. The anthropological literature and the popular legends are full of stories like mine, and those of you who have tasted the vine know what I am talking about. By the following morning, the lessons taught to me by Gaia, the lucidity and clarity that I had been shown by the goddess, became notes in my journal. All I could recall viscerally was the foul taste of the medicine, the violent retching, the beauty of the cosmos, and the now faint voice of Gaia. The plant opened the door, but I had to travel through it. Chemical ecstasy was not the way.

When the brain is broken

Today there are ayahuasca experiences offered everywhere, and many people report sublime visions from their urban ceremonies. And often, as the wisdom fades, and only the postcard insights are left. You remember something huge happened; yet the brain can not hold-on to the Oneness of the experience. Many-ness slowly crept back, and the luminous web of reality soon collapses into the everyday landscape where nothing seemed to be related to anything else. Like a postcard from a magical country we visited. The beach is beautiful, wish you were here. The reason we cannot hold the
experience of Oneness achieved during shamanic ecstasy is that our brains have been broken by the poisons in our food, the toxins in our water and air, and the processed grains in our diet. And the brain and nervous system is the vehicle thru which nature and Spirit communicates with man. At least while we are in a physical body. The ancient Egyptians mumified every part of the body except the brain, that they drained away through a straw. They understood that we would have no use for it in the afterlife.

When your brain is broken, you cannot enter shamanic ecstasy. You need the chemicals to take you there and then you forget. You cannot hear the voice of Spirit, or dialogue with nature. Yet even in those brief visits you can upgrade the quality of the information in your LEF. The LEF is the software that instructs the hardware, your DNA, to grow a new body that ages and heals differently. When your brain is broken you cannot upload the codes to heal and grow a new body. Instead, you are doomed to live out the genetic fate you inherited from your parents, and re-play their psychological dramas over and over again in your own life.

Do you have a damaged brain?

Answer these questions to learn if you have a damaged brain.

- I do not recall my dreams.
- I have a difficult time sleeping.
- I don’t have enough time.
- I stress out easily.
- People find me boring.
- I find myself boring.

How our Brains were Broken

If you answered yes to more than 3 of these questions, in all likelihood you have a brain that is in need of a tune up. Mitochondria are the energy factories at work within your cells. They impact your moods, your vitality, your aging process, and even how you might die. They are also in charge of the elimination of old cells and replacement with new cells. The mitochondria are influenced by the foods you eat, the amount of calories you ingest, and the availability of specific nutrients. Since they are oxygen breathers, they produce free radicals and are damaged by free radicals. Mitochondria regulate the switching on and off of the genes that produce malignancy, health, or disease.

Before agriculture, our diet was nutrient dense and calorie poor. Our mitochondria had all the nutrients it needed to thrive and maintain us healthy. Our diet was rich in antioxidant fruits and berries, as well as nutrient rich veggies. After agriculture, our diets became calorie rich and nutrient poor. The new calories came from grains- carbohydrates that the body quickly turns into sugars and then into fat. Our body does not recognize processed grains as ‘food’, particularly those rich in gluten, as it takes 40-50,000 years for our DNA to adapt to a change in our diet and grains have been around for less than 10,000 years. The grains can trigger an inflammatory response and a leaky gut, where food particles can pass into our bloodstream through the gut lining, a membrane only one cell thick. And while our blood is designed to transport nutrients, it is not intended to carry undigested food particles. These fermenting bits from our last meal trigger an auto-immune response, produce inflammation, mucus, brain fog, and lethargy. And the inflammatory cytokines they produce damage mitochondria in the brain.

The Agricultural Disaster

Anthropologist Jared Diamond describes agriculture as “the worst mistake of the human race.” Today we are living the dreadful effects of this colossal mistake. In the last fifty years the number of children diagnosed with autism has gone from one in 20,000 to one in one-hundred. Twenty percent of the population will die of heart disease. One in four Americans will die from cancer. And these diseases are largely preventable. Shortly after the last ice age, as the world religions start to flourish, humans in
Asia begin to cultivate rice; in the Americas, maize (corn); and in Europe and Mesopotamia, wheat was the favored grain. And the faithful prayed “give us this day our daily bread…”

For the first time since the dawn of humanity, our brain begins to run on sugars (carbohydrates) derived from abundant grain crops. Prior to farming we nourished our bodies and brains with the proteins and fats from a shore-based diet rich in Omega-3 fatty acids and other brain nutrients, as well as vegetables and nuts. The little beef we ate was organic and grass fed. All the vegies were fresh. It was only at the end of summer, when fruit was ripe, that we ate sugars. Then insulin stored these sugars as fat to help us through the long winter ahead.

With the domestication of grains we begin to live on sugars year round. And sugars, together with the inflammatory and allergic reactions caused by the new grain diet, damage the regions of the brain (the neocortex and prefrontal lobes) that are involved in shamanic ecstasy.

The worst blow came during the green revolution of the 1960’s when dwarf wheat species were introduced. This new variety of wheat has saved the lives of over one billion people around the world. Yet this genetically modified grain has nearly twenty times more gluten than earlier domesticated varieties. And gluten is the part of a story we are all familiar with, as we recognize our own sensitivities to bread, pasta, etc., and the rise in celiac disease. Meanwhile, the rulers and priests of Egypt, the Mayan lords and Inka kings, continued to eat a protein and fat rich, fish and plant based diet, while their pyramid building slaves and soldiers lived on bread, rice, and corn. The fats our Paleolithic ancestors consumed provided the superfuel the brain required to repair the hippocampus and achieve shamanic ecstasy, and their plant based diet protected them from the high incidence of dementia, cancer, diabetes and athero-sclerosis found in today’s civilized world. Our Paleolithic ancestors lived long, disease-free lives. Indeed, after the rise of agriculture human lifespan was dramatically shortened. Our recent ancestors did not live long past the age of forty, which suited their slave masters, as you did not get much good work out of a gray-haired slave or good fighting from an ageing warrior.

If the microorganisms in our gut track are out of balance, or if we have leaky gut, or food allergies, we are unable to digest properly and can develop autoimmune disorders. Meanwhile, 75% of our immune defense cells are located in the gut. Fruits and vegetables with a lot of color. Turn on the NRf2 detox pathway with broccoli, turmeric, and resveratrol.

Accompanying the change in the fuel for our brain when we switched from fats to grain was a profound change in our psychology. Man the hunter became man the warrior. Blood sports became popular in coliseums and bullrings. Men left the family for the wheat fields and for wars to protect their fields. The father became absent from the home and the women became the property of men and breeders of strong boys.

**Shamanic Energy Medicine**

Shamanic medicine works by upgrading the quality of the information of the luminous energy field (LEF) that surrounds your physical body and instructs your molecules, cells, and genes. The LEF is the software that informs DNA, through the hardware, which is the brain and nervous system. The LEF has been depicted as the aura around the Christ and the Buddha. It’s the blueprint that organizes the physical body the same way that a magnet organizes iron fillings on a piece of glass. If you change the blueprint, the body changes. When you repair the blueprint, the body heals.

The shaman understands that we are flesh and blood as well as the energy field that surrounds the physical body and infuses it, guiding its growth and repair. The mind-body connection that we have been talking about in the west was never forgotten by indigenous people, who understood that emotions, thoughts, the environment, and the invisible world affects our LEF and thus influence what is happening in our bodies. The way to heal the body is by upgrading the quality of the LEF. Then the body can stop manifesting disease and start creating health. But you need a healed brain to do this.
Connecting with Spirit in the Invisible World

In the West we have identified many different illnesses, and only two kinds of people, men and women. In contrast, the shaman believes that there are many different kinds of people, and only two kinds of ailments — the illnesses of God and the illnesses of man. The illnesses of man are the product of envy and jealousy, of ‘bad vibes’ and toxic energies as well as exposure to natural toxins, from snake bites to poison berries. The illnesses of God cover everything else. To heal these, you must go to God, sit across the dinner table from Him and negotiate for your health. You then will be shown the aspects of your life that need to return to harmony, and the lessons you must learn in order to heal. And you can best visit with God during shamanic ecstasy. Of course, we can pray at any time. But a face-to-face with God is something different. In the West only the prophets of old spoke directly with God. For the shaman, everyone with a little talent and a heroic discipline can do this, once they have detoxified their body and brain. Otherwise the shaman has to journey to meet Spirit on your behalf. But nothing beats meeting God personally, and we must learn to do this if we are to take charge of our health. But instead of going to God or Gaia we go to the doctor and who are experts in disease yet often know little about health.

We evolved over millions of years together with the plants. We did not co-evolve with the animals as we only domesticated them a few thousand years ago. Plants stayed put... animals ran away.

The plants were nutrient rich and calorie poor. This is the key to nutrition for healing. Nutrient rich and calorie poor foods. Fresh, organic green juice in the morning, for the colors and phytonutrients that “turn on” the genes for health and “turn off” the genes associated with cancer, heart disease, diabetes, and stave off the illnesses of civilization. A diet rich in good fats to repair mitochondria.

Next you need to detox, from the very inside of your cells. Broccoli seed extract, Curcumin, and resveratrol turn on the Nrf2 detox pathways and the innate production of free-radical scavengers like glutathione and SOD inside the cell. Then you have to repair and strengthen your mitochondria, the fuel factories and female life-force inside every neuron and cell in the body. Then you will be primed to experience shamanic ecstasy, and to create exceptional health. You do this using energy medicine to heal the luminous energy field that is the matrix of all life.

Juice! Start the morning with a green juice. Repopulate your gut with healthy probiotics. Eat high quality proteins, including nuts and fish. Eat colorful! Eat fruits and vegetables with a lot of color. Turn on the NRf2 detox pathway with broccoli, turmeric, and resveratrol.

The chemicals produced by our ordinary stressful lives, cortisol and adrenaline, are powerful steroids that will keep us trapped in a predatory mind-frame fighting for survival, mired in strife and away from ecstasy. And these steroids damage the hippocampus, which is rich in cortisol receptors. You need a healed hippocampus to turn on your brain’s innate production of DMT.

The first task is to repair your hippocampus with Omega-3 rich foods and supplements. We used to get our Omega 3’s from fish. Today our fish is farm raised and devoid of omega 3’s. You need three grams a day, mostly DHA. (Breast milk is 40% DHA, crucial for the developing brain.) Then you need to eliminate gluten and dairy from your diet, at least for a month to allow your immune system the opportunity to reset. If you are going through a healing process you need to be off all grains. This will reduce the inflammation in your body and turn on the Sirt-1 genes that are responsible for longevity and health.
Reselecting **Your Genetic Destiny**

Modern physics explains that interactions across time and space are possible. Shamans learned to put this into practice and employed imagery to program their genetic destiny during states of ecstasy, selecting genes from the gene pool for health and longevity.

So imagine that you could go back in time to the moment of your conception and select the biological traits that you wish you had inherited from your mother and your father. Perhaps you would choose your father’s heart because there was no incidence of heart disease in his side of the family. Or you might select your mother’s brain because there was no Alzheimer’s in her branch of the family tree. You likely would want the trait of longevity from either of them.

You received the entirety of your genetic makeup at the moment of your conception. You also received one half of each of your parents’ genetic code. This means that, while you received 50 percent of each of your parents’ hereditary information, their genotype, you also express only some of those select traits, your phenotype.

But that is only part of the story. While you may have inherited a predisposition for either heart health or disease, your beliefs, diet, and choice of lifestyle will influence your inherited risk factors. As the pharmaceutical industry knows, lifestyle modifications are often not enough, and seemingly healthy men and women can and do suffer heart attacks at a relatively young age.

So, what else can you do? You can look beyond your physical or genetic side to your spiritual side.

Ancient sages developed techniques that they believed allowed them to “journey back in time” to influence the effects of their ancestral heritage. The effectiveness of this exercise derived, at least in part, from their ability to influence the expression of their DNA. In other words, they used visualization techniques during states of shamanic ecstasy to modify genetic expression! When skilled practitioners journey back to the moment of conception to consciously select the traits they want to express, they look at other factors...beyond genotypes and phenotypes...that may have influenced their genetic makeup. The father may have consumed too much alcohol. The mother may have been afraid of getting pregnant. The environment may not have been infused with love, peace, and tranquility. Stress hormones easily cross the placental barrier and inform the child of every mood the mother is feeling.

But now, from your current perspective, you can go back and visit the moment of your conception. You can bring a meditative and sacred feeling to the moment of the comingling of your genes.
Exercise: **The Moment of Your Conception**

With your eyes closed, take a few deep, relaxing breaths. Count your breaths from one to ten, then back to one again, until you feel yourself entering a deep state of relaxation. You will notice that, at first, your mind will wander. You may find yourself counting past ten or chasing a thought about what you forgot to do yesterday or whom you must call still today. Let all of these thoughts go by like clouds that appear, then disappear, in the sky.

Now imagine your timeline, the chronological series of events in your life, poised in front of you. Perhaps you imagine a golden thread or a string with many beads or moments of time. Perhaps you simply see a road that leads in one direction to the past and another direction forward into the future. Begin traveling backward along your timeline, briefly revisiting events of the past few days. Then go further into the past, to your childhood, and to your earliest memories as a toddler. See the images as though they are in a movie that you can fast-forward or reverse at will. When you are no longer able to recall events or situations, use your imagination. Imagine yourself as a baby in your mother's arms. Imagine being inside her womb. Imagine the instant of your conception, when your mother's egg is surrounded by your father's numerous sperm, all trying to fertilize it. Imagine yourself sitting inside that luminous egg. It is a peaceful bubble. Bring your stillness and grace into that space. Know that you are filling it with your peace and luminosity.

Now sense the egg selecting and inviting the finest sperm to fertilize it. Imagine that as it enters into the ovum, you witness the most extraordinary alchemy that is the conception of you. You see proteins cross-link with each other, making the matrix of the egg hard and impermeable to other sperm. The nuclei of the sperm and the egg dissolve, and the father's DNA and the mother's DNA fuse. The egg divides and forms two tiny, identical cells. They begin to replicate, doubling, quadrupling, and exponentially adding to their numbers at an extraordinary rate.

As you watch this amazing process, you hold steadfast to your intention of forming and shaping yourself into your desired healthy, strong, and luminous being. You bathe these nascent cells with your great peace, your serenity, your light. You bless this holy union that is you regardless of what the “facts” of your conception may have been. And there, then, as the growing, forming you, you forgive your parents. You see them as the holy, glorious, innocent beings they are. You bathe them with your love, knowing that all is well. You sigh. And smile.

Then, you return along your timeline to the present, bringing with you...into the here and now...your feelings of peace and luminosity, your joy and exhilaration, that you experienced at that moment of your conception. And you allow this to inform your health for the rest of your life.
Hacking the Human Biofield

The 30-day plan to Grow a New Body and Mind
...And Avoid the Illnesses of Civilization

The following are extracts from my book One Spirit Medicine. Please order it online for the full science!

Nature invests in the longevity of the species by programing us to go crazy over sex from the perspective of biology. Just ask any 18 year-old.

Longevity of the individual is of no interest to Nature, but it is to you and me.

These programs are encoded in the biofield, an energy and information field that envelops the body.

Shamans of old discovered that they could hack the biofield to live long and healthy lives, to get their health span to equal their life span, and free their consciousness from their biology!

WOW. It’s not complicated, actually.

You have to detox the body and repair mitochondria - you must upgrade the hardware first. Then upgrade the brain so you can upload the new apps for longevity and the exploration of the ultimate frontier, the conscious Cosmos.

It’s an experiment of N=1, you! And shamans and Tibetan Buddhists have been doing it for millennia.

Step One:
The 30 day diet to Grow a new Body and Mind.

You can’t grow a new body on junk food. Have you noticed how no self-respecting critter will touch the fries that have fallen under the seat of your automobile, even after a year?

First, Clean it up.

Dairy: Get off it. If you must, use goat cheese and goat yogurt.

Grains: No gluten. Get off all grains if you can. Grains are a modern-day discovery and our systems are not adapted to process them. We have a hunter-gatherer genome. Processed grains turn into sugar in your system! Quinoa is fine (it’s not a grain!)
Beans: Limit to one-cup per day.

Sugar: Eliminate. No soft drinks, no artificial sweeteners. Mice addicted to cocaine and offered sugar as an alternative will choose the sugar 100% of the time!

Second, what to eat.

Fats and Protein: Make sure they are good fats, omega 3 rich foods, avocados, nuts (no peanuts) and coconut oil. No vegetable oils. Good quality proteins.

Eat Green: We co-evolved with plants, not with animals, who would run away! The key is nutrient-dense and calorie-poor.

Meat: Sparingly, and only free-range, grass fed.

Fish: Great for you. Eat small, wild caught. Watch for mercury toxicity.

Eggs: Are great for you, if you are not allergic. Great source of protein, and they don’t affect cholesterol.

Kale: Loaded with healthy info. Juice it in the morning, cook it lightly at lunch or dinner. Avoid too many root vegetables that are sugar rich.

A plant based diet (nutrient-dense, calorie-poor) will switch on more than 500 genes that create health and switch off more than 200 genes that create cancers.

Step Two:
Supplements to Upgrade the Brain and Grow a new Body

“Regenerative medicine is a game-changing area of medicine with the potential to fully heal damaged tissues and organs…” - Mayo Clinic

You can switch on the regenerative mechanisms in every cell to grow a new body that ages, heals, and dies differently. The codes are latent in your DNA, but are password protected. These supplements will give you access.

Daily for Four Weeks.

Morning

Vitamin B12 is essential for liver detoxification and for protecting DNA. Most of us are B12 deficient. Be sure to take sublingual methylcobalamin, an enhanced form of B12 that dissolves quickly under the tongue. Take 2,500 mcg.

Vitamin C is essential for detoxification processes. Take 2,000 mg.

Vitamin D3 can prevent or reduce depression, dementia, diabetes, and autoimmune disorders. Take 5,000 international units (IU) of vitamin D3 during the 7 day program, then 1,000 IU thereafter.

S-acetyl glutathione is the first truly bioavailable form of the free-radical scavenger glutathione. Take 1 gram in the morning on an empty stomach.

DHA and EPA, are omega-3 fatty acids important for brain health and preventing Alzheimer’s. Take 3 grams, from fish oil or algae.
Curcumin, the active ingredient in the spice turmeric, activates the genes that turn on powerful antioxidants in the brain. Take 1 gram. Be sure it is in liposomal form.

ProAlive Probiotic resettles healthy flora in the gut and facilitates digestion. Take five drops in water. (You can order at www.ascendedhealth.com.)

Coconut oil is jet fuel for the brain. Take 1 teaspoon in the morning and 1 teaspoon in the midafternoon.

Evening (two hours after dinner):

Alpha-lipoic acid helps eliminate toxins and heavy metals in brain tissue. Take one 600 mg capsule.

Magnesium Citrate helps with your bowel movement and to eliminate waste, as well as relax your muscles.

Take One Week On, One Week Off, One Week On, One Week Off...

Trans-resveratrol, triggers production of the brain’s antioxidants and down-regulates genes that activate apoptosis, programmed cell death. Take 500 mg.

Pterostilbene, found in blueberries and grapes, works with trans-resveratrol to prevent cancer and other diseases. Take 250 mg.

Some of the facts: Pterostilbene, Trans-resveratrol, and Curcumin regulate genes which oversee apoptosis, or programmed cell death. These products upgrade mitochondrial function and aid in the electron transport chain. They also decrease inflammation, and switch on the longevity genes (Sirt1) inside every cell.

Ok, so now you have the plan of what to avoid, what to eat, and what supplements to take.

The last step is to trigger autophagy, which is the recycling of cellular waste, the garbage build-up inside cells (and brain cells in particular) that interfere with mitochondrial function and mess with the death clock inside every cell. Our ancestors used to feast and then fast. When they fasted, autophagy cleaned out all the waste inside their cells. But we have been eating 3 meals a day since we were born.

Autophagy is triggered when there are no sugars in the system, and insulin signaling has been turned off.

It’s that simple. You already fast during your sleep. With the One Spirit Medicine program you want to stay away from sugars and carbs for 18 hours a day. This means you start the day with proteins and fats (like eggs and one half avocado) instead of with toast and fruit - you can eat your fruit at noon if you like. And dinner is at 6:00 with no desert!

NEXT....

Upload the new OS. Buddhists do this through the practice of compassion and Metta, loving-kindness. It reprograms the brain from scarcity, anger and fear and lets you be present in every moment. Shamans do it through the prayer Mitakuye Oyasin “All my relations...”

The Sun is providing us with the codes for a new human through the plasma storms striking the earth (X Class flares). If you have upgraded the brain and body, you will be able to upload the new codes. If you have not, they will make you crazy!

These codes have always come from the Sun, and are read by our mitochondria which communicate with bio-photons to all life around you.

Try the experiment. To your good health!
LONGEVITY & REPRODUCTION

Nature invests in the longevity of the species by programming us to procreate – to have sex. As enjoyable as that may be, many of us are equally interested in our longevity, which is of little use to Nature. Once our reproductive years are over, as we enter into our 40’s, human growth hormone production and free radical scavenging systems begin to wind down and we start to decline, with all the consequences that entails.

Shamans of old knew how to hack into Nature’s programming to get their health span to equal their life span, and free their consciousness from their biology. Through One Spirit Medicine, they found they could switch off the “death clock” inside each cell, and turn on the “immortality genes” hidden deep within DNA.

Diet was key to their good health and longevity. Primarily horticulturalists, they ate nuts, fruit and the occasional animal. Their diet was calorie poor and nutrient rich, with green plants abundant in essential phytonutrients. Most importantly, they knew the sacred plants that switched on the “immortality genes.”

OUR ANCESTOR’S TOXINS

In the west, we take care of the body without understanding it’s relationship with the spirit. We mistakenly think the body was created just to be able to eat, travel, move and inhabit this earth for a short while. But biology is really the most spiritual thing in the world. So we begin by healing the body, by getting our health span to equal our lifespan, repairing and priming the brain for enlightenment, and then we can upgrade the Luminous Energy Field that surrounds and informs the physical body.

Understand that 99% of the people living the Western lifestyle have a brain that has been damaged by pollutants and toxins. We are not aware our brain has been compromised until we do the repair protocols and notice how brain fog clears, we have clarity and lucidity, our mood issues go away and we begin to come back into relationship with all of life. The first thing we do is to repair the brain so that we can stop creating psychosomatic disease and begin to create psychosomatic health.

The minute we picked up a shovel to farm and started eating wheat or barley or corn, our lifespan was reduced by 50%. We began to raise animals, and feed from the meat of these animals. We began to eat carbs from heavily processed grains, giving rise to a new social class of masters and slaves, and religions that pray “give us this day our daily bread”.

Grow a New Body
Alberto Villoldo, PhD

Neuroscience of Enlightenment
The Four Winds Society
There is no archeological evidence of large scale warfare anywhere in the world prior to the invention of agriculture. That’s because land had no value for nomadic gatherers. They didn’t own the land, they were stewards of the land, keepers of the land – they were one with nature.

But when farmers settled the land, they started craving somebody else’s land, and that grew exponentially. If you look at the problems in the Middle East today, they are all about who owns the land. The arrival of agriculture also led to a shift in human temperament, thanks to the consumption of processed grains – carbs that turn into sugars as soon as we eat them. Sugar feeds the lower brain, the primitive, predatory brain that focuses on survival and is tremendously aggressive and competitive, not collaborative.

We became disconnected from nature – from our own nature, our own bodies, our instincts. When we upgrade the quality of the brain, we begin to reset our instincts.

**A PROCESS OF ELIMINATION**

We need to first eliminate certain foods from our diet, starting with sugar. A hundred years ago, the average American ate five pounds of sugar a year. Today the average American eats 200 pounds of sugar a year – and we wonder what’s gone wrong with society? You must also go off artificial sweeteners: no aspartame, no saccharin, no sucrulose.

Dairy is likewise off-limits. (If you must, eat goat cheese and goat yogurt.) Also, do not eat more than 1 cup of beans a day; and quit the coffee habit while you’re at it.

And then, there’s gluten, a protein found in grains that actually cleaves the tight junctions in the gut where we absorb nutrients, allowing flora from your GI tract to enter into your blood stream and create a huge inflammatory immune response. So get off the pasta and off the bread. I love the scent of fresh baked bread, but now I just smell it; I don’t eat it anymore.

The many foods we can and should eat are diverse and delicious: fats, proteins, leafy green (phytonutrient-rich) and cruciferous vegetables, nuts, seeds, avocados, berries, eggs, meat (sparingly), and small wild-caught fish. These foods will up-regulate the expression of the genes that create health, and down-regulate the genes that create disease.

And that is key because disease is rampant today. Just as an example, one in every two Americans who get to the age of 85 will have diagnosable Alzheimer’s. You really want to avoid that, because those are the years you will want to be living in bliss and communion with spirit, not wondering who these people are that you don’t recognize, when they’re your own children.

I spent 20 years in the Amazon as a medical anthropologist working with communities that had seldom seen a white man before. (The kids would run up to me and start rubbing my hands to see if the white “dirt” would come off.) What amazed me was that among these primarily horticulturalist communities was there was no heart disease, no Alzheimer’s, no cancer, no dementia and they were happy.

The people of the Amazon did not take supplements, but those of us in the rest of the world should. Omega-3, especially EPA and DHA are essential to repair the brain. Our bodies cannot produce Omega-3s naturally, so we have to get these fatty acids from food and supplements. A recommended dose of 3 grams (3000 mg) a day will repair the hippocampus within six weeks, but making Omega-3 a permanent part of your daily regimen will help reduce the risk for Alzheimer’s by more than 50%.

Other essential supplements for brain health include Vitamin D3, curcumin, the active ingredient in turmeric; trans-resveratrol, found in red wine and red grapes; sulforaphrane (from broccoli flowers) and coconut oil, which is jet fuel for the brain.
GOING RADICAL

You’ve got to go radical and extreme because it will take you six months to get there and every meal you ordinarily would eat you have to pass on. This means no processed carbs, no sugar, no dairy, no coffee, no eggs (if you are allergic to them.) No processed grains, and no gluten…The minute you start to eliminate these foods you will begin to clear toxins from your system. If you complement that with certain supplements, in six months you will have eliminated the last 40 years of toxins you have been exposed to. That includes chemicals, paints, asbestos, lead, pesticides and an incredible amount of other poisons we absorbed because back then our parents believed in better living through chemistry. And although many toxic substances have now been outlawed, we still need to eliminate them from our own bodies.

The next point we need to address is replenishing the flora in our GI tract, because we are a colony organism. More than 90% of our DNA belongs to microbes that we have a symbiotic relationship with – an incredible colony of 90 trillion cells that make up who we are. If you have taken antibiotics just once in your life, you decimated your intestinal flora.

FIGHT OR FLIGHT?

Most of us also need to reset our “fight or flight” system, which is responsible for the production of stress molecules. The fight or flight system is in a very primitive region of the brain that feeds on sugars and cannot tell time or calculate distances. When it hears about Ebola in Africa or terrorist attacks in Australia or France, this organ of the brain thinks these things are happening just outside the village gates. It can’t tell the difference between 2000 miles away or 20 blocks away, so it triggers cells into fight or flight, which produce the stress molecules cortisol and adrenaline. For many, the fight or flight system is stuck on overdrive and they have lived in a constant state of anxiety since they were born.

The alchemical laboratory in the pineal gland, on the other hand, was designed to flood the brain with endogenous bliss molecules, creating states of joy, oneness, and communion. When the brain is producing stress molecules, it cannot produce the molecules for bliss. It’s either one or the other.

The minute we are able to calm our fight or flight system, relax and breathe deeply throughout the day, the brain will kick-start the lab in the pineal to begin producing natural consciousness-expanding bliss molecules. So you don’t need to go to the ayahuasca ceremony down the block. You can produce it yourself, because ayahuasca is a dimethyltryptamine (DMT) which is analogous to the neurotransmitter serotonin, which the pineal gland can transform into a powerful bliss molecule.

ERASE TRAUMA FROM YOUR FIELD

The shamans greatest contribution to our understanding of life, death and our place in the cosmos is the Luminous Energy Field, and that’s where it really gets interesting. This infinite field of incandescent energy that surrounds our physical body is our link to the past, the future and the universe around us. It is an informational field that contains all of the stories of our life. During a healing session, the shaman clears the Luminous Energy Field, erasing the imprints of trauma from the field, so it doesn’t have to organize the body into disease.

Clearing your field also allows you to reselect your genetic destiny. For most of us, our genetic destiny was selected in a moment of wild or boring sex that our parents had, in which they merged their chromosomes and in that very moment, our entire genetic destiny was determined. But we are not slaves to our genetics. Today we know that we are not our genes, that we are our dreams. In a new field called epigenetics we are discovering how meditation, joy, compassion, forgiveness, and the food we eat, can switch on the genes that create health and switch off the genes that create disease. And when we meditate, the neural networks in our brains can determine how we can age gracefully, how we can heal rapidly, and how we can die consciously.

Once we have repaired our brain with the neuro-nutrients and superfoods, we select a different genetic destiny, so we don’t have to live out the illnesses and dramas of our families of their origin. You can reselect your genetic destiny for one that has a long life, a good brain, a strong heart and a joyous, healthy lifespan.
TEN PRINCIPLES FOR HEALTHY DETOXIFICATION

1. Drink 8-10 glasses of filtered water a day
2. Keep your bowels moving once or twice a day
   Use 2 TBSP of flax seeds, probiotics and magnesium citrate
   and buffered ascorbic acid (vitamin C) to keep regular
3. Eat organic produce and animal products
4. Eat 8-10 servings of colorful fruits and vegetables and include cruciferous veggies and garlic daily
5. Avoid caffeine, nicotine and moderate alcohol intake
6. Exercise 5 days a week with conditioning, strengthening and stretching exercises
7. Get rid of the white menace - white flour and sugar
8. Sweat profusely at least 3 times a week - Sauna, steam, detox bath
9. Take a high quality multi-vitamin and mineral, fish oil and vitamin D daily
10. Deeply relax daily

SEVEN KEY PRINCIPLES OF AN OPTIMAL DIET

1. Eat a rainbow assortment of fruits and vegetables – get your phytonutrients
2. Reduce exposure to pesticides by choosing organic and grass fed when possible
3. Eat to regulate your blood sugar – eat early and often and avoid sugars and flours – small
   frequent meals with protein (nuts, seeds, beans, lean animal protein)
4. Do not over consume meat and other animal foods
5. Eat the right types of fats (omega 3 fats, olive oil, nuts, avocados)
6. Keep your salt intake low and your potassium intake high (eat a plant based diet)
7. Drink enough water every day

PRACTICAL SUGGESTIONS

1. Take home at least one new thing, behavior or habit that makes you thrive.
2. Leave one thing here that does not agree with you.
3. Use saunas (try an infrared one – www.sunlighten.com
4. Find a meditation teacher or yoga class or get private sessions
5. Do one deep relaxation experience daily (yoga, breathing, meditation)
6. Resources: The UltraSimple Diet, UltraMetabolism, The UltraMetabolism Cookbook, Ultraprevention,
   The 10-Day Detox Diet by Mark Hyman, MD, and One Spirit Medicine by Alberto Villoldo PhD
7. Detox Baths: 2 cups Epsom salts, ½ to 1 cup baking soda, 10 drops of essential lavender oil and
   soak in hot water for 20 minutes, the go to bed
8. Avoid TV in the bedroom (or closet)
9. Avoid computers 2 hours before bed
10. Make broth and enjoy as a snack or drink
11. Take your vitamins and supplements daily
An Invitation to Healing and Detoxification

Consider Avoiding

- Dairy (milk, cheese, butter, ghee)
- Gluten (barley, rye, oats, spelt, kamut, wheat – see www.celiac.com for foods that include gluten)
- Eggs
- Corn
- Soy
- Peanuts
- Nightshades (tomatoes, eggplants, peppers, potatoes)
- Caffeine
- Alcohol
- Sugars (table sugar, honey, maple syrup, corn syrup)
- Sweets
- Yeasted products (wine, vinegar, breads)

Foods to Include

- Fresh Fruit (except citrus, pineapple or dried fruit for the first two weeks)
- Raw vegetables: salads – include artichokes, avocados, olives
- Sea vegetables: wakame, hijiki, arame, etc.
- Nuts (almonds, walnuts, pecans, macadamia nuts, hazelnuts, but no peanuts)
- Nut Butters (almond, cashew, macadamia nut)
- Fish (small wild fish – wild salmon, sardines (see www.vitalchoice.com for mercury free small wild salmon, excellent canned salmon and sardines), shrimp, scallops, small ocean fish that fits in a pan
- Organic chicken, Organic lamb and occasional beef
- Low allergy grains such as quinoa, buckwheat, millet
- Continue with vegetables, beans, rice, healthy oils (olive oil, cold pressed nut oils)
- Spices – include garlic, ginger, curry or turmeric, rosemary, cilantro
- Herbal teas - non-caffeinated
- Try 1 TBSP organic coconut butter a day in the morning straight or in shake or hot water

Eating Out

- Japanese, Thai, healthy Chinese (not fried, no MSG)
- Order fish and veggies at Italian restaurants
- Avoid bread

Phase Two Reintroduction of Foods

- Reintroduce one new group every 3 days. Eat it at least 2-3 times a day for the 3 days to notice a reaction (unless of course you notice a problem right away, then stop)
- Start with dairy, then gluten, then soy, then eggs, then corn, then nightshades, then peanuts and yeasted products
- Keep log of common symptoms that can occur from 6 to 72 hours later
- These include: fatigue, brain fog, headaches, digestive symptoms (bloating, gas, diarrhea, constipation), post-nasal drip, sinus congestion, mood changes, sleep problems, irritability and joint pains (although many other symptoms are possible)
- If you have a reaction, note the food and eliminate it for 12 weeks.
- Then try to reintroduce again. If you still have symptoms you may need to avoid this long term.
- I would recommend avoiding sugar for at least a month as an experiment, then try very good dark chocolate and savor it!
- Caffeine and alcohol are the two substances that we can enjoy from time to time and feel good about it
Detox Bath

1. Magnesium sulfate (Epsom salts) two cups
2. Baking soda, one half cup
3. Lavender oil, ten drops
4. Put all in hot bath and soak for 20 minutes

What Supplements Should I Take?

The basics that you should take daily include a multi-vitamin and mineral, a calcium magnesium formula, fish oil, Vitamin D3, and probiotics. Additional support may be needed with digestive enzymes, adrenal supportive herbs, or detoxification support from time to time. Magnesium is often needed and can help with stress, constipation, muscle cramps, headaches and more.

Detoxification Formulas and Energy and Mitochondrial Formulas can be taken for one week twice a year, as needed.

Multivitamins

1. Xymogen Active Nutrients with or without Iron or
2. Wellness Essentials (multivitamin, fish oil and detox formula) or Wellness Essentials for Women 1 packet a day (multivitamin, fish oil, calcium, vitamin E)

Omega 3 Fats

1. Xymogen Omega Pure 820 one twice a day – fish oil

Minerals

1. Magnesium glycinate or citrate by Pure Encapsulations 2-4 twice a day (citrate if you tend to be constipated), or CALM, in powdered form.

Vitamins

1. Vitamin D3 1000 or 5000 U a day by Xymogen
2. Buffered Ascorbic Acid (vitamin C) by Pure Encapsulations or Xymogen 1000 mg 1 or 2/ day
3. Vitamin K2 by Xymogen

Detoxification Formulas

1. Opticleanse GHI by Xymogen
2. Sulforaphane Complex by Xymogen
3. Resveratrin by Xymogen (trans-resveratrol)

Adrenal Formulas

1. Adrenal Rebuilder 2 tabs 2-3 times a day by Future Formulations

Relaxation and Sleep Formulas

2. Xymogen 5 HTP for sleep
3. Melatonin by Xymogen 2 to 3 mg
Energy and Mitochondrial Formulas

1. CoQ10 (Ubiquinol) 200 mg a day by Xymogen
2. ALAMax – Alpha lipoic acid 600 mg one or two a day by Xymogen

Digestive Health

3. Pro-Alive probiotics from Ascended Health (AscendedHealth.com)
4. Xymozyme by Xymogen (digestive enzymes)

To Order from Xymogen: www.xymogen.com
CODE: fourwinds
PRACTITONER: Villoldo

UltraBroth Recipe

For each three quarts of water, add:

1 large chopped onion
2 sliced carrots
1 stalk of peeled burdock root
1 cup of Daikon root and tops
1 cup of winter squash cut in large cubes
1 cup of root vegetables: turnips, parsnips, rutabagas for sweetness
1 ½ of chopped greens – either kale, parsley, beet greens, chard, dandelion, other greens except cilantro, which can added if you like it
2 celery stalks
1 cup of sea weed – any combination of nori, dulse, wakame, kelp, or kombu
1/2 cup cabbage
Sea salt to taste
If available, fresh or dried Shitake or Maitake mushrooms - one cup

Add all ingredients at once and place on low boil for 40 minutes
Strain and enjoy. Can be cooled and stored tightly sealed glass container for later use.

This article is by Mark Hyman, MD, from the materials given to participants in the detox programs directed by Dr. Hyman and Alberto Villoldo PhD. Used with permission.
Whole Health Source

Antioxidant supplements are probably ineffective. They may even be hazardous to your health. Many people take daily supplements that include antioxidants such as Vitamins A, C, and E; beta carotene, coenzyme Q10, and alpha lipoic acid. I used to be one of them, convinced of the theory that supplementation with antioxidants is an effective way to neutralize harmful free radicals. These free radicals, also called ROS or “reactive oxygen species”, can cause oxidative damage to cells and organs, and have been implicated in the pathogenesis of degenerative diseases such as cancer, heart disease, and Alzheimer’s disease.

However, study after study not only fails to show a consistent benefit, but in many cases documents positive harm from taking antioxidants. While I continue to believe that antioxidant supplementation is helpful in certain isolated cases of acute infection, tissue damage, or a damaged or aged metabolism, for most of us antioxidants are probably worthless. In fact, antioxidant supplements can interfere with and weaken the body’s inherent ability to mount an effective defense against oxidative damage and its contribution toward degenerative diseases.

I’ve resisted this conclusion because I could not make sense of it. That is…until I came across recent research into the biochemistry and genetic regulation of the antioxidant response element (ARE). Fortunately the ARE provides us with an in-built adaptive stress response that combats oxidative stress and inflammation. The ARE makes the need for antioxidants in the diet unnecessary — other than to keep our food fresh. Surprisingly, antioxidant supplements can impair our adaptive stress response.

But there’s much we can do to strengthen this response.

Fruits, vegetables and green tea. One of the strongest arguments for taking antioxidant supplements is the observation that consumption of fruits and vegetables reduces the levels of oxidative damage and associated degenerative diseases. This has been shown in both epidemiological studies and observational studies. Similar benefits have been associated with the consumption of certain herbal compounds rich in polyphenols, such as green tea, garlic and curcumin. The assumption has always been that these benefits can be attributed to the fact that many fruits, vegetables and herbs are rich sources of naturally occurring antioxidants. Therefore, it only makes sense that if you can’t get enough fruits and vegetables in your normal diet, supplementation with purified chemical forms of these antioxidants can boost those benefits. But it turns out that the protective effects of fruits and vegetables are most likely not due to their antioxidant content, which is probably too weak and inconsistent to explain the health benefits.

But before discussing the real reason that fruits and vegetables have health benefits, let’s review what is known about supplementation with antioxidants.

Antioxidant supplementation studies. It may surprise you that numerous of clinical trials and metabolic studies show no benefit, or even harm, from using antioxidant supplements:

- A 2004 American Heart Association meta-analysis of 20 clinical trials showed no benefits for the use of Vitamins C, E and beta carotene in the prevention of heart attacks or strokes, and no reduction in mortality. While they acknowledged that the scientific evidence from observational studies supports the conclusion that “a diet high in food sources of antioxidants and other cardioprotective nutrients” reduces the risk of CVD, they found no support for any benefits from the use of antioxidant vitamin supplements. They did indicate that antioxidant supplementation may be useful in certain critical medical procedures, but not for routine dietary supplementation.

- A 2008 Cochrane Institute meta-analysis of 7 randomised clinical trials on antioxidant supplements (beta-carotene, vitamin A, vitamin C, vitamin E, and selenium) versus placebo or no intervention found no evidence that antioxidant supplements prevent mortality in healthy people or patients with various diseases.

- A 2001 University of Washington randomized trial showed evidence of positive harm from antioxidants. A cocktail of antioxidants added to the course of patients with high cholesterol and using statin-niacin therapy led to reduced levels of HDL and increased levels of coronary blockage.

- A study at Cedars-Sinai Heart Institute showed that cardiac stem cells cells that were loaded with high doses of antioxidants developed genetic abnormalities that predispose to the development of cancer.
• A study comparing chemical Vitamin C with oranges containing an equivalent amount of Vitamin C given to test subjects showed that the blood from those who ingested the oranges could neutralize hydrogen peroxide (an oxidant) but those who ingested Vitamin C tablets failed to do so.

• A 2009 study by German and American researchers found that daily supplementation with 1000 mg Vitamin C and 400 IU Vitamin E during a 4-week exercise program by healthy young men suppressed improvements in insulin sensitivity observed in the non-supplementing control group.

These results were at first puzzling to me. How can it be that administering the same antioxidant chemicals ubiquitous in “protective” fruits, vegetables and herbs — the same chemicals which have been shown to neutralize oxidants in the test tube — appear to be ineffective or even harmful when taken as dietary supplements? What’s going on here?

The endogenous antioxidant defense. What is missing in the above picture is the role of our body’s own innate defenses system for handling toxic chemicals like free radicals. While our immune system handles invading organisms and large proteins, another system is needed to deal with chemical toxins. It’s called the xenobiotic metabolism; “xenobiotic” is from the Greek and Latin roots for “foreign to the organism”. It consists of three “waves” of protective enzymes which neutralize dangerous chemicals, designated: Phase I, Phase II, and Phase III.

In Phase I the “xenobiotic response element” (XRE) chemically modifies the foreign toxins, which can sometimes make them even more reactive oxidants.

In Phase II, a set of antioxidant enzymes known as the “antioxidant response element” (ARE) neutralizes these toxins, including free radicals.

Phase III involves further modifications and excretion.

The ARE is your body’s own endogenous antioxidant defense. And it is far more powerful and effective than any antioxidants you consume orally at mounting a defense against free radicals. The ARE system is activated by the presence of oxidants in specific tissues in the body. These oxidative toxins are detected by transcription factors, most importantly Nrf2 (Nuclear factor (erythroid-derived 2)-like 2).

Nrf2 has been called the “master redox switch”. It turns on a series of cytoprotective genes, which have been nicknamed “vitagenes” by U. Massachusetts toxicologist and hormesis researcher Edward Calabrese. These vitagenes upregulate the production of endogenous antioxidant enzymes that combat oxidative stress and inflammation.

Collectively, they are known as the Phase II antioxidant enzymes:

• glutathione transferase
• glutathione peroxidase
• glucuronysyl transferase
• quinone reductase
• epoxide hydrolase
• superoxide dismutase
• gamma glutamylcysteine

So how can it be that supplementing with antioxidants can actually dampen the body’s internal antioxidant defense system?

Homeostatic compensation. As we’ve seen time and again in this blog, the body is an adaptive system. The organism adjusts to maintain a relatively constant state: homeostasis. Provide it with external “help” and it will reduce the effort in building its own internal defenses. Just as using corrective lenses will weaken the eye’s inherent ability to focus, and avoiding exposure to allergens will prevent the adaptive immune system from developing, it turns out that chronic consumption of exogenous antioxidants reduces the “pressure” on your adaptive stress response — specifically your ARE system — to gear up its own endogenous antioxidant defense system by producing adequate amounts of the Phase II enzymes.
In biological terms, taking antioxidants leads to homeostatic downregulation of the antioxidant response element. This actually makes biological sense: Why should the organism expend precious energy and resources building a defense system if the defense is provided for “free” through diet or supplements?

A number of studies bear out this compensatory effect:

- A metabolic study in houseflies showed that administering Vitamin C (ascorbic acid), Vitamin E (alpha tocopherol) and beta-carotene caused a compensatory depression of activity of key endogeneous antioxidant enzymes including superoxide dismutase, catalase, and glutathione. The administration of vitamins C and E also reduced life span. Granted that humans are not the same as flies, but we use the same enzymes to detoxify.

- A study of supplementation of cells with the antioxidant lipoic acid showed that it inhibits the antioxidant adaptive response triggered by treatment with UV-B light. The added lipoic acid decreases the intracellular oxidative signals necessary to develop the adaptive response in human mononuclear cells.

- A 2008 study at the University of Valencia showed that Vitamin C supplementation hampered exercise endurance. While Vitamin C reduces ROS levels in short term, it impairs the adaptive response by reducing transcription factors that enable mitochondria production, and inhibiting expression of antioxidant enzymes superoxide dismutase and glutathione peroxidase.

- A 2010 study showed antioxidants can cause neurodegeneration by inhibiting autophagy — an important process for removing damaged cellular material. Inhibition of autophagy by antioxidants has a range of other potential negative consequences.

So it appears that, by consuming more antioxidants, we become dependent upon them and perversely reduce our innate ability to detoxify. With any let-up in the constant supply of external defenses, we become more vulnerable to oxidative and inflammatory attack. And the externally supplied antioxidants themselves are in any case much less effective than the endogenous ones.

But if the endogenous antioxidant defense system is so potent, what steps can we take to build it up?

**Plant toxins to the rescue.** Nature exhibits a wonderful phenomenon called “biological arms races”. To defend against predators, plants or animals develop defenses, and often this involves the production of biological “poisons”. To defend themselves against pests and parasites, plants have evolved a set of mildly toxic substances that discourage, sicken, or even kill predators, from microbes and insects to mammals. These toxic substances typically taste bad and can be irritating. However, predators evolve to be able to tolerate at least some of these plant toxins, at least in moderate amounts. They do this by developing detoxification systems. Which is exactly what the ARE is!

Some plant toxins are too poisonous and deadly. But, as Nietzsche said: “That which does not kill us makes us stronger”. Biologically speaking, this is the principle of hormesis advocated on this blog, the principle by which small amounts of a stressor activates and strengthens our internal defenses, but excessive levels of the same stressor overwhelms these defenses. Our ARE anti-toxin system will develop in response to virtually any toxic compound. In principle, you could strengthen it by ingesting all kinds of chemical poisons. But why play roulette?

Humans have grown up for eons consuming a fairly regular supply of certain plants to which they have become habituated, plants that contain tolerable amounts of toxins which moderately stimulate the adaptive stress response, but not sufficiently to kill us. Of course, there are still poisonous plants and mushrooms which exceed this threshold, so there is a continuum. And probably some people and populations can tolerate more than others of certain plant toxins. But some of these plant toxins are well enough tolerated by most of us to prove reliably beneficial.

**What are the good plant toxins? We refer to them as “phytochemicals” or “phytonutrients”.**

There are a nearly infinite number of phytonutrients, most of them unknown and uncharacterized. But a number of them have been studied for their impact on upregulating the Phase II enzymes of the ARE system, as Mattson...
et. al. have detailed... Many of these compounds fall into the chemical class of polyphenols, more specifically flavonoids. They are typically pigmented, bitter or spicy tasting molecules. A partial list includes:

- resveratrol – from red grapes, which turns on sirtuins and has broad cardiovascular, memory and anti-aging benefits
- sulforaphone – from broccoli, which turns on antioxidant and anticancer enzymes in the skin, arteries and stomach
- curcumin – from tumeric, inhibits transcription factors and kinases involved in cancer and inflammation
- green tea – a rich but variable source of bioflavinoids which have been shown to have anticancer and cardioprotective effects

Other polyphenolics that stimulate that Phase II enzyme system have been found in garlic, rosemary, ginko, bee propilis, and even...coffee!

What may have confused many researchers is that these polyphenolic flavonoid compounds in many cases have antioxidant properties. This fact may have led to drawing the mistaken conclusion that they work because they are antioxidants in their own right. And yet this antioxidant effect is not consistent — polyphenols and other phytochemicals sometimes function as pro-oxidants, dependent on the context and dosage. I believe the evidence for their being hormetic stimulants of the endogenous ARE system is stronger than the case for thinking of them as antioxidants.

For example:

- A review of cell culture experiments with various polyphenols shows that their mechanisms of action goes beyond their intrinsic antioxidant properties, by indirectly stimulating enzyme transcription through the ARE system.

- Resveratrol seems to have its optimal effect at concentrations too low to be explained by an antioxidant effect. A metabolic study of resveratrol in heart cells, showed that even at very low (micromolar) concentrations, it upregulates endogenous “cytoprotective factors” — antioxidants and phase 2 enzymes such as superoxide dismutase, catalase, glutathione, glutathione reductase, glutathione peroxidase, glutathione S-transferase (GST), and NAD(P)H:quinone oxidoreductase-1 (NOQ1).

- An Israeli study showed that caratenoids in tomatoes activate the ARE transcription system, upregulating the phase II detoxification enzymes in a manner that is not correlated with the antioxidant potential of the caratenoids. However, caratenoids appear to have an optimum level, above which they may be harmful.

In his article, Guyenet mentions the interesting phenomenon that the hormetic effects of polyphenols tend to be non-specific:

One of the most interesting effects of hormesis is that exposure to one stressor can increase resistance to other stressors. For example, long-term consumption of high-polyphenol chocolate increases sunburn resistance in humans, implying that it induces a hormetic response in skin. Polyphenol-rich foods such as green tea reduce sunburn and skin cancer development in animals.

Stephan Guyenet

I’m an obesity researcher, neurobiologist, and author. In addition to my research, I enjoy synthesizing and communicating science for a general audience. I have a BS in biochemistry (University of Virginia) and a PhD in neurobiology (University of Washington). Whole Health Source is a free resource for anyone who loves the science of health.
Curcumin Nanomedicine: 
A Road to Cancer Therapeutics

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Abstract
Cancer is the second leading cause of death in the United States. Conventional therapies cause widespread systemic toxicity and lead to serious side effects which prohibit their long term use. Additionally, in many circumstances tumor resistance and recurrence is commonly observed. Therefore, there is an urgent need to identify suitable anticancer therapies that are highly precise with minimal side effects. Curcumin is a natural polyphenol molecule derived from the Curcuma longa plant which exhibits anticancer, chemo-preventive, chemo- and radio-sensitization properties. Curcumin’s widespread availability, safety, low cost and multiple cancer fighting functions justify its development as a drug for cancer treatment. However, various basic and clinical studies elucidate curcumin’s limited efficacy due to its low solubility, high rate of metabolism, poor bioavailability and pharmacokinetics. A growing list of nanomedicine(s) using first line therapeutic drugs have been approved or are under consideration by the Food and Drug Administration (FDA) to improve human health. These nanotechnology strategies may help to overcome challenges and ease the translation of curcumin from bench to clinical application. Prominent research is reviewed which shows that advanced drug delivery of curcumin (curcumin nanoformulations or curcumin nanomedicine) is able to leverage therapeutic benefits by improving bioavailability and pharmacokinetics which in turn improves binding, internalization and targeting of tumor(s). Outcomes using these novel drug delivery systems have been discussed in detail. This review also describes the tumor-specific drug delivery system(s) that can be highly effective in destroying tumors. Such new approaches are expected to lead to clinical trials and to improve cancer therapeutics.

Keywords
Nanotechnology; curcumin nanomedicine; drug delivery; cancer therapy; chemo-prevention; and tumor targeting

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CONFLICT OF INTEREST
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DISCLOSURE
Declared none.
1. INTRODUCTION

Cancer is the most prevalent disease not only in the United States but worldwide. An estimated 1,596,670 new cancer cases and 571,950 cancer deaths occurred in 2011 in the United States [1]. Conventional therapeutic approaches such as chemotherapy, radiation, combinational chemotherapy, and surgical treatments are widely accepted to treat or eradicate tumor(s). While chemotherapy remains a highly successful weapon to treat cancer, it is often associated with limitations and major side effects. There is always a possibility of recurrence and these cancers can develop resistance to chemo- and radiation therapies. A drug or combinational drug(s) may not work in all types of cancers since therapeutic agents work on single or dual mechanisms which control growth or induce apoptosis in fast growing cells. Therefore, developing new treatment modalities is essential to precisely treat tumors and prevent progression of cancer to the metastatic stage. In order to overcome common major obstacles in conventional cancer therapeutics, scientists are searching for effective treatments within alternative medicine, complementary medicine, and supplements. The National Cancer Institute at the National Institutes of Health (USA) recognizes complementary and alternative medicine (CAM) as prevention/treatment options (http://www.cancer.gov/cancertopics/cam). Natural herbal compounds or daily food ingredients are widely studied to learn their specific roles in anticancer activities [2, 3]. Unlike first line cancer therapeutic drugs, herbal and natural compounds are capable of targeting cancer(s) via several pathways and are therefore, more valuable and reliable in producing superior therapeutic effects in a disease condition (multiple pathogenic factors) [4, 6]. It is believed that herbal medicine brings a new hope for cancer prevention due to the safety of herbs and lack of discernible toxicity to normal cells. This alternative approach has been used to treat a wide spectrum of cancers.

Curcumin (CUR) is a hydrophobic polyphenolic compound derived from the rhizomes of Curcuma longa. This natural compound has a long history of use as curry (turmeric) in East Asian countries. Commercially available curcumin consists of a mixture of three curcuminoids [diferuloylmethane (~77%), demethoxycurcumin (~18%), and bisdemethoxycurcumin (~5%)]. Curcumin exhibits keto-enol tautomerism, having a predominant keto form in acidic and neutral solutions and a stable enol form in alkaline media. Curcumin is “Generally Recognized as Safe (GRAS)” by the Food and Drug Administration (FDA). Curcumin is characterized by a wide range of antibacterial, antifungal, antiviral, antioxidative, antiinflammatory, and antiproliferative activities [5, 7, 9]. Curcumin has demonstrated strong cancer preventive activity, including prevention of tumor initiation, promotion, metastasis, and angiogenesis in experimental animal systems, against a wide range of tumor cells [8, 10, 11]. Curcumin has pleiotropic properties that modulate numerous targets including proteins (thioredoxin reductase, cyclooxygenase 2 (COX-2), protein kinase C (PKC), 5-lipoxygenase, and tubulin), transcription factors, growth factors and their receptors, cytokines, enzymes, and gene regulating cell proliferation and apoptosis [12–14]. Because of this multi-targeted behavior, curcumin can perform a wide spectrum of actions while smart drugs or therapeutic drugs have only one target and are eliminated from the cells if they do not reach the right compartment [9].

Several in vitro investigations demonstrate that curcumin inhibits cancer cells growth (IC_{50}, 50% cell growth inhibition) at concentration of 5–30 μM [3, 8, 12, 14, 15], resembling cisplatin and gem-citabine (chemotherapeutic drug) concentrations. Because of its exceptional medicinal value, a total of 68 clinical trials have been registered with clinicaltrials.gov (as of May 3, 2012) in which the majority of them are targeting cancer. Curcumin has an extremely safe profile in both animals and humans [16, 17]. A detailed discussion about the bioavailability and safety profiles of native curcumin can be found in many review articles [18–20]. So far all the preclinical and clinical results from oral...
administration of curcumin have revealed very poor bioavailability, typically in nanomolar concentrations [18–20]. A classic example is a pharmacokinetic study involving healthy humans which found that only 2.30 ± 0.26 and 1.73 ± 0.19 μg.mL⁻¹ of curcumin (Cmax) was present in serum levels even after a high oral dose of 10 and 20 g curcumin, respectively, was given [21]. This suggests curcumin undergoes extensive metabolic changes in the intestine and liver. Additionally, a clinical study comprised of 15 patients with colorectal cancer showed the cancer was nonresponsive to curcumin at a daily dose of 3.6 g (4 months) [22]. This study suggested there was no change in tumorigenesis or tumor markers. Overall, the studies concluded that while curcumin exhibits anti-cancer effects at a concentration of 5–30 μM for 1 or 2 days, achieving these concentrations at the tumor site in humans not been accomplished due to curcumin’s low bioavailability and higher metabolic activity. Therefore, curcumin must be formulated in such a way that it can overcome these critical issues. Various pharmaceutical and generic industries have developed and customized curcumin formulations with the aim of improving solubility and bioavailability (Fig. 1). Adjuvant, cyclodextrin, and some patented technologies were initially used to overcome this issue [23–25]. For instance, piperine is highly recommended because of its inhibitory effects of hepatic and intestinal glucuronidation, which promoted 154% and 2000% bioavailability in rats and humans, respectively [27, 28]. While these attempts can improve bioavailability they are unable to target the fate of curcumin to tumors. Therefore, this manifests the necessity of curcumin encapsulation in nanoparticles for cancer therapy with possible targeting moieties [15, 18, 20]. This review discusses and offers potential new avenues that are appropriate to translate curcumin into nanomedicine for cancer therapeutics. This review also presents a comprehensive list of important approaches that have been undertaken to make efficient cancer therapeutic curcumin nanoformulations.

2. LITERATURE SURVEY OF CURCUMIN AND CURCUMIN NANOFORMULATIONS

Curcumin is a widely studied molecule for a number of medicinal applications. A PubMed search justifies curcumin’s clinical importance and provides a rational why curcumin nanoformulations are needed for further investigation (Fig. 2). The database covering Jan. 2001 to Dec. 2011 with key words ‘curcumin’ in “title and abstract” demonstrates an exponential growth of investigations, i.e., over 4000 studies. The search results for curcumin based nanoparticle, liposome, nanotechnology, and nanomedicine, reveals very little. A total of ~ 220 reports delineate various aspects of curcumin medicinal benefits. These investigations suggest nanotechnology mediated delivery of curcumin is in the early stages of development. As per our knowledge, there are only 4 review articles primarily covering curcumin and its nanoformulations. The first review article by Aggarwal group [20] explains bioavailability of curcumin, drug delivery associated issues, and possible adjuvant and conjugate analogues and nanotechnology promises. Our review focuses on various types of nanoformulations based on their structural variability, function, and improved activity [15]. At the same time, Bansal et al. [18] present an outstanding review primarily focused on the chemo-preventive aspect of nanoformulations and curcumin stent technologies. Another review article describes curcumin induced mechanisms in cancer and implications of curcumin nanoformulations in chemoprevention and treatment [29]. In our opinion, to date there is no specific, detailed review documenting the important synthetic routes for preparation of curcumin nanoformulations, drug loading phenomenon and the critical role of nanoparticle uptake by cancer cells, anticancer activities, tissue and bioavailability, and blood compatibility. Therefore, this review aims to provide up-to-date contributions of curcumin nanoformulations to cancer therapeutics; and further, to discuss how novel trends benefit cancer therapeutics.

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3. CURCUMIN NANOFORMULATIONS

For the past few decades, nanoparticle technology has been widely employed in medicine, including for cancer therapy [30–32]. As drug nanocarriers, nanoparticles possess several attractive features: (i) improved encapsulation or solubilization of therapeutic drugs for protective and targeted delivery, (ii) high surface to volume ratio enable modifications to surface functional groups in order to obtain extensive stabilization and internalization, (iii) biocompatibility, superior pharmacokinetics and minimal clearance from body, and (iv) controlled, stimuli responsive, remote actuation and on demand drug release properties. A large number of anticancer drug nanoformulations are currently in clinical or preclinical development. Some of the nanoformulations have been approved by the FDA are currently available in the market. A detailed list of approved formulations is available [33, 35]. Among these, the albumin-bound paclitaxel (PTX) poly(lactide-co-glycolide) (PLGA) nanoformulation (Abraxane™, http://www.abraxane.com/dtc/) is highly successful in increasing the specificity and treatment efficiency of various cancer(s). Nanoparticle formulations of nutritional ingredients such as carotenoids, co-enzyme Q10, vitamins (A, D, E, K), phytosterols, minerals, and natural extracts are not new and have been available since 1960 [36].

The main principle in effective cancer therapy is to achieve the desired concentration of therapeutic agents at the tumor site to destroy specific cancerous cells while minimizing toxicity to normal cells [37, 40]. In our view, it is essential to develop curcumin nanoformulations that exhibit superior anticancer activity compared to native curcumin. Our recent review documented that various types of nanocarriers were being used to achieve superior properties with implications for cancer therapeutics [15]. Each method of preparation determines the formulation stability, efficacy and specificity in cancer therapeutics. In our opinion, poly(lactide-co-glycolide) (PLGA) or poly(caprolactone) (PCL) nanoparticles, liposomal and self-assembly formulations of curcumin should be given the highest priority for cancer therapeutic applications due to their biocompatibility. The majority of important curcumin nanoformulations will be discussed. Table 1 provides a number of curcumin nanoformulations and their preparative methods which regulate particle size of formulations.

Polymer nanoparticles based on PLGA, a biodegradable and biocompatible polymer, can produce curcumin nanoparticles of approximately 100–200 nm size [41–44]. This particle size range is small enough to allow intracapillary passage while suitable surface coating allows escape from macrophage uptake [45]. These curcumin nanoformulations are widely studied in cell culture and animal models. Dendrimers have been employed to prepare curcumin nanoformulations, resulting in a globular macromolecule defined by core and branched units and surface groups [46, 47]. This type of curcumin derivative formulation retains biological activity and has the ability to destroy human neurotumor cells in a selective manner. Such methodology provides a general strategy for attaching curcumin to various functional macromolecular chains. Gel nanoparticles (nanogels) offer an excellent depot for drug(s) and protein delivery [48]. Poly(N-isopropyl acrylamide)-curcumin (PNIPAM-CUR) gel nanoparticle formulations exist with different compositions [49–52]. Because of a hydrophilic-hydrophobic interchange opportunity within the structure, they make a unique model drug delivery systems. Additionally, high water retention, low interfacial tension with biological fluids and stimuli responsive drug release suggest pharmaceutical implications. Hydroxypropyl methylcellulose (HPMC) and poly(vinyl pyrrolidone) (PVP) alone or in combination with six different surfactants were utilized to screen for the best composition for achieving a low particle size formulation [53]. Pluronic F68 stabilized PVP-curcumin formulation exhibited the lowest particle size of 100 nm. The order of various PVP-curcumin formulations with surfactants is followed as: Pluronic F68
Pluronic F127 (500 nm) < Cremophor RH 40 (720 nm) < D-a-Tocopheryl polyethylene glycol 1000 succinate (TPGS) (~ 800 nm) < tween 80 (~ 1.75 μm) < Tween 20 (3.75 μm). Pluronic F68’s particle size is smaller than F127 due to its amphiphilic nature. The oil in water emulsion method is also a very popular method of producing curcumin nanoparticles [54–56]. This method strictly relies on the homogenization speed, number of cycles and pressure. A recent method subjected medium chain triacylglycerols (MCT) as oil and Tween 20 as emulsifier to high speed homogenization at 24,000 rpm, pressure of HP1500 for 40 cycles, and produced a mean droplet size of ~ 79.5 nm from 618.6 nm [57].

Micelles (di- or tri-block copolymers), liposomes and phospholipids are considered to be excellent drug delivery carriers because of their hydrophilic and hydrophobic units which bind curcumin through self-assembly process [56, 58–61]. Sou et al. [62] coined a new technique to assemble 40 mol% curcumin together with poly(ethylene glycol)-cholesteryl ether (PEG-Chol) (10 nm) which creates a highly stable formulation that can used in an injectable form. Curcumin and these micelles can cooperatively damage the cancer cells. Such type of carriers can improve the gastrointestinal absorption of curcumin and thus increase the curcumin levels in plasma [63, 64]. A recent study designed novel nanosized liposomes functionalized with curcumin through a conventional click chemistry technique [59]. A liposomal patented technology with various compositions has been identified for cancer therapeutics which exhibits very similar anticancer potential as free curcumin in BxPC-3, Capan-1, Capan-2, and HS766-T cells [65]. Phospholipid bilayers loaded with a curcumin formulation stabilized by apolipoproteins form a disk shape nanostructure (nanodisk) with a diameter <50 nm. This disk formulation showed enhanced cell growth inhibition in HepG2 and mantle cell lymphoma cells [66, 67]. Nanoplex is another type of self-assembly method to insert curcumin molecules in polyelectrolyte layers through electrostatic interactions. Biocompatible poly(allylamine hydrochloride) and dextran sulfate can form ternary phase diagrams of the curcumin-polymer salt complexes with ~80–85% curcumin loading (~300–500 nm). These complexes remain in an amorphous form and increase solubility up to 9-fold [68]. A soft gel-like formulation has been developed in a similar fashion as a layer by layer approach to embed natural polyphenols [69]. However, this sandwich approach involves the use of several polymers including poly(styrene sulfonate), poly(allylamine hydrochloride), poly(glutamic acid), poly(L-lysine), dextran sulfate, protamine sulfate, carboxymethyl cellulose, and gelatin.

Recently, approaches to prepare curcumin nanoformulations based on nanoprecipitation have received much attention [70, 71]. Such methods utilize three steps, i.e., nucleation, condensation, and coagulation, to achieve smaller particles. Curcumin nanoprecipitates with uniform size can be achieved by adjusting initial drug concentrations. For example, curcumin/ethanol solution (0.2–2 g/l) nanoprecipitation quickly formulates with particle size ranging from ~ 450 to 210 nm [70]. Using higher concentrations of curcumin in ethanol solution procures lower particle size. Anionic copolymers based on methacrylic acid and methyl methacrylate (EU-DRAGIT® S 100, Evonik Industries) modified particles have been widely investigated to improve colon-specific drug delivery of curcumin [72–76]. Dev et al. [77] developed a scalable process to obtain curcumin nanoparticles smaller than 50 nm using a continuous flow microfluid rotating tube processor. These formulations are stabilized by dimethyldioctadecyl ammonium bromide (DDAB) and Pluronic F127 polymer which enhances penetration through their higher cationic nature into cancer cells. In another method, 60–100 nm curcumin nanocrystals were obtained by the addition of polyelectrolyte under ultrasonic condition [78]. These polyelectrolyte layers control the drug release from nanoparticles. Many other important curcumin nanoformulations are provided in Table 1.
3.1. Curcumin Encapsulation and Release Characteristics

Curcumin drug loading is highly associated with the type of nanoparticle and preparative method used (Table 2). The drug loading can be determined by encapsulation efficiency which provides the percentage of drug added to the formulation that exists within the nanoformulations. Some estimation methods involve separating the curcumin nanoparticles from the medium and then quantifying the un-entrapped or unbound fraction of curcumin, giving an indirect quantification of curcumin encapsulated in the nanoparticles [52]. The most frequently utilized estimation method is breaking nanoparticles in organic solvent which results in an accurate curcumin encapsulated quantification [41, 50, 98]. It is interesting to note that many curcumin nanoformulations previously reported has achieved a loading capacity up to 25 wt./wt.% with 70–99% encapsulation efficiency. Based on the polymer structure, drug nature and their interactions by 3D molecular modeling drug loading information can be quickly predicted, minimizing not only the cost of the entire process but also reducing the time required for the optimization process [99]. The amount of curcumin released from nanoparticle formulations is important since the amount of curcumin release in its active form is responsible for the therapeutic effect. Therefore, complete drug loading information and release profiles of curcumin nanoformulations has been presented in Table 2. A curcumin encapsulated solid lipid nanoparticle demonstrates a simple Higuchi’s square root model up to 12 h [100]. The release profile of poly(butylcyanoacrylate) nanoparticles illustrate 34.74% in 2 h followed by a sustained release. According to the calculated two phases kinetics equation: 100 − Q = 4.5235e(−0.1724t) + 4.1641e(−0.0114t) [101]. Most of the nanoformulations follow in vitro release of curcumin in a biphasic pattern. Curcumin sustained release profile may vary depending on the type of nanoformulation, composition, location of entrapment and amount. One study suggests that the micro environment (in artificial gastric juice at pH 2.0 and in artificial intestinal juice at pH 7.4) drastically affects the release profile [102]. Although, initial release in 8 h does not vary much but 7 day sustained release from the nanoparticles found ~77% in the intestinal juice and 48% in artificial gastric juice. In some cases, the mechanism of release depends on various physical and chemical environmental conditions. Ultrasound is a powerful noninvasive modality for biomedical imaging, and holds great promise for noninvasive drug delivery enhancement and targeting. The release of curcumin entrapped in microemulsion droplets (20–35 nm) can be regulated by ultrasound frequency (40 kHz) and curcumin loading capacity [94]. Similarly, curcumin release from nanogel formulations can be controlled by diffusion and stimuli-responsive approaches [15, 52, 103, 104]. In addition, a magnetic field also promotes the release of curcumin from the formulations and thereby exhibits immediate therapeutic effects [105]. The controlled release of curcumin nanoformulations can promote accumulation of the drug in tumor(s) and slow release enhances the therapeutic outcome.

3.2. Cellular Uptake

There is a clear correlation that increased blood circulation time and accumulation of nanomedicine in target tissues improve therapeutic effects compared with free drugs. A stable nanoformulation can be determined by its cellular uptake which is one of the important parameters for drug delivery applications. Like any other nanoparticle mediated drug delivery system, curcumin nanoformulations also promote the uptake of tumor or cancer cells in passive targeting due to “Enhanced Permeation and Retention” (EPR) effect. Table 2 illustrates the preferential uptake of curcumin nanoformulations in various cancer cells. Similarly, some of these formulations show decreased uptake in macrophage or normal cells which suggests the reticuloendothelial system (RES) clearance of nanoparticles is avoided [51, 98, 106, 107]. Such a selective and improved intracellular accumulation or uptake of curcumin nanoformulations in cancer cells is an indication for a higher therapeutic index.

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The extent of cellular uptake of curcumin depends on the type of nanocarrier, particle size, surface charge, and cell line. For example, polyvinyl alcohol (PVA) coated PLGA nanoformulations of curcumin whose particle size varied between ~ 560 to 76 nm (6 formulations) have shown distinctly different uptake patterns [41]. The uptake is continuously increased with a decrease in particle size. This is evidence that low particle size is more easily and highly endocytosized than higher particle size. Additional coating of poly(L-lysine) (PLL) on these nanoparticles further increase the uptake due to positive charge which helps in penetrating inside the cells. Numerous reports of various drug nanoformulations support this phenomenon. Researchers [51, 107] have demonstrated that a chitogen based nanocarrier enhances the internalization in MCF-7 and PC-3 cancer cells as time increased from 1 h to 48 h. The curcumin levels are increased from 0.2 to 0.8% absorbance in UV-vis spectral study. We also learn that the uptake by various cancer cells is quite different and vary formulation to formulation [50, 108]. In a comparative study, PLGA, cellulose, β-cyclodextrin (β-CD), nanogel and dendrimer nanoformulations of curcumin were evaluated for uptake in SKBR-3, MDA-MB-231 (breast), and HPAF-II (pancreatic) cancer cells [50]. The order of uptake was found as MDA-MB-231 > SKBR-3 > HPAF-II. It is important to note that curcumin uptake through nanoformulations is at least 2–3 fold greater than free curcumin. In another comparative cellular uptake study, free curcumin diffuse across the melanoma cell membrane and observed fluorescence presence in cytoplasm, localization in the peri-nuclear region and microfilament displacement suggests curcumin interaction with cytoskeleton proteins [109]. When these melanoma cells were treated with magnetite nanoparticle the fluorescence intensity was lower. The reason was that curcumin inside the hydrophobic bilayers of the nanoparticles quench the fluorescence property. However, the targeting specificity of curcumin nanoformulations towards cancer cells can be improved via antibody, peptide, penetrating ligand or aptamer conjugation [15, 110].

After exposure of curcumin nanoformulations to cancer cells, many nanoparticles were localized in the cytoplasm and inside or around the nucleus [41, 51, 98, 107, 108]. Longer periods of exposure drastically changed the morphology (e.g., cell lysis and loss of spindle shape) of cancer cells and observed cell debris. This type of behavior is highly dependent upon typical physico-chemical properties such as amorphous, crystalline, particle size and morphology and surface charge of the nanoformulations. To study an effective internalization process of curcumin nanoformulations fluorescence or confocal microscope is commonly employed. These methods utilize the inherent fluorescence property of curcumin, however, it is interfered with some type of nanoparticles in some cases. Recent investigations also rely on transmission electron microscopy to further validate curcumin nanoformulations cellular uptake (internalization) [41, 51, 98, 107, 108]. These investigations provide clear evidence of the presence of nanoparticle internalization at higher magnification. Another convenient method established for the determination of cellular uptake is Prussian blue staining. This method provides both the qualitative and quantitative uptake of iron oxide based curcumin nanoformulations. The uptake of magnetic nanoparticles-curcumin (MNP-CUR) can be viewed via an accumulation pattern of nanoparticles; the accumulation increases as the MNP-CUR concentration increases. The internalized particles are localized in almost every cell and throughout the cell components. This type of nanoformulation showed very minimal uptake by macrophages which supports increased circulation time for a more effective therapy [98, 106]. It is also possible to improve the internalization capacity of this type of nanoparticle by a ligand/antibody/penetrating peptide [110].
3.3. Anticancer Properties

Anticancer properties of each curcumin nanoformulation depend on the mechanism of specific accumulation or affinity of released curcumin in cancer cells [15, 20, 44, 113]. The activity of the same formulation may vary in different cancer cell lines. For instance, the mPEG2000–curcumin conjugate formulation is active against Caco-2 (colon), KB (oral cavity), MCF-7 (breast), and NCI-H187 (lung) with IC_{50} values in the range of 1–6 μM, similar to that observed for curcumin itself [114]. The treated cells were much smaller in size when compared with untreated cells and had lost intercellular adhesion.

- Maitra and collaborators have developed a promising formulation (NanoCurc™) that can be implemented for future clinical translation in pancreatic and brain cancer(s) [52, 115–117]. Their initial study suggests NanoCurc™ *in vitro* efficacy and mechanisms of actions mirror that of free curcumin [52]. NanoCurc™ not only increases the bioavailability of curcumin in plasma and tissues but inhibits tumor growth in xenograft and athymic mice models of human pancreatic cancer [117]. It is interesting to note that tumor growth stops completely when NanoCurc™ was injected along with gemcitabine. These superior inhibiting effects were attributed to the attenuation of nuclear factor-kappaB (NF-κB) activity, reduction in expression of matrix metalloproteinase-9 and cyclin D1. This formulation has also shown a dose-dependent decrease in growth of multiple brain tumor cell lines: DAOY, D283Med (embryonic), HSR-GBM1, and JHH-GBM14 (glioblastoma neurosphere) [116]. The inhibition of cell growth is related to down-regulation of the insulin-like growth factor pathway and loss of STAT3. NanoCurc™ (5 mg/kg) localized up to 0.5% of formulation which is similar to when PLGA-curcumin or liposomal curcumin (20 mg/kg) is employed [115].

Nanocarriers made from ethylcellulose (EC) and methylcellulose (MC)/EC (ECMC) (a blend carrier) readily release curcumin into blood circulation by adhering to stomach mucosa. This property was initially detected using scanning electron microscopy analysis with *in vivo* experiments. These formulations have shown dose-dependent activity in MCF-7 and HepG2 hepatoblastoma cells [118]. Further, these curcumin nanoformulations were also applied in the form of lotions (oil in water, water in oil) which preferentially penetrated into porcine skin better than the water nanosuspensions [119]. Jayakumar group has established a novel formulation of curcumin loaded thermoresponsive chitosan-ε-poly (N-vinylcaprolactam) nanoparticles which exhibit extreme toxicity to MCF-7, KB and PC-3 cancer cells [51]. These specific attributions are related to the loss of mitochondrial membrane potential. It was noticed in many studies that nanocurcumin formulations are effective in down regulating pro-survival proteins, up regulating pro-apoptosis, activating caspases (3,7,8, and 9), and cleaving Poly (ADP-ribose) polymerase (PARP). This implies induction of apoptosis in cancer cells. Recent studies published from our group have demonstrated similar apoptosis characteristics with PLGA, CD assembly, cellulose, magnetic and dendrimer nanoformulations of curcumin [41, 50, 87, 106, 108, 120].

Excessive lysosomal activity or production of vacuoles is responsible for active apoptosis induction in cancer cells by curcumin nanoformulations as demonstrated by transmission electron microscopy (TEM) analysis [50, 108]. This activity is infrequently observed with free curcumin. The primary reason for greater apoptosis is that curcumin nanoformulations internalize in cancer cells by endocytosis and escape from the phagocytosis, which may result in the release of curcumin in active form which then efficiently acts on cancer cells, Chen et al. [121] have also verified this phenomenon with magnetoplasmonic nanoparticle-loaded drug formulations for such biological activity in HL60 cells. Their TEM results suggest clear characteristics of apoptosis such as blebbing, pyknosis, and damage of cell structure. This phenomenon most likely occurred due to the transport of drug to the nucleus of the cell which induced the activity of the telomerase. A core-shell curcumin-loaded
nanoparticle generated by amphiphilic methoxy polyethylene glycol-poly(caprolactone) (mPEG-PCL) block copolymers has shown similar effects in a rat C6 glioma cell line [122]. Curcumin encapsulation in a chitosan (CS) and silk fibroin (SF) blend polymer showed significantly lower IC₅₀ than SF-encapsulated curcumin Her2/neu (low and high) expressing breast cancer cells [96].

Numerous curcumin nanoformulations have exhibited very similar anticancer potential compared to free curcumin [42, 60, 62, 122, 123]. This can be explained by the release property of nanoformulations. Many formulations release curcumin in a sustained manner over a period of 15–30 days. In vitro cytotoxicity studies investigate the proliferation of cells in 2, 4 or 5 days. During this time curcumin release from the formulation is 1/3 that of free curcumin, yet nanoformulations still exhibit equivalent or slightly greater anticancer potentials. However, their improved efficacy can be observed in long term experiments (such as colony formation) [41, 62, 87, 88, 98] as well as in animal models [61, 117, 124–126]. Cationic poly(butyl) cyanoacrylate nanoparticles coated with chitosan mediated the release of curcumin efficiently which inhibited tumor growth and tumor angiogenesis [127]. Similarly, dendrosomal curcumin significantly reduced the tumor burden in BALB/c mice models in comparison with void curcumin and control samples. Additionally, this formulation increased the splenocyte proliferation and IFN-γ production and decreased IL-4 production [47]. Liposomal-cyclodextrin formulation of curcumin promoted autophagic cell death and is highly suitable to treat mesenchymal and epithelial origin cancers [128]. A complex of human serum albumin and curcumin not only transports 7.7-fold more curcumin than free curcumin but also confirms greater therapeutic effect, i.e., up to 66% tumor growth inhibition [129]. A recent treatment modality using curcumin nanoparticles enhanced the tumor reduction of HepG2 tumor xenografts, via decreased expression of Vascular endothelial growth factor (VEGF) as well as COX-2 [127]. Curcumin nano-disks (disk-shaped phospholipid bilayer formulations) demonstrated a dose-dependent increase in apoptosis through enhanced Fox03a and p27 expression, caspase-3, -9, PARP cleavage, and decreased cyclin D1, pAkt, and Bcl2 protein [67]. A recent formulation composed of cationic liposome, PEG and PEI complex exhibited 5 and 20-fold increases in the cytotoxic potential against curcumin-sensitive cells and curcumin-resistant cells, respectively [130]. This formulation is capable of inhibiting tumor growth 60–90% in mice bearing CT-26 or B16F10 cells.

Most of the tabulated formulations report that curcumin nanoparticles follow the passive targeting mechanism (Table 3) rather than the active targeting. Passive targeting is a key property of curcumin nanoparticles and this property promotes the accumulation in tumor(s). Passive targeting may depend on a few important parameters such as particle size, zeta potential, and solubility or dispersion of nanoparticles (Fig. 3). Nanoformulations with an optimal size only exhibit EPR effect which in turn increase levels of accumulation in tumor. Additionally, a hydrophilic coating with poly(ethylene glycol) reduces the protein-protein/cells interaction and thereby minimizes the opsonization process.

Folic acid (FA) is a well-known small molecule that binds to folate-receptors and facilitates receptor-mediated endocytosis in a variety of cancer cells and tumors. An optimized formulation of folate conjugated microemulsion (31.1 ± 0.99 nm) comprised of 57.5% Cremophor EL, 32.5% Transcutol, and 10% Capryol 90, increases the percentage of curcumin absorption from 58.41±7.26 to 73.38 ± 3.12 in the colon of rats [131]. Furthermore, this formulation efficiently targets HeLa and HT-29 cancer cells compared to plain curcumin and curcumin loaded microemulsions. A curcumin-loaded magnetic nanoparticle formulation with transferrin ligand exhibits active targeting of K562 cancer cells (myeloid leukemia) [131]. The active targeting of these curcumin nanoparticles results in significant down-regulation of the Bcr-Abl protein that effectively operates an intrinsic
apoptotic mechanism in myeloid leukemia cancer cells. Transferrin-mediated solid lipid nanoparticles demonstrate selective enhanced anticancer activity against MCF-7 breast cancer cells. This increased activity is due to increased cellular uptake, loss of mitochondrial membrane potential, and generation of excessive reactive oxygen species (ROS) [134]. A composite of PVP and hyaluronic acid (HA) curcumin formulation (six double layers) increased the hyaluronic acid receptor-mediated endocytosis to target cancer cells (glioma cells and Caco-2 cells) [135]. Additionally, this strategy also utilizes magnetic property to enhance the internalization. Manju and Sreenivasan [136] demonstrated enhanced efficacy of HA-conjugated curcumin with folate conjugated gold nanoparticles in HeLa cells, glioma and Caco 2 cells. Similarly, curcumin nanoformulations conjugated with Tet-1 peptide [79], apotransferrin [137], and apolipoprotein E (ApoE)-derived peptide [138] have improved the therapeutic value of curcumin.

A recent pre-clinical study reported for the first time using a targeted Prostate-specific membrane antigen (PSMA) nanoparticle containing the chemotherapeutic docetaxel in patients with solid tumors [139]. This formulation was developed from a combinatorial library of more than 100 compositions varying in particle size, drug loading and release, targeting efficiency and surface modifications. This further supports the premise that effective curcumin targeted nanoformulations can be developed for treatment of prostate cancer [15, 20]. Monoclonal antibody mediated delivery would improve targeting and binding efficacy to cancer cells which would significantly improve the curcumin anticancer activity. A number of monoclonal antibody conjugation techniques already exist for this purpose [32, 41, 98, 106, 133, 140]. It is anticipated that by selectively choosing particle size, zeta potential, stability and targeting moiety, curcumin nanoformulations can be targeted to specific cancer cells (Fig. 4).

3.4. Reversal of Multi Drug Resistance

Drug resistance or multidrug resistance is a phenomenon whereby tumor cells become resistant to primary anticancer drugs. Curcumin is known to sensitize cancer cells to chemo/radiation therapies [141]. Therefore, curcumin nanoformulations will have great therapeutic impact in cancer treatment. In our study, curcumin pre-treatment effectively induced chemo/radio-sensitization and considerably reduced the effective dose of cisplatin and radiation to inhibit the growth of cisplatin resistant ovarian cancer cells (A2780CP) [120]. This property can be induced more effectively using Nano-CUR with antibody conjugation capability. Co-encapsulated curcumin (CUR) and doxorubicin (DOX) in poly(butyl cyanoacrylate) nanoparticles prompted the highest drug resistance reversal and down-regulation of P-glycoprotein expression in MCF-7/ADR cell lines [142]. A new attempt has been made to leverage the therapeutic benefits of PLGA-CUR formulation in rats [143]. This study illustrates that a hypoxia condition considerably reduces the particle endocytosis and localization thereby lower tissue levels of curcumin are required compared to normoxic conditions. Such phenomenon can be altered by surface modification of nanoparticles. Similarly, curcumin and doxorubicin co-encapsulation in a lisosomal formulation supports the greater in vitro anti-tumor activities against A549 cells compared with that of free DOX [126]. A combined CUR/DOX nanoformulation would also facilitate the retention of DOX in the nucleus for a longer period of time as well as inhibit the expression of MDR1 and BCL-2 at the mRNA level in K562 cells. It is also true that when co-administered, curcumin and paclitaxel nanoformulations open up the drug resistance in cancer cells [144–146]. SKOV-3(Tr) human ovarian adenocarcinoma cells showed less growth with combination treatment and this co-therapy successfully inhibited the NF-κB activity and down regulated P-glycoprotein [144]. These study results demonstrate an enhancement of paclitaxel available for therapy (5.2-fold) and such approach results in no acute toxicity with better therapeutic outcomes in ovarian adenocarcinoma [145]. Another group proved that this...
approach greatly inhibits the cervical cancer growth in a xenograft mouse model. This higher therapeutic index was achieved due to down regulating the antiapoptotic factors downstream signaling of survival signals [146].

3.5. Pharmacokinetics and Bioavailability

Bioavailability is one of the key pharmacokinetic properties of a drug molecule. This behavior mainly depends on the solubility, stability, metabolism, and degradation of drug molecules. Drug bioavailability follows the administration route: intravenous > intramuscular > subcutaneous > oral > rectal > inhalation [147]. Bioavailability of curcumin indicates the extent of active compound that reaches the systemic circulation which is readily available at the site of action. Extensive research on in vivo investigations of curcumin nanoformulations is still limited. For the first time, Shaikh et al. proved that PLGA-curcumin nanoparticles are safe and beneficial in several ways. In vivo pharmacokinetics demonstrated a 9-fold increase in oral bioavailability compared to a curcumin combination (curcumin with piperine) [148]. Curcumin oral bioavailability was significantly improved with different compositions of various formulations using stabilizers or adjuvant [149]. For this experiment, 250 mg/kg equivalent curcumin was administered using oral gavages to male SD rats. The order of bioavailability was found to be: curcumin formulation of milk > aqueous suspension > micronized suspension > piperine > nanosuspension ≥ amorphous solid dispersion > inclusion complex (HP-β-CD). 200–500% enhancement of the curcumin area under the curve (AUC0-t) and maximum concentration (Cmax) was observed with the nano-suspension and inclusion complex (HP-β-CD). A brief summary of important formulations that significantly improved the pharmacokinetics and bioavailability is available in Table 4.

The hemo-compatibility is an index for therapeutic formulations that are immediately exposed upon administration in blood. Accessing their hemo-compatibility in animal or human blood would enhance translation of curcumin formulations from “bench to bed site”. A recent study suggests that PLGA, CD, cellulose, nano-gel, and dendrimer based curcumin formulations did not show any erythrocytes damage or occurrence of thrombus [50]. Similar observations were made with intravenous PLGA nanosuspensions, curcumin conjugated nanoparticles [149–153], gold- curcumin nanoparticles, and a layer-by-layer self-assembly curcumin formulation [115, 135, 136, 154]. Rejinold et al. [155] demonstrated the biocompatibility nature of a thermosensitive curcumin formulation by hemolysis assay. The hemolytic ratio of this formulation was determined to be 2.155% which is in the acceptable range for therapeutic applications. Approximately 5% is considered as the maximum safe hemolytic ratio for bio-materials according to ISO/TR 7406.

3.6. Challenges to Curcumin Nanopharmaceuticals

This section provides some key information on how curcumin nanoformulations can be utilized as pharmaceuticals. The first logical question to ask is whether the pharma industry has an interest to expand curcumin nanoformulations since curcumin is very inexpensive. The next question is which type of preparative method should be followed to obtain a more effective formulation with ensured reproducibility. We provide a schematic layout which proposes the basis upon which curcumin nanoformulations can be selected for future clinical application or clinical trials (Fig. 5). Liposomal formulations of drugs (Doxil, Myocet, Ambisome, and Depocyt), contrast imaging agents (gadolinium and iron oxide nanoparticles), PLGA formulations of paclitaxel (Abraxane), nanocrystal technology, nanomorph, nanoedge, nanopure, crititech and nanocochleate technologies are currently available in the market. Curcumin formulations developed by following these principle technologies would benefit from obtaining early approval from the FDA provided evident appropriate science, characterization tools, purity, stability, toxicity, safety profiles along

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with benefit to human health. However, curcumin formulations are considered to be as Abbreviated New Drug Applications (ANDAs) or New Drug Applications (NDAs). FDA is also authorized to inspect and examine records to nanotechnology, monitor the post-market safety and identify adverse events reporting. Based on such criteria FDA can pose ban if it is necessary.

Our laboratory is interested in identifying hybrid nanocurcumin formulations that can be applied for multi-functional applications in cancer therapeutics. Currently, we have developed theranostic curcumin nanoparticles that combine therapy and diagnosis in one platform [98, 105]. Such type of nanoformulation allows loading therapeutic drug(s), biomacromolecule(s) and diagnostic agents and provide not only real-time monitoring of therapeutic outcome but also offers stimuli therapeutic strategies (Fig. 6). Overall this review highlights important contributions and issues associated with curcumin nanoformulation translations for clinical use in the future. It also provides enormous opportunity for implementation of nanotechnology in curcumin delivery to cancer cells efficiently. Evidence of superior anticancer properties exist for all the strategies but further developments of these curcumin nanoformulations should follow commonly employed good laboratory and manufacturing practice (cGLP and GMP) using FDA approved compounds. Suitable curcumin nanoformulations can then be chosen based on appropriate priorities established for both the development of nanotechnology and subsequent therapeutic application.

4. CONCLUSION

Curcumin showed excellent anticancer properties yet its inherent poor solubility, higher metabolic activity and poor pharmacokinetics properties hamper its ability to emerge as a potent medicine for cancer. In addition, since curcumin is a natural compound, there would be some regulatory and intellectual property right issues in regard to using curcumin as a drug. However, through developing proper formulations, i.e., nanoformulations are possible to get approval. Nanoparticle technology of curcumin is one of the frontier areas in medicine which will improve human health care. Interest in this area has been emerging worldwide over the last few years. Curcumin nanoformulations may offer numerous advantages including improved efficacy, tumor targeting, reduced systemic toxicity, compliance and convenience. A simplified and standardized approach is necessary to obtain curcumin nanoformulations. The process should not be extensive which would make the formulation costs minimal. In this perspective, curcumin nanoformulations based on cyclodextrin assembly, PLGA and magnetic nanoparticle formulations are highly appropriate. Oral and intraperitoneal dosage nanoformulations are more preferred which reduces patient visits and also the cost burn enormously. Future pre-clinical and clinical investigations are required to gain in depth information about curcumin nanoformulations to translate as drug candidates to treat cancer(s) alone or in combination with other therapeutic modalities.

Acknowledgments

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References


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Fig. 1.
Commercially available and proposed curcumin formulations to improve bioavailability and activity.
Fig. 2.
Basic and clinical significance of curcumin and nanocurcumin formulations in the field of medicine over a ten-year period. The number of peer-reviewed publications was collected using PubMed (data was collected for the 10 year period from Jan. 2001–Dec. 2011).
Fig. 3.
Various clearance mechanisms of curcumin nanoformulations based on their physico-chemical properties. Note: Reticuloendothelial system (RES) is an older term for mononuclear phagocyte system.
Fig. 4. Schematic representation of targeted approach of curcumin nanoformulations for cancer treatment.
Fig. 5.
Schematic flow chart delineates the step by step process for the selection of curcumin nanoformulations for clinical applications.
Fig. 6.
Magnetic curcumin nanoformulations for theranostic and multi-functional applications.
### Table 1

Various approaches to Prepare Curcumin Nanoformulations, their Composition, and Particles Evaluation

<table>
<thead>
<tr>
<th>Curcumin Nanoformulation</th>
<th>Method/Technique of Preparation</th>
<th>Composition</th>
<th>Particle Size (nm) and Zeta Potential (mV)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>Solid/oil/water (S/O/W) technique</td>
<td>30 mg of PLGA polymer, 2% poly(vinyl alcohol) (PVA) and ethanol (1:1) solution, and curcumin 0.5–2 mg</td>
<td>30–50 nm (TEM) ~ 100 nm (Confocal microscopy)</td>
<td>[42, 43]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Single emulsion–solvent evaporation</td>
<td>200 mg of PLGA in 2 ml of ethyl acetate, 20 mg of curcumin, 4 ml of PVA (5%/v), and 100 ml of PVA (0.3%/w/v)</td>
<td>150–200 nm (TEM and SEM) ~30 to ~20 mV (DLS)</td>
<td>[79]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Nanoprecipitation</td>
<td>PLGA–PEG (100 mg), drug (5 mg), and acetonitrile (10 mL) in the presence of 0.1% Pluronic F8</td>
<td>25–75 nm (SEM) ~24.2 mV (DLS)</td>
<td>[44]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Single-emulsion/solvent-evaporation method</td>
<td>20 mg of curcumin, 4 ml of 5% w/v of PVA solution, and 100 mL of 0.3% w/v PVA solution</td>
<td>77±16 nm (SEM)</td>
<td>[80]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Single emulsion (o/w)/solvent evaporation method</td>
<td>100 mg of PLGA and 10 mg of curcumin in dichloromethane and acetone (w/v, 10:1) in the presence of 1% (w/v) PVA aqueous solution.</td>
<td>129.7±9.6nm (SEM)</td>
<td>[81]</td>
</tr>
<tr>
<td>poly(lactide)-vitamin E TPGS (PLA-TPGS) copolymer</td>
<td>Ring-opening polymerization</td>
<td>Curcumin solution in methanol was added to the solution of PLA-TPGS in dichloromethane in a polymer ratio of 1:100</td>
<td>100 to 400 nm (SEM) The small particles are 20–40 nm in size but micrometer-sized group of several clusters</td>
<td>[82]</td>
</tr>
<tr>
<td>Alginate nanoparticles</td>
<td>Alginate pre-gel nanoparticle hardening</td>
<td>Calcium chloride 7.5 ml of 18 mM and 0.063% of sodium alginate and chitosan 0.15% in the presence of Pluronic F127</td>
<td>100 ± 20 nm (SEM and AFM)</td>
<td>[83]</td>
</tr>
<tr>
<td>Soy protein nanoparticles</td>
<td>Isoelectric precipitation and diffusion</td>
<td>Soy protein isolate (SPI) (60 mg/mL) and curcumin (3 mg/mL) stock solution and curcumin/SPI ratio of 1:20, 1:50, or 1:100 (w/w)</td>
<td>200–1000 nm (DLS) depending on the ethanol and glutaraldehyde concentrations</td>
<td>[84]</td>
</tr>
<tr>
<td>Poly(vinyl pyrrolidone) (PVP) conjugate micelles</td>
<td>Chemical conjugation</td>
<td>1.5 g of PVP, 0.5 g of 4-dimethylaminopyridine, 1 mL of triethyl amine, and 100 mg of curcumin</td>
<td>22.4 nm and 20 mV (DLS) 18.94±4.35 nm (TEM)</td>
<td>[85]</td>
</tr>
<tr>
<td>α-cyclodextrin (α-CD) derivatives</td>
<td>Chemical conjugation</td>
<td>CD derivatives and their 2:1 and 4:1 complexes with Curcumin</td>
<td>In between 268±16 nm and 692±53 nm depending on the ratios of conjugates and curcumin</td>
<td>[86]</td>
</tr>
<tr>
<td>β-cyclodextrin-self assembly</td>
<td>Inclusion complexation and self-assembly</td>
<td>5, 10, 20 and 30 wt.% of curcumin in β-cyclodextrin</td>
<td>50 nm small clusters to 500 nm self-assemblies (TEM)</td>
<td>[87]</td>
</tr>
<tr>
<td>Poly(β-cyclodextrin)-self assembly</td>
<td>Inclusion complexation and self-assembly</td>
<td>5,10,20 and 30 wt.% of curcumin in poly(β-cyclodextrin)</td>
<td>Individual complex or assembly about 50 nm and clusters can reach up to 1 μm (TEM)</td>
<td>[88]</td>
</tr>
<tr>
<td>Casein micelle</td>
<td>Micelle or complexation</td>
<td>Casein (10 μM) in the presence of 0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 μM curcumin</td>
<td>166.3±33.1 nm (DLS) and the same was verified with SEM and AFM</td>
<td>[60]</td>
</tr>
<tr>
<td>Dextrin nanogels</td>
<td>Self-assembly process at 50 °C</td>
<td>DexC16 is composed of a hydrophilic dextrin backbone with grafted acrylate groups, which are partially substituted with long alkyl chains (SC16).</td>
<td>61.1 nm in water and 59.2 in PBS solution (DLS) (freshly prepared samples)</td>
<td>[89]</td>
</tr>
<tr>
<td>Curcumin Nanoformulation</td>
<td>Method/Technique of Preparation</td>
<td>Composition</td>
<td>Particle Size (nm) and Zeta Potential (mV)</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------------</td>
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<tr>
<td>Thermosensitive polymer nanoparticles</td>
<td>Redox-free radical polymerization</td>
<td>1.8 g monomer, cross-linker (N,N′-methylene bisacrylamide), 100 mg PEG-ester, initiator/activator and curcumin 20 wt% loading</td>
<td>~ 122 nm and -1.46 mV (DLS) [50, 90]</td>
<td></td>
</tr>
<tr>
<td>Thermosensitive polymer nanoparticles</td>
<td>Free-radical polymerization</td>
<td>Curcumin (5 mg in 0.1 ml ethanol) and polymer (chitosan-PNIPAM, 50 mg in 5 ml 1% acetic acid) with 100 µl 0.05% TPP solution</td>
<td>100–300 nm (DLS) SEM analysis of curcumin loaded TRC-NPs revealed a size range of 180–220 nm [51]</td>
<td></td>
</tr>
<tr>
<td>O/W nanoemulsions</td>
<td>High-pressure homogenization</td>
<td>Medium chain triacylglycerols (oil), tween 20, and curcumin</td>
<td>79.5–174.3 nm (DLS) [57]</td>
<td></td>
</tr>
<tr>
<td>Sub-micrometer dispersions</td>
<td>Moschwitzer’s method by high-speed homogenization</td>
<td>Curcumin suspensions in water (1%) were subjected to premilling treatments to reduce curcumin particle sizes to the micrometer range according to Moschwitzer’s method by high-speed homogenization at pressure levels ranging from 50 to 200 MPa and for up to 40 HPH cycles</td>
<td>2000, 1000–600 nm (SEM) [91]</td>
<td></td>
</tr>
<tr>
<td>Self-emulsifying drug delivery system</td>
<td>Self-emulsification</td>
<td>57.5% surfactant (emulsifier OP: Cremorphor EL, 1:1), 30% co-surfactant (PEG 400) and 12.5% oil (ethyl oleate). It improves curcumin solubility to 21 mg/g</td>
<td>~ 3.3 nm (DLS) [56]</td>
<td></td>
</tr>
<tr>
<td>Nanoprecipitation</td>
<td>Syringe driven filter nanoprecipitation</td>
<td>Curcumin/ethanol solution (0.2, 0.4, 0.8, 1.6, and 2.0 g/l)</td>
<td>The nanoprecipitate first formed as amorphous 30–40 nm nanoparticles, then their amorphous aggregates (~140 nm after 10 min and ~ 200 nm after 90 min), and finally became dendritic aggregates of needle-shaped curcumin crystals (SEM) [71]</td>
<td></td>
</tr>
<tr>
<td>Nanoprecipitation</td>
<td>Droplet controlled nanoprecipitation</td>
<td>Curcumin/ethanol solution (0.2, 0.4, 0.8, 1.6, and 2.0 g/l)</td>
<td>450–210 nm (SEM) [70]</td>
<td></td>
</tr>
<tr>
<td>Lipid nanospheres</td>
<td>Vesicle formation</td>
<td>Soybean oil (10 mg/ml) and DMPC:PEG-DSPE (10/1/0.06 molar ratio)</td>
<td>187±53 to 217±93 nm (DLS) [58]</td>
<td></td>
</tr>
<tr>
<td>Liposomal formulation</td>
<td>Curcumin decoration on liposomes using click chemistry</td>
<td>Dipalmitoylphosphatidylcholine/Chol/2:1 liposomes incorporating 10–20% curcumin conjugate</td>
<td>52.8±5.5 to 207.2 ± 8.0 with zetapotential between -7.6±1.7 and -24.3±1.7 mV depending on the liposome modifications (DLS) [59]</td>
<td></td>
</tr>
<tr>
<td>Superparamagnetic silica reservoirs</td>
<td>Composite</td>
<td>Fe3O4, nanoparticles (37% wt) and curcumin (30% wt) into the porous silica matrix</td>
<td>Fe3O4 core diameter 7.13 nm (variance = 1.89 nm) curcumin shell 2.59±0.07 nm (SAXS) Curcumin and Fe3O4 nanoparticles containing silica particles were ellipsoidal in shape and the size of the particles ranged from 200 nm to 1 µm. [92]</td>
<td></td>
</tr>
<tr>
<td>Magnetic nanoparticles</td>
<td>Nanoparticle coating with stabilizer or polymers</td>
<td>Fe3+/Fe2+ ratio of 2:1, chitosan or oleic acid</td>
<td>300 nm and 500 nm (DLS and TEM/SEM). [93]</td>
<td></td>
</tr>
<tr>
<td>Magnetic poly(lactic acid) microspheres</td>
<td>Oil-in-water emulsion</td>
<td>1% (w/v, 50 ml) of PVA, Fe3O4 nanoparticles (5 mg), PLA (50</td>
<td>0.55 to 0.75 (µm (DLS and SEM) [94]</td>
<td></td>
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</table>

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<table>
<thead>
<tr>
<th>Curcumin Nanoformulation</th>
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<th>Particle Size (nm) and Zeta Potential (mV)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Hollow capsules</td>
<td>Layer by layer assembly</td>
<td>Melamine formaldehyde templates coated with six double layers of poly(sodium 4-styrene sulfonic acid) and poly(ethylene imine) and 4.5 mg/mg of microcapsules</td>
<td>2.2 to 2.8 μm (DLS)</td>
<td>[95]</td>
</tr>
<tr>
<td>Silk fibroin and chitosan blend</td>
<td>Capillary microdot technique</td>
<td>Silk fibroin: chitosan with compositions of 100:0; 25:75; 50:50; 75:25</td>
<td>&lt;100 nm (TEM) 50:50 SFCS (130 ± 4.2 nm) (TEM)</td>
<td>[96]</td>
</tr>
<tr>
<td>Dendrosome</td>
<td>Diffusion</td>
<td>Dendrosome and curcumin ratio 25:1</td>
<td>200–500 nm (UV-microscope)</td>
<td>[47]</td>
</tr>
<tr>
<td>Albumin nanosuspension</td>
<td>Solvent evaporation</td>
<td>Not available</td>
<td>245.2 nm (DLS)</td>
<td>[97]</td>
</tr>
</tbody>
</table>

AFM - Atomic force microscopy; DLS - Dynamic light scattering method; DMPC - l,2-Dimyristoyl-sn-glycero-3-phosphocholine; PEG-DSPE - l,2-distearoyl-sn-glycerol-3-phosphoehanolamine-N-[monomethoxy poly(ethylene glycol); PLA - Poly(lactic acid); PVA - Poly(vinyl alcohol); SA - L-glutamic acid, N-(3-carboxyl-l-oxopropyl)-, 1,5-dihexadecyl ester; SAXS – Side angle X-ray spectroscopy; SEM – Scanning electron microscopy; TEM - Transmission electron microscopy.
<table>
<thead>
<tr>
<th>Curcumin Nanoformulations</th>
<th>Curcumin Loading</th>
<th>Curcumin Release</th>
<th>Uptake/Internalization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>2 mg loading in 30 mg batch of PLGA formulation with 90.88% encapsulation efficiency</td>
<td>10-13% release was observed within 1 hour and then a sustained curcumin release of about 65% was noted for 10 days</td>
<td>Robust uptake in DU145, PC-3, and LNCaP cells</td>
<td>[42]</td>
</tr>
<tr>
<td>PLGA</td>
<td>4 μg/mg of particles encapsulated with 97.5% encapsulation efficiency</td>
<td>Not available</td>
<td>4.5 to 1 fold change with curcumin and 4.9 to 1.4 fold change with nano-curcumin formulation in 0 to 60 min incubation in KBM-5 cells</td>
<td>[44]</td>
</tr>
<tr>
<td>PLGA</td>
<td>7.6 w/w% loading</td>
<td>A biphasic release profile is observed with an initial burst release during the first several hours followed by a sustained uniform release (~65% of curcumin release in 20 days)</td>
<td></td>
<td>[80]</td>
</tr>
<tr>
<td>Dendrosome</td>
<td>4 w/w% loading</td>
<td>Not available</td>
<td>6 fold increased uptake found in A431 cancer cells by dendrosomal curcumin formulation</td>
<td>[47]</td>
</tr>
<tr>
<td>Lauroyl sulphated chitosan</td>
<td>Encapsulation efficiency and drug loading content were 50.3% and 9.31%, respectively</td>
<td>16 mg release in 30 days and 82% stable curcumin in chitosan-CUR formulation for 30 day</td>
<td>Uptake similar to free curcumin in Caco-2 cells was observed</td>
<td>[111, 112]</td>
</tr>
<tr>
<td>Alginate-chitosan pluronic composite nanoparticles</td>
<td>5–10 fold increase in encapsulation in the presence of Pluronic polymer</td>
<td>36% in 12 h, 51% in 24 h and 96 h about 75% of curcumin determined</td>
<td>No significant difference in uptake detected between curcumin and curcumin nanoformulation</td>
<td>[83]</td>
</tr>
<tr>
<td>Curcumin/mono-methoxy poly(ethylene glycol)-poly(ε-caprolactone) (MPEG-PCL)</td>
<td>5-25 % by weight loading with &gt; 97% encapsulation efficiency</td>
<td>About 54.6% curcumin release found in 9 days</td>
<td>Not available</td>
<td>[61]</td>
</tr>
<tr>
<td>β-cyclodextrin self-assembly</td>
<td>Loading content in the range of 6.17–26.21 by weight of formulation</td>
<td>80% of curcumin retention in 6 days in the formation</td>
<td>2–9 fold increase was noticed in DU145 prostate cancer cells</td>
<td>[87]</td>
</tr>
<tr>
<td>Poly(β-cyclodextrin)-self assembly</td>
<td>The order of loading capacity (μg of CUR per mg of PCD) is PCD5 (48.5) &lt; PCD10 (115.2) &lt; PCD20 (163.4) &lt; PCD30 (223.2)</td>
<td>The order of stability for 72 h found to be PCD30 (~77.4%) &gt; PCD20 (~88.7%) &gt; PCD10 (~82.5%) &gt; PCD5 (~71.2%).</td>
<td>3–4 fold increased uptake of CUR was noticed in PCD20 or PCD30 treated prostate cancer cells</td>
<td>[88]</td>
</tr>
<tr>
<td>Albumin nanoemulsions</td>
<td>The encapsulation efficiency was up to 42.39 ±0.91% depending on the ratio albumin to curcumin</td>
<td>96% curcumin release in 72 h</td>
<td>Not available</td>
<td>[97]</td>
</tr>
<tr>
<td>Curcumin Nanoformulations</td>
<td>Curcumin Loading</td>
<td>Curcumin Release</td>
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<tr>
<td>Superparamagnetic Silica Reservoirs</td>
<td>A high loading of Fe3O4 nanoparticles (37% wt) and curcumin (30% wt) into the porous silica matrix</td>
<td>The curcumin entrapped inside the silica capsules diffuses out through passive diffusion processes</td>
<td>Not available</td>
<td>[92]</td>
</tr>
<tr>
<td>Hollow capsules</td>
<td>4.5 mg of curcumin/mg of microcapsules</td>
<td>Only 1.11% (0.26 μg/ml) of curcumin release was observed in 24 h, followed by a sustained release for about 1 week</td>
<td>Not available</td>
<td>[95]</td>
</tr>
<tr>
<td>Silk fibroin</td>
<td>Up to 96% encapsulation efficiency depending on the ratio of chitosan/silk fibroin/curcumin ratios</td>
<td>SF formulations released &gt; 0.3 and 0.6 μg of curcumin in 6 days while chitosan blend composition released only &lt; 0.1 μg</td>
<td>Chitosan-SF-curcumin formulation exhibited superior uptake in MCF-7 and MDA-MB-231 breast cancer cells</td>
<td>[96]</td>
</tr>
<tr>
<td>Thermosensitive nanoparticles</td>
<td>Higher loading efficiency and higher affinity of curcumin noticed between curcumin and thermosensitive nanoparticles</td>
<td>About 15-25% of the drug is released in about 10 h. Then, a much slower and almost constant release rate is observed.</td>
<td>Up to 2 fold increase in accumulation of curcumin nanoparticles in PC-3 and L929 cells</td>
<td>[51]</td>
</tr>
</tbody>
</table>

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Table 3
In Vitro and In Vivo Anticancer Potential and Mechanism of Action of Various Curcumin Nanoformulations

<table>
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<tr>
<th>Curcumin Nanoformulations</th>
<th>In Vitro Cytotoxicity Profile</th>
<th>Molecular Mechanism</th>
<th>In Vivo Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA</td>
<td>IC₅₀ (50% cell growth inhibitory concentration) of curcumin-loaded PLGA nanoparticles was between 20 μM and 22.5 μM while free curcumin ranged from 32 μM to 34 μM, in LNCaP, PC-3, and DU145 cancer cell lines</td>
<td>Inhibition of NF-κB function</td>
<td>Not available</td>
<td>[42]</td>
</tr>
<tr>
<td>PLGA</td>
<td>IC₅₀ of curcumin nanoparticles was less than 5 μM in human leukemia (KBM-5 and Jurkat), prostate (DU145), breast (MDA-MB-231), colon (HCT116) and esophageal (SE-51) cancer cells.</td>
<td>Do not induce NF-κB activation expression of cyclin D1, MMP-9, and VEGF</td>
<td>Half-life of curcumin NPs (2.5 mg/Kg mice) was 1.75 longer than that of curcumin</td>
<td>[44]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Nanocurcumin is as effective as curcumin in HeLa cells, SKBr3, and A549 cells</td>
<td>Increased Annexin V staining Cleaved PARP expression</td>
<td>Not available</td>
<td>[81]</td>
</tr>
<tr>
<td>MPEG-PCL micelle</td>
<td>IC₅₀ of free curcumin and Cur-MPEG-PCL micelles was 3.95 mg mL⁻¹ and 5.78 mg mg mL⁻¹, respectively</td>
<td>Not available</td>
<td>Up to 2-fold increase in CUR concentration was observed in plasma of rats Inhibited the growth of subcutaneous C-26 colon carcinoma in xenograft mouse model</td>
<td>[61]</td>
</tr>
<tr>
<td>β-cyclodextrin self-assembly</td>
<td>IC₅₀ of self-assemblies of curcumin was 16.8 μM and 17.6 μM(C4-2 cells and DU145 cells, respectively) which is slightly lower than free curcumin</td>
<td>Increased cleaved PARP expression</td>
<td>Improved CUR levels serum concentrations up to 2-fold (Unpublished data with Subhash Chauhan Lab)</td>
<td>[87]</td>
</tr>
<tr>
<td>Poly(β-cyclodextrin) self-assembly</td>
<td>Very close IC₅₀ values for both self-assembly and free curcumin in C4-2, DU145 and PC3 cancer cells</td>
<td>The PARP cleavage caused by PCD30 is much greater than free curcumin</td>
<td>Not available</td>
<td>[88]</td>
</tr>
<tr>
<td>poly(butyl cyanoacrylate) nano-particles</td>
<td>IC₅₀ was observed approximately 15 μg/mL for HepG2, Bel7402 and Huh7 cells</td>
<td>Down regulation of COX-2 and VEGF expression</td>
<td>2.2 fold decrease in tumor volume in HepG2 xenograft-bearing mice</td>
<td>[127]</td>
</tr>
<tr>
<td>Dendosome</td>
<td>2-fold reduction in IC₅₀ with dendosome curcumin in WEHI-164 (16.8 μM and 7.5 μM) and A431 cells (19.2 and 14.3 μM) in 24 and 48 h time</td>
<td>Increased Annexin V stain Cleaved PARP (apoptosis)</td>
<td>Tumor growth was significantly suppressed in mice treated with dendrosoomal curcumin</td>
<td>[47]</td>
</tr>
<tr>
<td>Thermo-sensitive nanocarrier</td>
<td>Formulation showed a specific toxicity cancer cell lines (MCF-7, KB, and PC-3) and non toxic to L929.</td>
<td>Increase apoptosis (PI and Annexin-A binding) Loss of mitochondrial membrane potential</td>
<td>Not available</td>
<td>[51]</td>
</tr>
<tr>
<td>Curcumin Nanoformulations</td>
<td>In Vitro Cytotoxicity Profile</td>
<td>Molecular Mechanism</td>
<td>In Vivo Results</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
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<tr>
<td>Folate-modified self-microemulsifying drug delivery system</td>
<td>18.27, 36.69, 30.4 μM and 20.57, 38.59, 25.62 μM in Hela and HT-29 cancer cells for folate CUR-nanoemulsion, CUR-emulsion and free curcumin, respectively</td>
<td>Not available</td>
<td>In situ colon perfused rats showed absorption of curcumin increased from 58.41% to 73.38% in 6 h with folate conjugated formulation.</td>
<td>[131]</td>
</tr>
<tr>
<td>NanoCurc™</td>
<td>IC₅₀ ranged between 10–15 μM for BxPC3, ASPC-1, PL-11 and XPA-1</td>
<td>Blocks the activation of NF-κB Downregulation of steady state transcripts of multiple pro-inflammatory cytokines</td>
<td>5 fold increased concentration was observed in pancreas. 3-fold or no growth in tumor was observed in mice with NanoCurc™ in combination with gemcitabine</td>
<td>[52, 117]</td>
</tr>
<tr>
<td>PEG-cholestrol</td>
<td>Cm/PEG-cholesterol based curcumin system showed IC₅₀ 1 μM more than free curcumin</td>
<td>Not available</td>
<td>Not available</td>
<td>[62]</td>
</tr>
<tr>
<td>NanoCurc™</td>
<td>Almost no growth was observed in DAOY and D283Med, and the glioblastoma neurosphere lines HSR-GBM1 and JHH-GBM14</td>
<td>Blocked the STAT3 and Hedgehog signaling G(2)/M arrest and apoptotic induction</td>
<td>0.5% of the injected material was localized in the brain</td>
<td>[115, 116]</td>
</tr>
<tr>
<td>Amphiphilic mPEG-palmitic acid polymer</td>
<td>IC₅₀ of curcumin, 14.32 μM, and nanocurcumin, 15.58 μM, were observed in HeLa cells</td>
<td>In vitro enzyme-catalyzed drug release enhances the anticancer activity</td>
<td>Not available</td>
<td>[132]</td>
</tr>
</tbody>
</table>

*Curc Pharm Des. Author manuscript; available in PMC 2014 January 01.*
Table 4
Pharmacokinetics Properties and Bioavailability of Various Curcumin Nanoformulations

<table>
<thead>
<tr>
<th>Curcumin Nanoformulations</th>
<th>Comment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPEG-PCL micelles</td>
<td>Tmax (min): 5 and 5 t(1/2) (time): 34.2 and 19.6 AUC(0–t) (mg L(^{-1}) min(^{-1})): 47642.1 and 7933.2 AUC(0–t) (mg L(^{-1}) min(^{-1})): 47864.6 and 7944.6 Cmax (mg mL(^{-1})): 430.5 and 305.7 for curcumin and micelle-curcumin, respectively</td>
<td>[61]</td>
</tr>
<tr>
<td>Dibenzoylmethane (DBM) nanoemulsion</td>
<td>3-fold increase in oral bioavailability</td>
<td>[19]</td>
</tr>
<tr>
<td>Curcurain-loaded solid lipid nanoparticles (C-SLN)</td>
<td>Curcumin levels in plasma were significantly increased i.e., 39 times at 50 mg/kg; 155 times at 1 mg/kg; and, 59 at 12.5 and 32 times at 25 mg/kg, respectively</td>
<td>[150]</td>
</tr>
<tr>
<td>PLGA</td>
<td>Nanoformulation significantly increased the retention time of curcumin by 96% in the cerebral cortex and 83% in the hippocampus</td>
<td>[151]</td>
</tr>
<tr>
<td>Theracurcumin</td>
<td>C(max) for 150 and 210 mg: 189 ±48 and 275 ± 67 ng/ml AUC (24 h) for 150 and 210 mg: 2,649 ± 350 and 3,649 ± 430 ng/ml x h t(1/2) for 150 and 210 mg: 9.7 ± 2.1 h and 13.0 ± 3.3 h</td>
<td>[152]</td>
</tr>
<tr>
<td>Nanosuspension</td>
<td>Area under the curve in plasma: 3.8-fold greater than curcumin the mean residence time: 11.2-fold longer than curcumin</td>
<td>[153]</td>
</tr>
<tr>
<td>(PLGA-PEG-PLGA) copolymer nanoparticles</td>
<td>AUC(0–∞): 1.31 fold greater than curcumin t(1/2α), t(1/2β): 2.48 and 4.54 fold increase than curcumin Mean residence time: 2.67 fold longer than curcumin</td>
<td>[125]</td>
</tr>
</tbody>
</table>

AUC: Area under the curve

C\(_{\text{max}}\): Peak concentration

(T\(_{\text{max}}\)): Time to peak concentration

(t\(_{\text{lag}}\)): Absorption lag time

AUC(0–t) and AUC(0–∞) of the test (e.g. generic formulation) to reference (e.g. innovator brand formulation)
Efficacy and safety of the probiotic \textit{Saccharomyces boulardii} for the prevention and therapy of gastrointestinal disorders

Theodoros Kelesidis and Charalabos Pothoulakis

\textbf{Abstract:} Several clinical trials and experimental studies strongly suggest a place for \textit{Saccharomyces boulardii} as a biotherapeutic agent for the prevention and treatment of several gastrointestinal diseases. \textit{S. boulardii} mediates responses resembling the protective effects of the normal healthy gut flora. The multiple mechanisms of action of \textit{S. boulardii} and its properties may explain its efficacy and beneficial effects in acute and chronic gastrointestinal diseases that have been confirmed by clinical trials. Caution should be taken in patients with risk factors for adverse events. This review discusses the evidence for efficacy and safety of \textit{S. boulardii} as a probiotic for the prevention and therapy of gastrointestinal disorders in humans.

\textbf{Keywords:} efficacy, gastrointestinal disorders, probiotic, Saccharomyces boulardii, safety

\textbf{Introduction}

There is increasing evidence that the gastrointestinal microflora is a major regulator of the immune system, not only in the gut, but also in other organs [Gareau \textit{et al.} 2010]. The nonpathogenic yeast \textit{Saccharomyces boulardii} has been prescribed in the past 30 years for prophylaxis and treatment of diarrheal diseases caused by bacteria. Importantly, \textit{S. boulardii} has demonstrated clinical and experimental effectiveness in gastrointestinal diseases with a predominant inflammatory component, indicating that this probiotic might interfere with cellular signaling pathways common in many inflammatory conditions. The goal of this study is to review the clinical evidence for efficacy and safety of \textit{S. boulardii} in the prevention and treatment of gastrointestinal disorders with diverse etiology.

\textit{Saccharomyces boulardii} as a probiotic

An increasing number of potential health benefits are being attributed to probiotic treatments [Gareau \textit{et al.} 2010; Szajewska \textit{et al.} 2006]. However, only a limited number have been confirmed in well-designed and conducted randomized controlled trials (RCTs) and even less in the pediatric population. \textit{S. boulardii} is a live yeast used extensively as a probiotic and often marketed as a dietary supplement [McFarland, 2010]. Several mechanisms of action have been identified directed against the host as well as pathogenic microorganisms and include regulation of intestinal microbial homeostasis, interference with the ability of pathogens to colonize and infect the mucosa, modulation of local and systemic immune responses, stabilization of the gastrointestinal barrier function and induction of enzymatic activity favoring absorption and nutrition (Tables 1 and 2) [Czerucka \textit{et al.} 2007; Im and Pothoulakis, 2010; Pothoulakis, 2009].

The multiple prophylactic and therapeutic effects of this probiotic yeast in inflammatory gastrointestinal diseases underline the efficacy of \textit{S. boulardii} in enteric diseases. This efficacy in the prevention and the treatment of acute and chronic gastrointestinal diseases is determined by many factors (Table 3) and has been assessed in several clinical trials (Table 4).

Factors that determine efficacy of \textit{Saccharomyces boulardii} as a probiotic

The efficacy of \textit{S. boulardii} as a probiotic involves many factors, including the intrinsic properties of the yeast (Table 3), its pharmacokinetics (Table 3),
Table 1. Mechanisms of action of *Saccharomyces boulardii*.

<table>
<thead>
<tr>
<th><strong>Action of Saccharomyces boulardii</strong></th>
<th><strong>References</strong></th>
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<tbody>
<tr>
<td><strong>Luminal action</strong></td>
<td></td>
</tr>
<tr>
<td><strong>A) Antimicrobial activity</strong></td>
<td></td>
</tr>
<tr>
<td>1) Inhibition of growth of bacteria and parasites</td>
<td>[Chen et al. 2006; Czerucka et al. 1994; Czerucka and Rampal, 2002; Dahan et al. 2003; Dalmasso et al. 2006a; Gedek, 1999a; Rigothier et al. 1994; Rodrigues et al. 1996; Mumy et al. 2008; Wu et al. 2008]</td>
</tr>
<tr>
<td>2) Reduction of gut translocation of pathogens</td>
<td>[Herek et al. 2004; Geyik et al. 2006]</td>
</tr>
<tr>
<td>3) Neutralization of bacterial virulence factors</td>
<td>[Buts et al. 1994; Jahn et al. 1996]</td>
</tr>
<tr>
<td>4) Suppression of host cell adherence that interferes with bacterial colonization</td>
<td>[Czerucka et al. 2000; Rodrigues et al. 1996; Wu et al. 2008]</td>
</tr>
<tr>
<td><strong>B) Antitoxin effects</strong></td>
<td></td>
</tr>
<tr>
<td>1) Inhibition of toxin receptor binding sites</td>
<td>[Buts et al. 2004; Castagliuolo et al. 1996, 1999; Czerucka et al. 2000; Tasteyre et al. 2002; Wu et al. 2008]</td>
</tr>
<tr>
<td>2) Stimulation of antibody production against <em>Clostridium difficile</em> toxin A</td>
<td>[Brandao et al. 1998; Qamar et al. 2001]</td>
</tr>
<tr>
<td>3) Direct proteolysis of the pathogenic toxins/Secretion of enzymatic proteins</td>
<td>[Buts et al. 2006; Castagliuolo et al. 1996; Pothoulakis et al. 1993]</td>
</tr>
<tr>
<td>a) Produces a serine protease that cleaves <em>C. difficile</em> toxin A</td>
<td>[Pothoulakis et al. 1993]</td>
</tr>
<tr>
<td>b) Produces 63 kDa phosphatase that destroys the endotoxin of pathogenic <em>Escherichia coli</em></td>
<td>[Buts et al. 2006; Castagliuolo et al. 1996]</td>
</tr>
<tr>
<td>c) Produces a 120 kDa protein that reduces the effects of cholera toxin</td>
<td>[Czerucka et al. 1994]</td>
</tr>
<tr>
<td><strong>C) Cross-talk with normal microbiota</strong></td>
<td></td>
</tr>
<tr>
<td>When <em>S. boulardii</em> is given to antibiotic-exposed mice or patients with diarrhea, normal microbiota is re-established rapidly</td>
<td>[Buts et al. 1986, 1999, 2006; Buts, 2009; Swidsinski et al. 2008]</td>
</tr>
<tr>
<td><strong>Trophic action on the intestinal mucosa</strong></td>
<td></td>
</tr>
<tr>
<td>1) Reduces the number of infected cells and stimulates the growth and differentiation of intestinal cells in response to trophic factors</td>
<td>[Barc et al. 2008; Swidsinski et al. 2008]</td>
</tr>
<tr>
<td>2) Prevents apoptosis and synthesis of TNFα</td>
<td>[Czerucka et al. 2000; Dahan et al. 2003; Dalmasso et al. 2006b]</td>
</tr>
<tr>
<td>3) Reduces mucositis</td>
<td>[Buts et al. 1986, 1999, 2006; Buts, 2009]</td>
</tr>
<tr>
<td>4) Restores fluid transport pathways</td>
<td>[Schneider et al. 2005]</td>
</tr>
<tr>
<td>5) Stimulates protein and energy production and restores metabolic activities in colonic epithelial cells</td>
<td>[Czerucka et al. 2007; Szajewska et al. 2007; Zanello et al. 2009]</td>
</tr>
<tr>
<td>6) Secretes mitogenic factors that enhance cell restitution</td>
<td>[Canonici et al. 2011]</td>
</tr>
<tr>
<td>8) Stimulates the production of glycoproteins in the brush border</td>
<td>[Buts et al. 1990]</td>
</tr>
<tr>
<td>10) Restores normal levels of colonic short chain fatty acids (SCFAs)</td>
<td>[Buts et al. 1994; Sezer et al. 2009; Breves et al. 2000]</td>
</tr>
<tr>
<td>11) Stabilizes gastrointestinal barrier function and strengthens enterocyte tight junctions</td>
<td>[Czerucka et al. 2007; Dahan et al. 2003; Szajewska et al. 2007; Wu et al. 2008; Zanello et al. 2009]</td>
</tr>
<tr>
<td>12) Reduces crypt hyperplasia and cell damage in colitis models</td>
<td>[Wu et al. 2008]</td>
</tr>
<tr>
<td>13) Decreases intestinal permeability in Crohn’s disease patients</td>
<td>[Garcia et al. 2008]</td>
</tr>
</tbody>
</table>
Action of Saccharomyces boulardii

References

Regulation of immune response

A) By acting as an immune stimulant

**S. boulardii effects on innate immunity**

1) Triggers activation of complement and migration of monocytes and granulocytes
   
   [Caetano et al. 1986]

2) Enhances the number of Kupffer cells in germfree mice
   
   [Rodrigues et al. 2000]

**S. boulardii effects on adaptive immunity**

1) Enhances the mucosal immune response and secretory IgA intestinal levels
   
   [Buts et al. 1990; Czerucka et al. 2007; Generoso et al. 2011; Szejewska et al. 2007; Zanello et al. 2009]

2) Enhances systemic immune response and levels of serum IgG to *C. difficile* toxins A and B.
   
   [Czerucka et al. 2007; Qamar et al. 2001]

3) Contributes to earlier production of IFN-γ and IL-12
   
   [Rodrigues et al. 2000; Thomas et al. 2009]

4) Stimulates regulatory T cells
   
   [Jahn et al. 1996]

5) Inhibits dendritic cell-induced activation of T cells
   
   [Dalmasso et al. 2006a]

6) Modifies migration of lymphocytes in a chronic inflammatory bowel disease model
   
   [Dalmasso et al. 2006a]

7) Modifies lymphocyte adherence to endothelial cells, improves cell rolling and adhesion
   
   [Dalmasso et al. 2006a]

B) By reducing pro-inflammatory responses and promoting mucosal anti-inflammatory signaling effects

1) Decreases expression of pro-inflammatory cytokines (IL-8, IL-6, IL-1β, TNF-α and IFN-γ)
   
   [Dahan et al. 2003; Dalmasso et al. 2006a, 2006b; Mumy et al. 2008; Sougioultzis et al. 2006]

2) Increases expression of the anti-inflammatory cytokine IL-10
   
   [Generoso et al. 2011]

3) Interferes with NF-κB-mediated signal transduction pathways in immune and colonic epithelial cells
   
   [Buts, 2009; Dahan et al. 2002; Mumy et al. 2008; Pant et al. 2007; Sougioultzis et al. 2006]

4) Blocks activation of ERK1/2 and MAP kinases
   
   [Chen et al. 2006; Kynes et al. 2001; Mumy et al. 2008; Sougioultzis et al. 2006]

5) Decreases NO and inhibits production of inducible NOS
   
   [Girard et al. 2005]

6) Modulates T cell migratory behavior and increases trapping of T helper cells into mesenteric lymph nodes
   
   [Dalmasso et al. 2006a; Fidan et al. 2009; Sougioultzis et al. 2006; Thomas et al. 2009]

7) Stimulates production of anti-inflammatory molecules in human colonocytes such as PPAR-γ
   
   [Chen et al. 2006; Lee et al. 2005, 2009; Mumy et al. 2008]

**ERK**, extracellular signal-regulated kinase; **IL**, interleukin; **INF-γ**, interferon gamma; **IgA**, Immunoglobulin A; **IGF**, insulin growth factor; **MAP**, mitogen-activated protein; **NF-κB**, nuclear factor kappa B; **NO**, nitric oxide; **NOS**, nitric oxide synthase; **PPAR-γ**, peroxisome proliferator-activated receptor-gamma; **TNFα**, tumor necrosis factor alpha

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There are many different Saccharomyces products commercially available sold as probiotics and *S. boulardii* is usually available in capsules of either lyophilized or heat-dried preparations [McFarland, 2010]. The choice of a high-quality probiotic product is one of the most important factors that determine efficacy of the probiotic. The quality of these products from different sources may vary and many of the commercially available products may lack regulated quality control programs [Marcobal et al. 2008; Martins et al. 2005; Masco et al. 2005; Weese, 2003]. Even if the label states it contains *S. boulardii*, a variation in efficacy may occur due to lower than stated dose or inaccurate strain composition [Martins et al. 2009]. Selecting high-quality probiotic products can be difficult without access to specific quality control assays for commercially available probiotic products. Selecting products from companies that sponsor original clinical trials may indicate a higher degree of commitment to high-quality products [McFarland, 2010].

The stability of the probiotic product may significantly affect its potency over time. Lyophilized products are stable at room temperature, have the...
Table 2. Mechanisms of action of *Saccharomyces boulardii* in specific infections.

<table>
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<tr>
<th>Action of Saccharomyces boulardii</th>
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<tr>
<td><strong>Clostridium difficile</strong> infection</td>
<td></td>
</tr>
<tr>
<td>1) Inhibits toxin A-mediated diarrhea, intestinal inflammation and histological damage by reducing toxin A-receptor binding</td>
<td>[Castagliuolo et al. 1996; Pothoulakis et al. 1993]</td>
</tr>
<tr>
<td>2) Releases a protease that cleaves <em>C. difficile</em> toxins and toxin intestinal receptors</td>
<td>[Castagliuolo et al. 1996; Pothoulakis et al. 1993]</td>
</tr>
<tr>
<td>3) Stimulates specific intestinal antitoxin A immunoglobulin levels</td>
<td>[Castagliuolo et al. 1996, 1999; Pothoulakis et al. 1993]</td>
</tr>
<tr>
<td>4) Inhibits IL-8 production and activation of the MAP kinases Erk1/2 and JNK/SAPK induced by <em>C. difficile</em> toxin A in human colonocytes</td>
<td>[Chen et al. 2006; Qamar et al. 2001]</td>
</tr>
<tr>
<td>5) Significantly fewer animals challenged with <em>C. difficile</em> died if given <em>S. boulardii</em> compared with controls</td>
<td>[Castex et al. 1990; Elmer and Corthier, 1991; Rodrigues et al. 1996; Toothaker and Elmer, 1984]</td>
</tr>
<tr>
<td><strong>Helicobacter pylori</strong> infection</td>
<td></td>
</tr>
<tr>
<td>Alters the structure of <em>H. pylori</em></td>
<td>[Vandenplas et al. 2009]</td>
</tr>
<tr>
<td><strong>Vibrio cholerae</strong> infection</td>
<td></td>
</tr>
<tr>
<td>1) Inhibits the effect of <em>V. cholerae</em> toxin and hydroelectrolytic secretions by reducing cAMP activity</td>
<td>[Vidon et al. 1986; Czerucka et al. 1994]</td>
</tr>
<tr>
<td>2) <em>S. boulardii</em> and the mammalian CT receptors could be structurally and functionally similar and the yeast binds CT</td>
<td>[Brandao et al. 1998; Czerucka et al. 1994]</td>
</tr>
<tr>
<td><strong>Amebic dysentery</strong></td>
<td></td>
</tr>
<tr>
<td>1) Reduces the number of red cells adhering to amoebae</td>
<td>[Rigothier et al. 1994]</td>
</tr>
<tr>
<td>2) Decreases the number of amoebae bearing red cells</td>
<td>[Rigothier et al. 1994]</td>
</tr>
<tr>
<td><strong>Infection with EHEC</strong></td>
<td></td>
</tr>
<tr>
<td>1) <em>S. boulardii</em> modifies host signaling such as NF-κB-associated pathways activated by bacterial invasion with EHEC</td>
<td>[Dahan et al. 2002, 2003]</td>
</tr>
<tr>
<td>2) Addition to T84 colonocyte monolayers diminishes MLC phosphorylation and decreases transepithelial resistance in response to EHEC</td>
<td>[Dahan et al. 2002, 2003]</td>
</tr>
<tr>
<td><strong>Infection with EPEC</strong></td>
<td></td>
</tr>
<tr>
<td>1) Modifies EPEC infection and acts as a receptor decoy for EPEC</td>
<td>[Buts et al. 2006; Canil et al. 1993; Czerucka et al. 2000; Gedek, 1999b]</td>
</tr>
<tr>
<td>2) Reduces the number of intracellular EPEC</td>
<td>[Buts et al. 2006; Canil et al. 1993; Czerucka et al. 2000; Gedek, 1999b]</td>
</tr>
<tr>
<td>3) Blocks transepithelial resistance and permeability changes, reverses impaired ZO-1 distribution and delays apoptosis of epithelial cells in response to EPEC</td>
<td>[Buts et al. 2006; Canil et al. 1993; Czerucka et al. 2000; Gedek, 1999b]</td>
</tr>
<tr>
<td>4) Dephosphorylates LPS from <em>Escherichia coli</em> strain O55B5</td>
<td>[Buts et al. 2006; Canil et al. 1993; Czerucka et al. 2000; Gedek, 1999b]</td>
</tr>
</tbody>
</table>

CT, cholera toxin; EHEC, enterohemorrhagic *E. coli*; EPEC, enteropathogenic *E. coli*; ERK, extracellular signal-regulated kinase; LPS, lipopolysaccharide; MAP, mitogen-activated protein; MLC, myosin light chain; NF-κB, nuclear factor kappa B; ZO-1, zonula occludens 1
Portability and maintain high viability counts over prolonged periods [Graff et al. 2008a]. Heat-dried preparations must be refrigerated and may not be stable at room temperature [McFarland, 2010]. A study of *S. boulardii* products found a lyophilized product outperformed three heat-killed *S. boulardii* preparations in terms of pharmacokinetics and higher number of viable cells [Schwenzer, 1998].

All of the RCTs using *S. boulardii* have utilized a single-strain preparation. Although mixtures of probiotics containing *S. boulardii* are available on the market, no RCTs have been performed showing that these mixtures are superior to the single-strain preparations. Preclinical studies in animal models have found promising results in probiotic mixtures containing *S. boulardii* [Bisson et al. 2010]. However, possible antagonism between the different probiotics may attenuate the therapeutic responses of the probiotic strains [Kajander et al. 2008].

Finally, the dose of *S. boulardii* used can also affect the efficacy of this probiotic [McFarland, 2010]. Different doses of *S. boulardii* used in different studies may explain some of the discrepancies in the efficacy and outcomes between these studies. Unfortunately, the dose of *S. boulardii* used is not reported consistently in all studies while in other studies the dose used is reported heterogeneously between different studies (e.g. number of organisms per 100 ml or number of organisms per day or colony forming units [cfu] per day or grams per day) [McFarland, 2010]. This heterogeneity limits meta-analyses and further analysis of the effect of dose of *S. boulardii* on its efficacy.

### Table 3. Properties of *Saccharomyces boulardii* that can determine the efficacy of *S. boulardii*.

<table>
<thead>
<tr>
<th>Properties of <em>Saccharomyces boulardii</em></th>
<th>References</th>
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<tbody>
<tr>
<td>1) Survives passage to its target organ (most commonly the colon): although much of the oral dose is destroyed (usually stool levels are 100–1000 times lower than the oral dose), surviving oral doses have been found to be effective (usually at levels over 10⁶ organisms/gram stool)</td>
<td>[Gorbach, 2000]</td>
</tr>
<tr>
<td>2) Survives at body temperature (37°C): unique advantage of being one of the few yeasts that do best at human body temperatures</td>
<td>[Graff et al. 2008b]</td>
</tr>
<tr>
<td>3) In lyophilized form, <em>S. boulardii</em> survives gastric acid and bile</td>
<td>[Graff et al. 2008b]</td>
</tr>
<tr>
<td>4) As is the case with all yeasts, <em>S. boulardii</em> is naturally resistant to antibiotics</td>
<td>[Graff et al. 2008b]</td>
</tr>
<tr>
<td>5) <em>S. boulardii</em> is resistant to proteolysis</td>
<td>[Buts, 2009]</td>
</tr>
<tr>
<td>6) <em>S. boulardii</em> exists in the competitive milieu of the intestinal tract</td>
<td>[Buts, 2009]</td>
</tr>
<tr>
<td>7) <em>S. boulardii</em> levels are higher in patients with disturbed intestinal microbiota (due to antibiotic exposure) compared to patients without antibiotic exposure</td>
<td>[Klein et al. 1993]</td>
</tr>
<tr>
<td>8) When given orally, achieves steady-state concentrations within three days and is cleared within 3–5 days after it is discontinued</td>
<td>[Blehaut et al. 1989; Elmer et al. 1999b]</td>
</tr>
<tr>
<td>9) Some types of fiber (psyllium) increased <em>S. boulardii</em> levels by 22%, while other types of fiber (pectin) showed no effect.</td>
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### Table 4. Clinical efficacy of *Saccharomyces boulardii* in acute and chronic diseases.

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Clinical efficacy of *Saccharomyces boulardii* as a probiotic in acute gastrointestinal conditions

*S. boulardii* has been tested for clinical efficacy in several types of acute gastrointestinal conditions, including antibiotic-associated diarrhea (AAD), *Clostridium difficile* infection (CDI), acute gastrointestinal infections (AGIs), and *Helicobacter pylori* infection (H. pylori).

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diarrhea, enteral nutrition-related diarrhea, traveler’s diarrhea and *Helicobacter pylori* infection.

**Antibiotic-associated diarrhea**

AAD is defined as otherwise unexplained diarrhea that occurs in association with the administration of antibiotics [Bartlett, 2002]. Measures to prevent AAD include the use of probiotics. Of the 10 controlled trials in adults using *S. boulardii* for the prevention of AAD, 8 (80%) showed significant efficacy for the prevention of AAD [McFarland, 2010]. The protective effect of *S. boulardii* and the significant relative reduction in AAD compared with controls ranged between 7.4% and 25% [McFarland, 2010]. Other studies failed to demonstrate a significant protective effect of *S. boulardii* and this may be secondary to short or no follow-up after antibiotic exposure [McFarland, 2009]. Two RCTs have assessed the ability of *S. boulardii* to prevent AAD in children and the relative significant increase in prevention of AAD in the *S. boulardii* group compared with controls ranged between 7.6% and 30.1% [Can et al. 2006; Kotowska et al. 2005].

A recent meta-analysis of the 10 randomized, controlled trials in adults found that *S. boulardii* was significantly protective for AAD with a pooled relative risk (RR) of 0.47 (95% confidence interval [CI] 0.35–0.63, *p* < 0.001) [McFarland, 2010]. Finally, in another meta-analysis from five trials involving 1076 subjects, a significantly protective effect of *S. boulardii* was found (pooled RR = 0.43, 95% CI 0.23–0.78) [Szałewska and Mrukowicz, 2005]. However, although many meta-analyses have concluded that probiotics are effective for preventing AAD [McFarland, 2009], most probiotic meta-analyses have focused on one type of disease indication (e.g. antibiotic-associated diarrhea) with a variety of probiotic strains. Thus, we summarized data from meta-analyses that are focused only on the use of *S. boulardii* in preventing AAD and conclude that this probiotic is efficacious for this indication.

**Clostridium difficile infection**

Probiotics represent a promising approach as an adjunctive therapy for CDI. A meta-analysis of six RCTs of different probiotics, including *S. boulardii* showed that probiotics had a significant efficacy to prevent subsequent recurrences of CDI (RR = 0.59, 95% CI 0.41–0.85, *p* = 0.005) [McFarland, 2006]. However, due to the limited number of trials, no meta-analysis was conducted for one probiotic strain.

Several pieces of evidence suggest that *S. boulardii* represents the most effective probiotic that can prevent or, together with other agents, treat antibiotic-associated diarrhea and recurrent CDI [McFarland, 2006] through many mechanisms (Table 2).

Animal models of CDI respond to this yeast and case reports or small case series of patients with recurrent CDI treated with *S. boulardii* showed improvement [McFarland, 2010].

The significant relative reduction in recurrent CDI in adults taking *S. boulardii* compared with controls was evaluated in two RCTs and ranged between 19% and 33.3% [McFarland et al. 1994; Surawicz et al. 2000]. There are only very limited data from one small observational trial in children suggesting that *S. boulardii* may be effective in CDI [Buts et al. 1993]. However, according to guidelines no compelling evidence exists to support routine use of probiotics for prevention or treatment of CDI [Cohen et al. 2010] especially since some of these studies did not control the dose or duration of either vancomycin or metronidazole for treatment of CDI [McFarland et al. 1994] and since scarce data exist on the use of *S. boulardii* for recurrent CDI in humans.

**Acute diarrhea**

Two RCTs using *S. boulardii* showed that this probiotic may be effective in treating acute adult diarrhea due to a variety of causes and can significantly lower diarrhea severity score compared with controls [Hochter et al. 1990; Mansour-Ghanaei et al. 2003]. Unfortunately, since the number of trials in this area is small and the etiologies were different in the two trials, only limited conclusions can be reached.

A recent RCT conducted in 100 hospitalized children showed that *S. boulardii* treatment for 5 days significantly reduces the mean duration of acute diarrhea and frequency of stools, and normalizes stool consistency [Hwe et al. 2008]. One RCT regarding the efficacy of *S. boulardii* for the prevention of acute diarrhea involved 100 children with acute watery diarrhea and reported a significant difference in the incidence of diarrheal episodes in the group receiving *S. boulardii* compared with the control group during 2 months follow up [Biloo et al. 2006].
A meta-analysis based on 5 RCTs (619 participants) [Billoo et al. 2006; Kurugol and Koturoglu, 2005; Villarruel et al. 2007] indicated that *S. boulardii* significantly reduces the duration of acute childhood diarrhea and the risk of prolonged diarrhea compared with control [Szczejewska et al. 2007]. A meta-analysis of seven RCTs (944 participants) showed a reduction in the duration of acute childhood diarrhea by approximately 1 day in those treated with *S. boulardii* compared with placebo [Szczejewska and Skorka, 2009]. The absence of blinding as well as other factors such as ambulatory care may explain why *S. boulardii* had no effect in a European RCT [Canani et al. 2007]. In summary, the findings from RCTs and guidelines from professional pediatric societies indicate that *S. boulardii* may be an effective adjunct therapy in managing acute gastroenteritis in children [Guarino et al. 2008].

**Persistent diarrhea**

Results from two clinical trials indicate that *S. boulardii* improves outcomes in children with persistent diarrhea [Castaneda et al. 1995; Gaon et al. 2003]. The relative significant reduction in persistent diarrhea in the *S. boulardii* group compared with controls was approximately 50% [Castaneda et al. 1995]. These results indicate that *S. boulardii* is useful in the management of persistent diarrhea in children. However, studies with larger populations are needed to determine whether *S. boulardii* therapy alone is also effective in children with persistent diarrhea.

**Enteral nutrition-related diarrhea**

Diarrhea is a significant problem in patients on total enteral nutrition (TEN) and may involve changes in intestinal short chain fatty acids (SCFAs) [Schneider et al. 2005]. Schneider and colleagues reported a significant increase in SCFAs in 10 enteral-fed patients receiving *S. boulardii* compared with 15 healthy controls [Schneider et al. 2005]. *S. boulardii*-induced increase of fecal SCFA concentrations may explain the preventative effects of this yeast on TEN-induced diarrhea [Schneider et al. 2005]. In three RCTs the relative significant reduction in enteral nutrition-related diarrhea in the *S. boulardii* group compared with controls ranged between 5% and 8.2% [Bleichner et al. 1997; Schlotterer et al. 1987; Tempe et al. 1983]. More studies are needed to elucidate the mechanisms of how *S. boulardii* can prevent TEN-induced diarrhea.

**Traveler’s diarrhea**

A meta-analysis of 12 RCTs of various probiotics (including *S. boulardii*) for the prevention of traveler's diarrhea found a significant reduction in the risk of traveler's diarrhea when probiotics are used (RR = 0.85, 95% CI 0.79–0.91) [McFarland, 2007]. The relative significant reduction in traveler's diarrhea in the *S. boulardii* group compared with controls in two RCTs ranged between 5% and 11% [Kollaretich et al. 1989, 1993]. These limited numbers of studies indicate that probiotics may be more effective in preventing traveler’s diarrhea, rather than treating diarrhea once it becomes symptomatic.

**Helicobacter pylori infection**

A recent meta-analysis involving 14 RCTs (1671 patients) evaluated the role of probiotics in *H. pylori* eradication [Tong et al. 2007]. In patients with *H. pylori* infection, probiotic supplementation improved eradication rates and reduced treatment-related side effects and individual symptoms [Tong et al. 2007]. In this meta-analysis, only one RCT evaluated *S. boulardii* and found that it decreased the risk of diarrhea when given concomitantly to patients receiving triple eradication therapy for *H. pylori* [Duman et al. 2003]. *S. boulardii* induces morphologic changes in *H. pylori* cells consistent with cellular damage [Vandenplas et al. 2009] and was shown to cause reduction in *H. pylori* colonization in infected children by 12% [Gotteland et al. 2005]. Of four RCTs testing *S. boulardii* in *H. pylori* infections, two were in children [Gotteland et al. 2005; Hurduc et al. 2009] and two in adults [Cindoruk et al. 2007; Cremonini et al. 2002]. Although there was no significant difference in *H. pylori* eradication between the *S. boulardii* and placebo groups, a significantly lower relative rate of AAD (16.1–25%) was observed. In a recent meta-analysis, the *H. pylori* eradication rate in the triple therapy group was 71% and increased significantly to 80% with *S. boulardii* supplementation [Szczejewska et al. 2010]. Thus, *S. boulardii* may not be effective in eradicating *H. pylori* itself, but it is effective in reducing the side effects of the standard triple therapy.

**Clinical efficacy of *Saccharomyces boulardii* as a probiotic in chronic diseases**

*S. boulardii* has been tested for clinical efficacy in several types of chronic diseases including Crohn’s disease, ulcerative colitis, irritable bowel syndrome (IBS), parasitic infections and human immunodeficiency virus (HIV)-related diarrhea.
**Crohn’s disease**

Recently, the use of probiotics for maintaining remission from active disease in patients with Crohn’s disease was given a ‘C’ recommendation rating level by a panel of experts evaluating the efficacy of the supplements, mostly due to a scarcity of data [Floch et al. 2008]. In a small pilot study of 31 patients with Crohn’s disease in remission all patients continued their maintenance medications and were randomized to either *S. boulardii* for 3 months or placebo [Garcia et al. 2008]. Those treated with *S. boulardii* were found to have a significant reduction in colonic permeability compared with those given placebo, thus reducing the risk of bacterial translocation in these patients [Garcia et al. 2008]. Two RCTs tested *S. boulardii* for patients with Crohn’s disease [Guslandi et al. 2000; Plein and Hotz, 1993]. In a small randomized study of 20 patients with Crohn’s disease all patients continued their maintenance medications and were randomized to either *S. boulardii* for 7 weeks or placebo. Patients treated with *S. boulardii* were significantly improved compared with the placebo group [Plein and Hotz, 1993]. Finally, in a study of 32 patients with Crohn’s disease who were in remission, significantly fewer patients treated with *S. boulardii* (6%) relapsed than the control group (38%) [Guslandi et al. 2000]. Further studies to establish the efficacy of *S. boulardii* in treatment of Crohn’s disease are needed.

**Ulcerative colitis**

Probiotics have been used as an adjunct treatment in an attempt to induce remission in patients with active ulcerative colitis flares [Cain and Karpa, 2011]. In a small pilot study of 25 adults with mild to moderate ulcerative colitis that were treated with a combination of mesalazine and *S. boulardii* for 4 weeks, most (68%) of the patients responded to the probiotic treatment [Guslandi et al. 2003]. This pilot study had a promising result, but the implications were uncertain as patients were treated for only a short time, were not followed up for subsequent disease flare ups, and no control group was included. In a small pilot study of 6 patients with ulcerative colitis, a therapeutic regimen including *S. boulardii* and rifaximin for 3 months seemed effective in preventing early flare ups of ulcerative colitis [Guslandi, 2010]. Further controlled studies on a larger number of patients treated for longer periods with this probiotic agent are warranted. Overall, based upon current consensus, the level of evidence for use of probiotics either to maintain remission or induce remission of ulcerative colitis symptoms is presently limited to a ‘C’ rating [Floch et al. 2008].

**Irritable bowel syndrome**

Recent evidence suggests a role of the microflora in IBS pathogenesis [Parkes et al. 2008]. A meta-analysis of 20 RCTs including 1404 subjects found a pooled RR for improvement in global IBS symptoms in 14 probiotic treatment arms (RR = 0.77, 95% CI 0.62–0.94) [McFarland and Dublin, 2008]. In a double-blind trial of *S. boulardii* versus placebo in the treatment of IBS patients, the probiotic agent significantly improved the quality of life, but did not improve intestinal symptoms [Choi et al. 2011]. These findings are inconsistent with those reported in double-blind, RCTs performed earlier in France [Bennani, 1990; Maupas et al. 1983]. Along these lines, a recent retrospective analysis suggested that addition of *S. boulardii* to mebeverine can provide superior results in IBS treatment and that the probiotic agent does exert beneficial effects on the quality of life and IBS symptoms [Guslandi, 2011]. More trials using *S. boulardii* for IBS are required to allow solid conclusions for its use in this condition.

**Parasitic infections**

Little is known about the efficacy of *S. boulardii* against protozoal infections but this probiotic seems to have a beneficial effect in amebiasis, giardiasis and infection with *Blastocystis hominis*. In adults, co-administration of lyophilized *S. boulardii* with conventional treatment in acute amebic colitis significantly decreased the duration of symptoms and cyst carriage after 4 weeks [Mansour-Ghanaei et al. 2003]. A prospective RCT in patients with amebic colitis showed that addition of *S. boulardii* to metronidazole enhanced clearance of cysts and decreased the mean duration of diarrhea, fever and abdominal pain [Dinleyici et al. 2009].

In a small clinical study of symptomatic children with *Blastocystis hominis* infection *S. boulardii* had potential beneficial effects in symptoms and number of parasites [Dinleyici et al. 2011].

The combination therapy of *S. boulardii* in addition to metronidazole in patients with giardiasis resulted in a disappearance of *Giardia* cysts 2 weeks after start of the treatment in contrast to
17.1% of patients treated with 10 days metronidazole as monotherapy who still had Giardia lamblia cysts in the stool [Besirbellioglu et al. 2006]. In another clinical trial of 40 children who received tinidazole for giardiasis of 3 or 4 weeks duration, the percentage of children with only one to three bowel movements per day was significantly higher in the S. boulardii group compared with the placebo group (65% versus 15%) [Castaneda et al. 1995]. However, all of the studies regarding use of S. boulardii for treatment of parasitic infections are small and the reported results need to be confirmed by larger studies.

**HIV-related diarrhea**

Patients with HIV-associated diarrhea seem to be one group that requires a higher than typical dose of S. boulardii. In a blinded, placebo-controlled study in 11 HIV-positive patients who had chronic diarrhea, lower doses of S. boulardii were not as effective compared with 6 patients who reported that diarrhea was controlled while taking 3 g/day S. boulardii after 1 month [Elmer et al. 1995]. In a RCT of 35 adult patients with acquired immune deficiency syndrome (AIDS) and chronic diarrhea, 61% of patients given S. boulardii had their diarrhea resolved compared with patients on placebo (12%) [Saint-Marc et al. 1991]. Further confirmation on whether higher doses of S. boulardii may benefit patients with HIV-related diarrhea is needed since this observation is based on very limited data.

**Safety of Saccharomyces boulardii as a probiotic**

Although no adverse effects were observed in any of the clinical trials with S. boulardii, the administration of S. boulardii is not without risk. A recent systematic review documented that probiotic products such as S. boulardii, have been shown to increase the risk of complications in specific patient groups such as immunocompromised subjects [Whelan and Myers, 2010]. Saccharomyces fungemia is the most severe complication secondary to administration of the probiotic especially in patients with severe general or intestinal disease who had an indwelling catheter [Hennequin et al. 2000].

Up to now, almost 100 cases of S. boulardii-associated fungemia have been reported [Vandenplas et al. 2009]. The origin of the fungemia is thought to be either a digestive tract translocation or a contamination of the central venous line by the colonized hands of health workers [Hennequin et al. 2000; Herck et al. 2004]. Indeed, the simple act of opening a packet of S. boulardii can produce air contamination that may pose environmental risk especially for immunocompromised patients [Hennequin et al. 2000]. Once the diagnosis is made, fungemia with S. boulardii can effectively be treated with antimycotic medication, although treatment failure with fluconazole has been reported [Burkhardt et al. 2005].

A rare gastrointestinal allergic reaction was also recently reported after S. boulardii was given to an infant with a prior diagnosis of food protein-induced enterocolitis syndrome [Hwang et al. 2009].

Overall, S. boulardii is safe for use in otherwise healthy populations and fungemia with S. boulardii has not been reported, to the best of the authors’ knowledge, in immunocompetent patients. Caution should be taken in patients with risk factors for adverse events (e.g. patients with central venous catheters or increased bacterial translocation) [Venugopalan et al. 2010]. Institutional guidelines are needed to address the potential safety issues related to S. boulardii use [Venugopalan et al. 2010].

**Conclusions**

Several clinical trials and experimental studies displayed the role of S. boulardii as a good biotherapeutic agent allowing to prevent and/or treat several gastrointestinal diseases. S. boulardii mediates effects which resemble the protective effects of the normal healthy gut flora. Although the administration of S. boulardii can be associated with fungemia, no adverse effects were observed in any of the clinical trials. Caution should be taken in patients with risk factors for adverse events, such as immunocompromised patients. Larger prospective, placebo controlled clinical trials could elucidate the mechanisms of action of the yeast and suggest new therapeutic applications.

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**Conflict of interest statement**

Dr Kelesidis has no conflicts of interest to declare. Dr Pothoulakis was a paid consultant with Merck and Optimer Pharmaceuticals and a paid speaker for the Postgraduate Institute for Medicine.
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Review Article

Extending life span by increasing oxidative stress

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Abstract

Various nutritional, behavioral, and pharmacological interventions have been previously shown to extend life span in diverse model organisms, including Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster, mice, and rats, as well as possibly monkeys and humans. This review aims to summarize published evidence that several longevity-promoting interventions may converge by causing an activation of mitochondrial oxygen consumption to promote increased formation of reactive oxygen species (ROS). These serve as molecular signals to exert downstream effects to ultimately induce endogenous defense mechanisms culminating in increased stress resistance and longevity, an adaptive response more specifically named mitochondrial hormesis or mitohormesis. Consistently, we here summarize findings that antioxidant supplements that prevent these ROS signals interfere with the health-promoting and life-span-extending capabilities of calorie restriction and physical exercise. Taken together and consistent with ample published evidence, the findings summarized here question Harman’s Free Radical Theory of Aging and rather suggest that ROS act as essential signaling molecules to promote metabolic health and longevity.

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Calorie restriction

Calorie restriction (CR), i.e., a reduction in ad libitum calorie uptake by 10 to 50%, represents the most convincing intervention to retard aging and attenuate age-related disease in multiple species. Since 1935, when McCay initially described the influence of CR on life expectancy, it has been frequently demonstrated that CR is able to increase the median and maximal life span in a variety of organisms, suggesting a conserved underlying mechanism [1,2].

Although CR clearly reduces risk factors associated with aging in humans, including type 2 diabetes and cardiovascular diseases, it is still a matter of debate whether CR is capable of increasing life expectancy of humans [3–5]. A recent study in nonhuman primates found no significant effect of CR on overall mortality. However, arbitrarily defined “age-related mortality” (which moreover explained only 54% of deaths) was decreased in those monkeys. Most interestingly and contrasting with ad libitum-fed animals, monkeys on CR did not show any impairment in glucose homeostasis, strikingly reducing the prevalence of metabolic disorders such as type 2 diabetes [6]. Thus, it seems possible that CR is also sufficient to improve the life span of humans, which is also supported by additional findings [3–5,7,8].

The concept of CR was initially based on the assumption that lowering caloric intake would result in a subsequent reduction of the
metabolic rate. Hence, it was postulated at the beginning of the 20th century that the maximum life span of an organism is inversely proportional to the nutritive energy metabolized [9]. Consequently, Pearl’s Rate-of-Living Hypothesis, formulated soon after, suggests that increased metabolic rate results in decreased life span in eukaryotes [10].

A feasible molecular cause for this hypothesis was proposed in 1956 by Harman, who connected metabolic activity, especially that of respiratory enzymes, with the formation of potentially harmful reactive oxygen species (ROS) [11]. Accordingly, increased metabolic rate would promote ROS formation, which subsequently causes damages within the cell and beyond. The accumulation of these damages results in age-related decline of cellular functions and ultimately to death of the organism [11]. Up to now, this so-called Free Radical Theory of Aging (FRTA) has become a popular and frequently cited theory in aging research [12].

However, more recent findings regarding the question whether CR actually decreases metabolic rate are, at least in part, inconsistent with FRTA. Hence, it has been reported that CR increases metabolic rate (quantified by both oxygen consumption and heat production) in the nematode and well-established model organism for aging research, Caenorhabditis elegans [13]. Furthermore, a positive correlation between low metabolic rate and enhanced life span could also not be observed in the fruitfly Drosophila melanogaster [14].

Despite the fact that CR has been extensively investigated in a broad range of species, the underlying mechanisms are still elusive. As mentioned above, it is commonly accepted that CR is able to retard the onset of a variety of diseases related to aging, including cardiovascular diseases, type 2 diabetes, and cancer. Therefore, CR-mediated prevention of chronic and ultimately life-threatening disorders that reduce longevity could be the reason for the life-span-extending effects of CR. Additionally, it has been shown that CR itself stimulates molecular processes that diminish age-associated disease as well as improving life expectancy. Accordingly, it was frequently reported that CR induces defense mechanisms, especially those that are involved in ROS detoxification such as radical-scavenging enzymes [15–22] and possibly beyond, including phase II response enzymes. This association of CR on the one hand and increased antioxidant defense on the other has been commonly misinterpreted as being caused by a primarily decreased ROS production in states of CR. Conversely, and as explained in more detail below, more recent investigations suggest that adaptive response mechanisms seem to be the cause of the aforementioned beneficial alterations unquestionably initiated by CR [23–27].

Reduction of specific macronutrients

Macronutrients are represented by carbohydrates, triglycerides, and proteins, which, after experiencing enzymatic breakdown, are ultimately metabolized as monosaccharides (such as glucose), fatty acids, and amino acids, respectively. They provide the bulk of energy required by the organism. In this regard it should be noted, however, that only glucose can be metabolized in the absence of oxygen. In contrast, ATP generation using fatty acids and some amino acids requires mitochondrial oxidative phosphorylation (OxPhos) and therefore oxygen. Inversely, only metabolism of glucose can generate ATP independent of mitochondrial organelles and hence without promoting ROS production.

So far, only a few studies have investigated the question whether restricting a single macronutrient can cause a response comparable to that seen in states of general CR. Whereas restriction of triglyceride uptake in invertebrates has not been examined yet, restriction of lipids in mice without CR does not influence life span [28].

The influence of dietary protein levels on life span has been investigated primarily in D. melanogaster and rodents. Accordingly, it was shown that reduction of nutritive protein content results in extension of life expectancy in mice [29–31]. Similarly, casein restriction prolongs life span in D. melanogaster [32]. On the other hand, supplementation of essential amino acids, especially methionine, abolishes the life-span-extending effect of CR in flies [33]. Interestingly, methionine restriction in rodents has been shown to exert antiaging properties and improves tissue-specific mitochondrial biogenesis as well as aerobic capacity [34–36], whereas high protein intake results in increased lipid peroxidation and reduced superoxide dismutase activity [37]. Consistently, impaired peptide transport extents life span in C. elegans [38].

In apparent contrast to the above-mentioned fact that ATP generation from glucose is capable of avoiding ROS production, glucose restriction has been found to be beneficial in various lower organisms as well as in rodents. In D. melanogaster, for instance, restriction of sugar reduces mortality and extends life span [39]. The same applies for the model organism Saccharomyces cerevisiae, in which depletion of glucose results in life-span extension dependent on induction of respiration as well as on sirtuins [40,41]. However, whether sirtuins are involved is still a matter of debate [42–45]. Accordingly, sirtuin-independent pathways have been discussed [22,46].

Although it is generally difficult to restrict dietary glucose in eukaryotic organisms such as C. elegans or rodents, the use of 2-deoxyglucose (DOG) is frequently reported to achieve depletion of glucose metabolism [47]. DOG is a synthetic glucose analogue that inhibits glycolysis in a competitive manner due to its inability to be further metabolized after conversion into deoxyglucose 6-phosphate [48]. Application of DOG was shown to mimic a ketogenic diet (very low carbohydrate diet) as well as metabolic hallmarks of CR in rodents [49–51]. It is therefore commonly accepted that DOG represent a powerful CR-mimetic compound [52–55].

DOG exposure results in decreased glucose availability and life-span extension in C. elegans [23], whereas it does not extend life span in rats [56]. Notably, and similar to the above-mentioned findings in S. cerevisiae, glucose restriction in C. elegans not only promotes life span but also increases oxygen consumption [23]. However, and in contrast to yeast, in nematodes sirtuins seem not to be involved [23]. It was suggested instead that the underlying mechanism in regard to life-span prolongation is dependent on AMP-activated kinase (AMPK) [23]. AMPK is assumed to be a central key regulator of energy metabolism within the cell [57]. Functionally similar AMPK orthologues have been found in lower organisms such as worms and flies, suggesting a highly conserved mechanism [58–60]. Metabolic stress, e.g., cellular lack of energy, activates AMPK, which in turn up-regulates energy-producing processes such as mitochondrial biogenesis leading to neutralization of the energy deficit, possibly with additional health-promoting implications [57]. Consistently, applying metformin, a long-standing antidiabetic drug, to C. elegans activates AMPK and subsequently promotes adaptive processes involved in CR and oxidative stress response, culminating in extended life span [61].

As an alternative approach to influencing intracellular glucose concentrations in mammals, mice with impaired GLUT-4 transporters in muscle and adipose tissue were established. These mice show typical metabolic switches such as fasting hyperglycemia, glucose intolerance, increased fatty acid turnover, and utilization. However, life span (examined up to 18 months of age) was not affected [62]. Increased cellular glucose availability due to overexpression of GLUT-4, on the other hand, was also shown to lack any effect regarding extension of life span [63]. In addition, increased glucose abundance in C. elegans, examined in three independent studies, reduces life span significantly [23,64,65].

In humans, varying the relative amounts of macronutrients within diets has been postulated to be health beneficial in regard to obesity and cardiovascular disease prevention. Although low-carbohydrate/high-protein diets are as efficient as low-fat/high-carbohydrate diets in regard to weight loss, serum parameters known to determine...
cardiovascular risk were shown to be positively influenced by a reduction in dietary carbohydrate consumption [66–68]. Very low carbohydrate diet has been also demonstrated to reduce several inflammation markers in overweight men and women with atherogenic dyslipidemia [69]. However, more research, especially long-term studies, is needed to evaluate the putative effect of low-carbohydrate diets on human health.

**Impaired insulin/IGF-1 signaling**

In mammals, insulin and IGF-1 represent peptide hormones produced in pancreatic β-cells and liver, respectively. Insulin is a regulator of the peripheral glucose metabolism, most notably glucose uptake. In addition, insulin is also involved in other metabolic processes such as fat metabolism. IGF-1 is produced as a consequence of growth hormone (GH) (also called somatotropin) release from the pituitary gland, which stimulates subsequently IGF-1 production in the liver. IGF-1 is therefore a mediator for some of the GH functions, thus involved in growth and anabolism. Insulin, IGF-1, and GH mediate their effects by binding to specific and distinct receptors in mammals.

Mice with reduced GH and/or IGF-1 signaling exhibit dwarfism with a phenotype that is comparable to those of mice exposed to CR [70]. As shown for CR, those mice are also long-lived [71]. Conversely, increasing GH availability leads to improved body size and diminishes life expectancy [72,73]. Furthermore, heterozygote impairment of the IGF-1 receptor signaling in the entire animal, as well as impairment of the IGF-1 receptor in neurons, results in life-span extension in mice by preventing neurodegenerative processes [74,75]. Conversely, long-term IGF-1 exposure leads to mitochondrial dysfunction and reduced cell viability in human cell culture [76].

Down-regulation of insulin receptor activity in humans is assumed to be a cause for insulin resistance. This state is defined as an inappropriate reduction in the intracellular response to extracellular insulin [77]. Consequently, a reduction in GLUT-4-mediated glucose uptake, which represents a key insulin response, occurs. Therefore, intracellular glucose availability is reduced in subjects suffering from insulin resistance [78].

However, despite the fact that global disturbance of the insulin receptor in mice results in a prenatally lethal phenotype, musclespecific knockout mice experience neither hyperglycemia nor diabetes. Instead, a remarkable rise in fatty acid turnover has been observed [79]. Although life-span data on these mice are unavailable, disruption of the insulin receptor in adipose tissue only causes prolongation of life span [80]. Moreover, disruption of the insulin receptor substrate 1 (IRS-1), which is localized downstream of both the insulin and the IGF-1 receptors, is associated with murine longevity as well as knockouts of neuronal IRS-2 and heterozygous global IRS-2 [81,82].

Moreover, and as initially published more than 20 years ago, impaired insulin/IGF-1 signaling strikingly prevents aging in invertebrates. Whereas in mammals insulin and IGF-1 bind to specific and distinct receptors, in *C. elegans* and *D. melanogaster* insulin and IGF-1 signaling is limited to one receptor. Hence, mutations in the corresponding receptor orthologues as well as in downstream components were shown to be life-span extending in worms and flies in a manner even more pronounced than in mammals [83–87]. *C. elegans* daf-2 mutants, which show impaired activity of the orthologue of the mammalian insulin/IGF-1 receptor, live twice as long as wild-type nematodes [84]. Although it is not known whether glucose uptake or intracellular glucose availability is affected in this regard, a very recent work on daf-2 mutants indicates that the age-associated decline in mitochondrial activity, e.g., mitochondrial protein content and energy supply, is delayed in comparison to wild-type animals [88].

In summary, it seems that reduction of the insulin receptor as well as insulin receptor substrate below a certain threshold contributes to longevity in a variety of organisms, including worms, flies, and mice. This may be also relevant to humans because mutations of insulin/IGF-1 signaling have been linked to regulation of life expectancy in various cohorts [89,90].

Whether reduced insulin/IGF-1/GH signaling lengthens life span in the same manner as CR is an ongoing matter of debate. Although several studies have demonstrated independent mechanisms, others have proposed that similar pathways and processes are initiated by both interventions [59,91–101]. Based on the assumption that mutations associated with impaired insulin/IGF-1 signaling cause reduced intracellular glucose availability, it seems likely that subsequent effects are comparable to those seen in glucose-restricted model organisms, at least in regard to metabolic shifts and also possibly life-span-extending mechanisms. Although to date direct evidence is missing, some studies provide support for this hypothesis [102–106].

**Induction of mitochondrial metabolism by calorie/glucose restriction**

In general, mitochondria are cellular organelles that provide the bulk of energy within the cell. ATP generation due to mitochondrial OxPhos is considerably more efficient in comparison to nonoxidative metabolism of glucose and some amino acids. Whereas glycolytic breakdown of 1 mol of glucose generates 4 mol of ATP, its oxidative metabolism produces 30 mol of ATP. Mitochondria also produce ROS as a by-product of OxPhos. Thus, being the main producer of cellular energy as well as a source of potentially harmful ROS, mitochondria appear to exert a central role in physiological and pathophysiological processes.

Accordingly, mitochondrial dysfunction is associated with the onset of age-related diseases such as diabetes, cancer, and neurodegeneration [107–110]. Furthermore, impairment of mitochondrial activity is assumed to be a main cause of the aging process [111,112]. Whether this decrease in mitochondrial capacity is linked to altered production of mitochondrial ROS seems questionable.

Although a few studies suggested that overall net calorie uptake during the lifetime is unaltered in CR [39,113], it is commonly accepted and agreed upon that by definition calorie/glucose restriction causes a reduction in available nutritive energy. This short-term energy deficit has been proposed to induce mitochondrial activity to counteract the energy depletion. Accordingly, calorie/glucose restriction causes an increase in mitochondrial respiration in yeast and worms [23–25,40]. Enhanced mitochondrial activity is, as shown in these studies, associated with life-span extension [23–25,40]. Furthermore, CR promotes mitochondria biogenesis and OxPhos in rodents as well as enhancement of respiratory capacity in mammalian cells [114,115]. These results are in line with the observation that energy expenditure as a function of body mass is unexpectedly increased in calorie-restricted rats [116]. Moreover, as mentioned before, reduced insulin/IGF-1/GH signaling stimulates mitochondria metabolism in rodents [102,104–106]. In addition, an abundant supply of branched-chain amino acids increases mitochondrial biogenesis and promotes longevity in yeast and mice [117,118]. Finally, further interventions that induce mitochondrial activity, such as pharmacological treatments and physical exercise, are capable of improving life span [119–123].

In contrast, and as mentioned before, reduced mitochondrial activity has been shown to decrease life span in various organisms such as *S. cerevisiae*, *C. elegans*, and rodents [124–126].

In regard to proposed mechanisms involved in the activation of mitochondrial metabolism some key cellular regulators have been frequently reported, including the previously mentioned sirtuins and AMPK. Activation of these proteins is associated with increased mitochondria activity. In contrast, impairment of another nutrient-sensing pathway, mTOR (mammalian target of rapamycin), was
shown to extend life span in *S. cerevisiae* by inducing mitochondrial metabolism [127,128]. Consistently, the translational inhibitor 4E-BP, which is repressed by TOR, regulates mitochondrial activity in CR flies [129]. Furthermore, TOR signaling has been shown to be regulated by AMPK, suggesting that both nutrient-sensing pathways are located upstream of mitochondria function, thereby representing key regulators of mitochondrial metabolism [130].

Taken together, there are numerous studies linking mitochondrial activity with prolongation of life expectancy, suggesting that a metabolic switch to oxidative metabolism seems to be beneficial in regard to delay aging and the onset of age-related diseases.

**Oxidative stress and mitochondrial hormesis (mitohormesis)**

Increased ROS formation as a consequence of increased metabolic rate has been postulated to be the major determinant of life span [11]. Because mitochondria are an intracellular source of ROS, the theory was extended to the mitochondrial free radical theory [131], without the knowledge that meanwhile the fact that increased metabolic rate does not necessarily result in increased ROS formation had been established. Thus, significant research has been done to prove this hypothesis with inconsistent and partly contradictory results [132]. However, a considerable number of findings in various organisms suggest that reduction of oxidative stress is associated with prolongation of life expectancy [133–147]. Consequently, ROS-lowering interventions were widely proposed as an antiaging strategy in humans. Antioxidants, a group of synthetic or naturally occurring substances, which are capable of scavenging free radicals, were extensively examined in that regard. Unexpectedly and in contrast to some of the above-mentioned work in lower organisms, several prospective clinical intervention studies were unable to show a positive association between supplementation with antioxidants and health-beneficial effects. Whereas most studies found a lack of effect in regards to health promotion in humans [148–162], other reports even suggest that antioxidants may promote cancer growth [163–168]. Moreover, supplementation with antioxidants has been linked to increased incidence of a number of diseases with adverse effects on human longevity [169–175].

Not surprisingly, these findings question Harman's FRTA and require a different point of view concerning the role of mitochondrial ROS formation. Accordingly, numerous findings have emerged in recent years indicating that ROS may evoke cellular signaling that promotes metabolic health and longevity. It has been assumed that they serve as essential signaling molecules delivering messages from the mitochondria to other cellular compartments in response to physiological or pathophysiological changes [23,176–190]. Moreover, and given the increased levels of oxidative damage during increasing age, intrinsic aging may be considered an insufficient ability to respond to endogenous ROS signals.

Interestingly, exposure of *C. elegans* to hyperbaric conditions results in stress resistance and prolongation of life expectancy, whereas such conditions cause an increase in mitochondrial ROS formation [191–194]. Hypothermia, a state that is associated with extend life span in mice and *C. elegans* [195,196], has been recently shown to induce mitochondrial ROS production as well [197]. Moreover, it was shown that CR also induces low-level stress leading to the same adaptive processes, such as increased stress resistance and longevity [21,26,198–200].

These findings insinuate that so-called adaptive response processes may explain how increased ROS formation culminates in promotion of health and life span. Interestingly, low doses of ROS seem to exert such effects, whereas higher doses are unquestionably detrimental. Such biphasic responses to a potentially harmful compound are commonly named hormesis, a concept that was initially postulated in 1943 by Southam and Ehrlich and which was shown to have significant impact on aging with a variety of stressors described [201–205]. Later, this term was extended to mitochondrial hormesis or mitohormesis, with regard to mitochondrial ROS as a hypothetically sublethal stressor [206].

In agreement with this concept, it has been frequently reported that rodents exposed to CR exhibit elevated antioxidant defense capacities [15–20,207]. Furthermore, life-extending glucose restriction in yeast was shown to be accompanied by a decrease in ROS production, whereas respiration was enhanced [22]. On the other hand and in conflict with these data, it was also reported that the same intervention in the same model organism increases ROS production as well as respiration [23–25,43,208,209]. Moreover, antioxidant enzyme activity was found to be elevated as well [24,43,208,209], suggesting a potential involvement of increased respiration, enhanced ROS formation, and the induction of ROS defense mechanisms in regard to regulation of longevity.

Consistently, numerous studies using various model organisms were unable to find any evidence to support that lowering ROS is beneficial in regard to longevity, nor that increasing antioxidant capacity extends life span [210–227]. Moreover, life-span-extending mutations in *C. elegans* are commonly accompanied by increased stress resistance and sometimes paralleled by enhanced metabolic activity [228–233]. Furthermore, in the field of neuroprotective research, similar hormetic results were achieved with CR as well as DOG application in rodents [234]. Depletion of mitochondrial NADH kinase, an enzyme crucial for antioxidant defense, causes life-span extension and DNA stability due to adaptive mechanisms in *Podospora anserine* [235]. Finally, human subjects on a carbohydrate-depleted diet (i.e., a ketogenic diet) show improved ROS defense capacity presumably due to elevated oxidative metabolism [236].

Taken together, all these findings provide indirect evidences for the hypothesis that ROS production and subsequent induction of ROS defense are essential contributors to longevity. To prove this hypothesis, the previously described inhibitor of glycolysis, DOG, was applied to *C. elegans*, resulting in a decrease in glucose availability followed by a compensatory increase in respiration [23]. The increase in oxygen consumption was associated with an increase in ROS formation and a consequent induction of antioxidant enzyme activity, finally leading to life-span extension [23]. Most importantly, simultaneous treatment with various antioxidants completely abolished this life-span-extending effect of DOG, suggesting that an increase in ROS formation is essential for CR-induced promotion of longevity [23].

These findings were corroborated by very recent studies that examine the effect of CR in *S. cerevisiae* and *Schizosaccharomyces pombe* [24,25,27]. Correspondingly, an increased mitochondrial respiration and/or a subsequent enhanced ROS production after CR were observed [24,25,27]. Hence, similar to the above-mentioned observations in *C. elegans*, activation of stress response pathways as well as induction of defense mechanisms has been discussed as representing the underlying life-span-extending mechanisms [24,25,27,188–190]. It should be noted that endogenously produced ROS presumably not only induce ROS defense enzymes, but also increase activities of phase II response enzymes that protect from damage beyond ROS. On a hypothetical basis this would explain the clearly opposite effects of supplementation with exogenous antioxidants and/or genetic overexpression of antioxidant enzymes, on the one hand, and endogenous response to endogenous ROS production on the other hand. Future research will also have to investigate whether response mechanisms to stressors such as endogenous ROS may be less likely to be activated at higher age.

**Physical exercise**

Consistent with the concept of mitohormesis, glucose restriction leads to an increase in mitochondrial activity accompanied by an increase in respiration-derived ROS formation that serves as a mild stressor (Fig. 1). This ROS signal is able to induce conserved downstream processes (such as activation of specific oxidative stress-
that may in physical activity share, at least in part, common mechanistic features support the conclusion that CR, glucose restriction, and moderate physical activity, an intervention that is known to be health beneficial in a broad spectrum [120,121,237–239], is assumed to cause induction of mitochondrial metabolism and ROS production [240–242]. Moreover, health-promoting effects were demonstrated to be reduced if subjects exposed to physical activity were cotreated with antioxidant supplements [186,243].

Conclusions

 Taken together, the data summarized and discussed in this review support the conclusion that CR, glucose restriction, and moderate physical activity share, at least in part, common mechanistic features that may influence the aging process, i.e., enhanced mitochondrial activity and subsequently increased ROS formation that ultimately induce an adaptive response (increased defense mechanisms and improved stress resistance), which culminates in metabolic health and extended longevity.

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