Mitochondria Inspire a Lifestyle

Peter Kramer and Paola Bressan

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P. Kramer
Dipartimento di Psicologia Generale, University of Padova, Padova, Italy
ORCID: 0000-0003-4807-7077
e-mail: peter.kramer@unipd.it

P. Bressan
Dipartimento di Psicologia Generale, University of Padova, Padova, Italy
ORCID: 0000-0003-2763-0902
e-mail: paola.bressan@unipd.it

Abstract

Tucked inside our cells, we animals (and plants, and fungi) carry mitochondria, minuscule descendants of bacteria that invaded our common ancestor two billion years ago. This unplanned breakthrough endowed our ancestors with a convenient, portable source of energy, enabling them to progress towards more ambitious forms of life. Mitochondria still manufacture most of our energy; we have evolved to invest it to grow and produce offspring, and to last long enough to make it all happen. Yet because the continuous generation of energy is inevitably linked to that of toxic free radicals, mitochondria give us life and give us death. Stripping away clutter and minutiae, here we present a big-picture perspective of how mitochondria work, how they are passed on virtually only by mothers, and how they shape the lifestyles of species and individuals. We discuss why restricting food prolongs lifespan, why reproducing shortens it, and why moving about protects us from free radicals despite increasing their production. We show that our immune cells use special mitochondria to keep control over our gut microbes. And we lay out how the fabrication of energy and free radicals sets the internal clocks that command our everyday rhythms—waking, eating, sleeping. Mitochondria run the show.

Keywords: Mitochondria, Free radicals, Antioxidants, Circadian rhythms, Dietary restriction, Longevity

> Now, here, you see, it takes all the running you can do, to keep in the same place.
> — *Lewis Carroll, Through the Looking-Glass and What Alice Found There*

1 Pump or Perish

Mitochondria were once bacteria that invaded one-cell creatures from another domain of life, and that happened to let out as waste precisely those gasses that the host creatures used to sustain themselves (Martin et al 2016). The cohabitation went spectacularly well and the guest bacteria ended up relinquishing part of their genetic material to the host cell—which out of it built a control center, a "nucleus"—while keeping only a few genes to themselves. This unplanned breakthrough endowed the hosts with a convenient, portable source of energy, making it possible for them to evolve into more ambitious forms of life (of which we are one).

From their bacterial ancestor, mitochondria have inherited the ability to produce energy via an intricate mechanism whose details differ little across the various kingdoms. Digested food is burned to fuel a machine that, from ADP (adenosine *di*phosphate) and an extra phosphate, forges ATP (adenosine *tri*phosphate). The freshly coined ATP is then dispatched from the mitochondrion to the host cell. Wherever the cell needs it, energy is released from ATP by stripping it of a phosphate. This leaves behind ADP and phosphate, that within the mitochondrion can be recharged to ATP again. What is exquisitely elaborate is how mitochondria put the plan into effect.

Each mitochondrion features a fairly smooth outer membrane, that separates the mitochondrion from the rest of the host cell, and a much more convoluted and interesting inner membrane; together they enclose the intermembrane space (Figure 1). The inner membrane is important enough that an average human carries 14,000 m^2 of it (Rich 2003). Plugged through the membrane are *complexes*, minuscule pieces of machinery dedicated to the job of ATP generation; a single mammalian mitochondrion accommodates legions of them, perhaps in the order of 30,000 (Lane 2005). These are organized into sets of four consecutive stations. In the space in the middle of the mitochondrion, the matrix, equal numbers of protons and electrons are stripped from the molecular remains of digested food; the electrons are separated from the protons and fed into the first machine, called complex I. From there, the electrons are shuttled along the inner membrane through two further stations[1] and finally disgorged back into the matrix, where they are sucked up by oxygen.

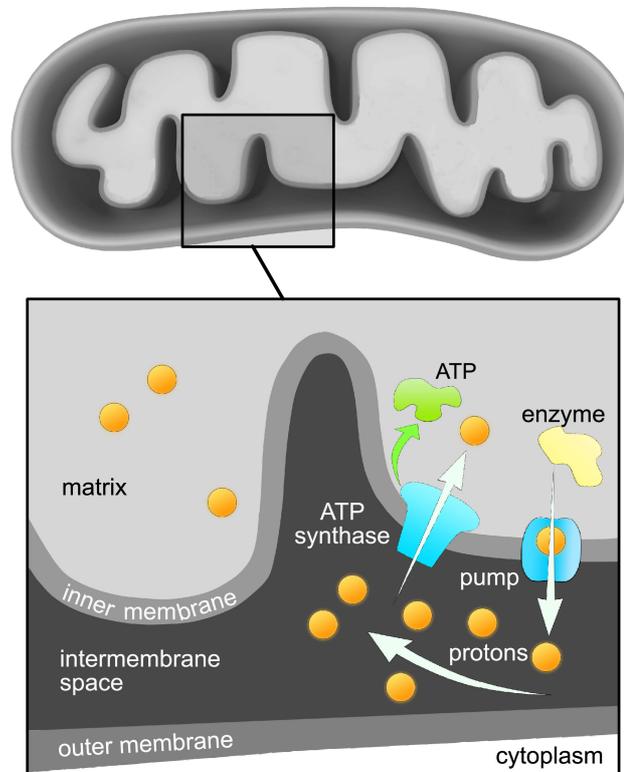[1] Only two, because one of the four stations is just an alternative entry point for electrons.

Fig. 1 Schematic, simplified representation of a mitochondrion. Inset: in the matrix (light gray), an enzyme (yellow) delivers protons (orange) and electrons stripped from digested food to a proton pump (blue shape on the right; electrons and additional stations are not shown). The flow of electrons within the pump provides the energy to make it transfer protons from the matrix to the intermembrane space (dark gray). The protons can then return from the intermembrane space to the matrix via the ATP synthase (mushroom-like blue shape on the left). In the process, the ATP synthase is pressed into service and produces ATP (green). Image source: Bressan (2018).

The passage of electrons *along* the membrane only serves to power the passage of protons *across* it—which is indeed the whole point of the arrangement. Making its way within each station, an electron creates disturbances that end up opening, one after the other, a series of channels across the membrane. Each channel stays open just long enough to let a proton pass through and shuts closed right behind it. The end result is that the protons are transferred from one side (the matrix) to the other (the intermembrane space) of a membrane that is otherwise sealed off to them. For every pair of electrons that reaches the end of the chain to be snatched up by oxygen, ten protons have been pumped across the membrane.

Each proton carries one positive charge. Thus, the amassing of protons on one side of the membrane produces a large difference in electrical charge, with the outside (the

intermembrane space) becoming strongly positive relative to the inside (the matrix). The voltage difference across the membrane amounts to one tenth of that of an alkaline watch battery, but because the membrane is extraordinarily thin, we are talking about an electric field strength in the order of 30 million volts per meter, much like a bolt of lightning (Lane 2005). As the number of protons builds up, the difference in proton concentration and electrical charge between the two sides tends to pull the protons from the intermembrane space to the matrix. Protons are held back by the membrane at all places, however, except at specific stations[2]. Each of these stations accommodates one *ATP synthase*, a molecular machine impressively similar to man-made rotary motors, except that, with a velocity of up to several hundred revolutions per second (Ueno et al 2005) and an efficiency of very nearly 100% (Omote et al 1999), it outperforms them all. An ATP synthase removed from a bacterium and integrated in a nickel nanopropeller has been shown to power it splendidly (Soong et al 2000), suggesting that the fabrication of nanomachines from live biological materials is the stuff of science fiction no longer (Strong 2004).

The ATP synthase looks a bit like a mushroom with a rotating stalk embedded within the mitochondrial inner membrane and a head protruding into the matrix. Protons make the stalk rotate by flowing through channels in it, and this movement turns—a third of a full rotation at a time—a stalk-connected rotor sitting within the head. This sequence of turns prompts the head to perform a fixed sequence of operations. At the first turn the head clasps ADP and phosphate; at the second it forces them together to make ATP; at the third it releases the newly-formed ATP (Boyer 1975). A full rotation requires 10 protons and delivers 3 molecules of ATP. The ATP can now leave the matrix, through a dedicated transporter embedded in the membrane that grabs the ATP molecule on the matrix side and deposits it on the other side—a result similar to that achieved by a bank's sliding security doors. The portal is now ready to do the same job in reverse: receive ADP via the open external door, lock it in and release it on the matrix side by opening the internal door. A separate entrance admits phosphate; upon reaching the matrix, ADP and phosphate are on call to be recharged to ATP again.

When all is well, the electrons flow smoothly down the route to oxygen by hopping from one site to the next, in single file, within each complex. But if they are too slow, the chain becomes clogged up with them; each is unable to move on because the next site is already taken. This can happen, for example, when there is no ADP left in the cell to be converted into ATP, perhaps because ATP is not being consumed fast enough and is thus not broken down into ADP again. An electron stuck in the chain can escape from it onto an oxygen molecule or another chemical nearby. All chemicals contain one or more nuclei, made of protons and neutrons, and a number of electrons orbiting around them. While

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[2] Some protons are allowed to leak back into the matrix through pores in the membrane. This transfer reduces the difference in proton concentration on either side of the membrane and dissipates as heat part of the energy that was pent up in it. In warm-blooded animals, this comes in handy to maintain body temperature (Brand et al 1991).

circling around, each electron spins around its own axis and typically has a companion electron that spins in the opposite direction. By sucking up an extra electron from the electron transport chain, the chemical acquires an unpaired electron, and this turns it into a free radical.

Free radicals are extraordinarily corrosive, first and foremost to the mitochondrion that produces them but also to the cell that hosts the mitochondrion and, by extension, to the organism that hosts the cell. Free radicals have a way of reacting with whatever molecules happen to be nearby, including mitochondrial genes which in this way accumulate damage by collecting mutations. An avalanche of free radicals thus bodes no good, but mitochondria have evolved to use it as a smoke alarm—a signal that electrons are not being transferred properly along the chain. In fact, complex I might be deliberately set up so that electrons can leak from it at the slightest hint that the chain is blocked (Lane 2015). In response to a massive release of free radicals within it, a mitochondrion strives to take countermeasures. For example, it can increase the number of respiratory complexes so as to raise ATP production and tailor it to demand, or it can divide and multiply. If demand cannot be met, the inept mitochondrion may go on to commit suicide, freeing space for newcomers, or, if its host cell has incurred too much damage, it may proceed to kill the entire cell. The vital feedback role of free-radical leakage in improving mitochondrial function can help explain, as we shall later see, why antioxidants supplements not only fail to prevent disease but can be downright dangerous.

2   All About Eve

Despite that 99% of mitochondria's components are manufactured by around 1500 nuclear genes and 1% by 13 mitochondrial ones (Rahman and Rahman 2018), it is mitochondrial genes that oversee the control system of energy production (Johnston and Williams 2016). Just like local matters are best left to local rather than national governments, local diversity in energy requirements is best addressed by genes in the locally active mitochondria rather than by those in the faraway nucleus. In response to a smoke alarm, for example, the cell nucleus would have no clue as to which particular mitochondrion is responsible and thus needs to be upgraded or replaced.

Mitochondrial genes do sit very close to where energy is locally produced. Unfortunately, this means they also sit very close to where free radicals are locally released, hence they might accumulate mutations faster than they would if they were stationed in the nucleus. ("Might", because mitochondrial genes appear to be physically shielded and painstakingly defended from such assaults: Kauppila and Stewart 2015.) Because mitochondria do get heavily tarnished with use, it comes as something of a surprise that those that are inherited by each new generation tend to carry no damage whatsoever. Every bit as surprisingly, the mitochondria that do get passed on—in the vast majority of species

including ours—come entirely from mothers (Pyle et al 2015). Together, human mothers and fathers hand down a set of about 20,000 nuclear genes; in addition, the fertilized egg houses 100,000 live mitochondria (Jansen 2000) exclusively from the mother, each featuring multiple sets of 37 mitochondrial genes.

To make sense of these two surprises, it has been proposed that the mitochondria in egg cells are prevented from ever producing any energy and are simply kept in storage (Allen 1996; Allen and de Paula 2013; de Paula et al 2013). They will be used as templates for producing new mitochondria after transmission to the offspring; in female embryos, some of them will be sequestered away in the eggs sitting in the tiny, still developing ovaries. These pristine mitochondria will then be passed on to the next generation—and so on and so forth, from mother to daughter. Eggs may afford to waive active mitochondria because they do nothing much, and can thus subsist on energy coming from less efficient sources or from the mitochondria of adjacent cells in the ovary. Sperms cells, instead, cannot just sit around; they must swim as fast as they possibly can to arrive first and get to fertilize the egg cell. Their mitochondria must deliver a flawless performance, at the cost of getting corroded by free radicals in the process. Thus, whereas egg cells can keep their mitochondria pure and fresh, sperm cells cannot.

To be prevented from being passed on, sperm mitochondria are labeled as unworthy right from the start and are promptly destroyed when they run into the egg's destruction machinery (Sutovsky et al 1999). Apparently, the harm that they would do to the offspring is so great that fathers cooperate biologically in having them all dismantled upon arrival in the egg. Across species, indeed, a staggering diversity of techniques (Sato and Sato 2013) has evolved to dispense with male mitochondria (including that of defecating them soon after birth: Pitnick and Karr 1998), suggesting that selection pressures favoring uniparental inheritance appeared on the scene repeatedly and independently. One wonders if the problem is only with paternal mitochondria being subpar, or if inheriting two different sets of mitochondrial genes is undesirable in and of itself. Through manipulation and selective breeding it has been possible to answer this question by forcing mice to inherit two perfectly healthy, but different, sets of mitochondrial genes. Interestingly, these mice turn out to be physiologically, emotionally, and cognitively worse off than mice that, other things being equal, inherit just one set (Sharpley et al., 2012).

Assisted reproductive technology may unwittingly force human babies to inherit dissimilar mitochondrial DNA too (e.g., Hiendleder 2007; Sutovsky et al 1999). Some of these techniques aim to prevent the transmission of mitochondrial disease from mother to child by introducing a twist in the practice of inserting the eggs of a healthy donor into the womb of an unhealthy recipient. Before implantation, the nucleus of each donated egg is replaced with the nucleus removed from an egg of the recipient (Herbert and Turnbull 2018). The recipient can thus pass along to her children all of her own healthy nuclear genes, but none (ideally, because perfection is not of this world) of her unhealthy mitochondrial ones. Babies born with the help of these techniques have effectively three biological parents—

having inherited only nuclear DNA from their father, (hopefully) only nuclear DNA from their mother, and only mitochondrial DNA from another, donor mother.

Even disregarding that these babies are likely to get some of their mitochondria from the wrong mother, the trouble is that the nuclear genome from one mother and the mitochondrial genome from the other might not cooperate properly (Hiendleder 2007). The apparently risible percentage of mitochondrial components encoded by the mitochondrial genome includes most of the building blocks of the electron transport chain; the remaining are produced by the nuclear genome (Johnston and Williams 2016). Thus, a less than perfect interaction between the two genomes could alter the nanoscopically precise distances between the sites through which the electrons pass down the route to oxygen, impairing their flow—with more or less catastrophic consequences (Lane 2011). Only time will tell how the health and lifespan of people with three parents measure up to those with just two.

Incidentally, the fact that mitochondrial (unlike nuclear) DNA never gets mixed with that of fathers makes it uniquely suitable for tracing people's ancestry along the female line. Following our progenitors' mitochondrial footprints back from mother to grandmother to great-grandmother, one arrives at Mitochondrial Eve, a title currently held by a woman who gave birth to her own daughters around 200,000 years ago (Cann et al 1987) and from whom all people alive today descend in an unbroken female line[3].

3   All Creatures Great and Small

How much an organism needs to eat depends on how fast it converts food into energy. This metabolic rate depends on whether the organism is moving or not and on what its characteristics are, such as whether it is cold or warm blooded, what sexual strategy it likes better, and how big it is (Lane 2005). Yet not all body parts demand an amount of energy that is proportional to the organism's overall size (Haldane 1926). In larger land mammals, for example, bones—which require little energy to maintain—are relatively wider, but the blood vessel system is less dense, with each capillary feeding a greater number of cells, and this allows the heart to beat more slowly, saving energy (Lane 2005). Indeed, larger species spend energy more efficiently: food and oxygen consumption per gram of body weight diminish with size, along with the breathing, heartbeat, and metabolic rates (Lane 2005). The lower the metabolic rate, the lower the production of free radicals per cell and per mitochondrion—and thus the lower the risk of early degeneration and death. Indeed, large species tend to outlive small ones (Blagosklonny 2013): a lucky mouse has a life expectancy of two or three years, an elephant of sixty or seventy.

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[3] Other women were around at the time and also earlier, of course, but their own mitochondrial lineages happened to become extinct. Some of these women may well have descendants alive today, yet not in a direct female line but passing, at some point or other, through a male (a dead end for mitochondria).

Large species spend their energy more efficiently than small ones, grow to maturity more slowly, and—perhaps counterintuitively—feature lower levels of growth hormones (Stuart and Page 2010; Swanson and Dantzer 2014; Tian et al 2017). Within a species, however, all individuals mature about equally fast, thus larger adults must have grown faster than smaller ones. Individuals' final size is likely to reflect an inclination towards promoting or inhibiting the hormonal mechanisms that sustain growth; indeed, larger adults have higher levels of growth hormones (Bartke 2017; Swanson and Dantzer 2014).

The problem with growth is that it depends on cell divisions and each division requires the copying of DNA. Every round of copying increases the probability of mistakes (mutations) and, with it, the probability that growth spins out of control, leading to cancer. Larger species, that in principle would be most vulnerable to cancer, actually invest more than do smaller species in mechanisms dedicated to fighting it (Tian et al 2017). For example, elephants have 20 copies of a gene that forces cancer cells to commit suicide, whereas humans and mice have 1 copy only (Sulak et al 2016). As it happens, genetically modified mice with an extra copy develop fewer tumors (Matheu et al 2007).

Whereas individuals of larger species are better protected against cancer than those of smaller ones—which cancels the costs of being big—larger and smaller individuals of the *same* species have identical defenses in place. In line with this, taller men are more likely to suffer from cancer, and tend in general to die earlier, than shorter men (He et al 2014). Tellingly, the manufacturing of an important growth hormone appears downregulated in both human centenarians (Suh et al 2008) and their offspring (Vitale et al 2012). If growth hormones are diminished artificially, an experiment that has been tried in a kind of roundworm (Zarse et al 2012), cells suck in less glucose from the blood; starved of sugar, they must switch to less preferred fuels, such as those derived from fat. So, they have no choice but to rely even more heavily on mitochondria than they usually do, because only mitochondria can make ATP by burning fat. This surge in mitochondrial activity is accompanied by a modest increment in free radical levels, which in turn prompts antioxidant defenses that reduce these levels to values below those seen before the intervention. The net effect is that the worms age more slowly and end up living twice as long as usual (see also Miller et al 2018; Schulz et al 2007).

One can achieve similar results in creatures other than worms by simply offering them no more food than necessary. In rodents, for example, restricting food intake reduces the level of the chemical that feeds electrons into the electron transport chain, the escape of electrons from the chain, and the formation of free radicals (Barja 2013). As a rule, eating adequately but less than desired postpones the diseases of aging and prolongs life in organisms ranging from yeast to fish, from spiders to humans (Fontana et al 2010). In humans, these benefits also apply to people who are not considered overweight to start with (Redman et al 2018).

Eating less than preferred is very hard. With this truth in mind, dietary regimes have been proposed that do not restrict calories but still lower the levels of glucose, like ketogenic

diets, or those of growth hormones, like diets that curtail the consumption of protein or some critical component of it. Ketogenic diets feature plenty of fats such as cream and butter but very little carbohydrates, our main source of glucose, and thus conveniently mimic fasting (Longo and Mattson 2014). Lack of sugar pushes the liver to break down stored fat into molecules called ketone bodies, which mitochondria can use for fuel (e.g., Newman and Verdin 2014). Ketone bodies, likely as an adaptive response to nutrient scarcity, also intensify the recycling of damaged cell components and similar debris, which may serve as emergency food for the cell (Camberos-Luna et al 2016). Perhaps because it removes potentially lethal garbage, this frenetic housekeeping might be another reason why ketogenic diets appear to ameliorate a variety of afflictions, ranging from neurological diseases to cancer (Branco et al 2016; Gano et al 2014; Kramer and Bressan 2018; Miller et al 2018).

Ketogenic diets drastically cut out carbohydrates but reduce protein too, on the grounds that protein could otherwise be converted into glucose. This measure may have an accidental further advantage, because restricting protein—or at least two of its most common component amino acids—appears to promote health and longevity in itself (Dong et al 2018). These two amino acids are methionine and cysteine (methionine restriction being largely ineffective if cysteine is not removed too: Wanders et al 2015). Methionine is an essential amino acid, one that cannot be produced by the body but must be introduced with the diet, and the ability to sense its deficiency and promptly respond to it is of course indispensable to survival. As is often the case with indispensable activities, multiple mechanisms appear to be in place to achieve the same purpose. A major one involves the liver, that within hours of a methionine-restricted meal increases 5-fold its production of a fundamental metabolic regulator (Forney et al 2017a).

This regulator proceeds to orchestrate a suite of adaptive responses that include a coordinated increase in energy intake (likely on the lookout for extra methionine, one eats more) and expenditure (Anthony et al 2013). The latter is achieved mainly by making the mitochondrial inner membrane leakier to protons, so that some of the energy pent up in the intermembrane space, rather than being used to make ATP, is wasted as heat (Hasek et al 2010). As a consequence, the production of free radicals drops, and with it drops the damage that free radicals inflict on tissues and DNA (e.g., López-Torres and Barja 2008). It may be no coincidence that species that live longer, such as the pigeon, happen to carry less methionine in their flesh than species that live shorter, such as the rat. In mammals—from mice to horse, through rabbit and pig—the negative correlation between methionine content in the heart and maximum lifespan is nearly perfect (Pamplona and Barja 2006).

Despite ingesting more food, animals on methionine-restricted diets stay thin because the cost of growth increases: more energy is required for maintaining existing tissue (Hasek et al 2010). Yet a cost is a cost, and while the degree of restriction that compels individuals to take in more food proves salutary, going just a bit further down that road causes excessive weight loss: animals become thinner and thinner but stop consuming extra food, as though methionine is now so low that eating more would make no difference (Forney et al 2017b).

An even stricter dilution of any essential amino acid creates (within minutes: Hao et al 2005) an aversion to the unbalanced food, and, if no alternative source can be tracked down, simply fails to sustain life. More of a good thing is not always a better thing.

4   Have Kids, Die Early

Periodic famines may be one reason why we animals eat more than we need. Another may be that stern diets inhibit the immune system, impairing wound healing and recovery from infection; reduce the response to cold, increasing mortality in small animals when temperature drops; and diminish bone and muscle mass, potentially hindering one's ability to fight or escape from predators (Adler and Bonduriansky 2014). All of this saves energy, but—except under lab conditions—bodes ill for survival. Yet the most important reason why we desire more food than necessary may be that eating is not just about sustaining ourselves but also about producing offspring. The hormones and enzymes that encourage growth in response to nutrient abundance reduce lifespan but also upregulate hormonal mechanisms that bolster fertility and sexual activity (follicle-stimulating hormone, luteinizing hormone, estrogen, and testosterone); hormones and enzymes that reduce growth do the opposite (Ellison 2017; Templeman and Murphy 2018).

Within species, individuals that invest more in acquiring mates and producing offspring tend to die earlier (Lemaître et al 2015). This is also the case in humans: reproductive success (measured as either a larger number of children or shorter birth intervals) is associated with reduced survival in both women and, to a smaller extent, men—even after adjusting for socioeconomic status and other potential confounds (Penn and Smith 2007). Rounding off this evidence, both male and female centenarians have fewer children than other people and had them later in life (shown in Ashkenazi Jews: Tabatabaie et al 2011). Thus, the survival costs of reproduction go beyond the physical burdens of pregnancy, childbirth, and breastfeeding.

Achieving reproductive maturity requires development and growth, and the amount of growth that has taken place is reflected in individuals' size. Indeed, dwarf people resemble centenarians (who, as mentioned earlier, manufacture less growth hormone) in that they reproduce later in life (Bartke et al 2013; Hull and Harvey 2014). In dwarfs, the odds of developing diabetes and cancer are also lower than average, whereas in giants they are higher and accompanied by a reduced life expectancy (Bartke 2017). In mice bred to be dwarfs, which do not have enough of a particular growth hormone, fertility is either abolished or delayed, but lifespan is stretched by up to 50% (Bartke et al 2013). Female hyenas with low levels of growth hormone in their youth tend to be smaller as adults, but even when they are not, they breed later and live longer (Lewin et al 2017).

Intriguingly, in humans (like in several other animals), low maternal food intake has been associated with a greater likelihood of giving birth to females than to males, and women

who gain less weight during gestation produce fewer sons (Navara 2014; see also Myers 1978; Trivers and Willard 1973). The main reason appears to be that sons tend to be larger than daughters and hence require more resources, even in the womb: more male than female fetuses are spontaneously aborted when food is scarce. Women who became pregnant during the great Chinese famine from 1957 to 1961, which killed 30 million people, were less likely to give birth to sons than expected (Song 2012; Song 2015); the same happened during the 1974 famine in Bangladesh (Hernández-Julián et al 2014).

Growth, reproduction, and longevity are also interrelated in another interesting way. Different species have different strategies regarding the quality and quantity of their offspring (Lane 2015). Those enforcing the strictest quality control discard as defective the most egg cells and spontaneously abort the most of their potential offspring. This results in fewer but healthier offspring that can survive longer. Those producing more offspring are forced to lower their quality control standards, but then their offspring tend to die sooner. It is a species' circumstances that appear to dictate its strategy. For example, creatures that fly require more energy than those that do not, and this calls for better mitochondria and therefore for higher quality control of offspring. A pigeon delivers 15 young per year and a rat 60, but then the pigeon's lifespan is ten times longer than the rat's.

Demonstrating that mitochondria play a critical part in such survival strategies, beetles that are artificially selected for either long or short lives, and respectively breed later or earlier, end up with distinctly different types of mitochondrial DNA (Stojković et al 2017). From an evolutionary point of view, of course, it is better to live a short life yet reproduce than to live a long one but leave no descendants. In this respect, the fact that mitochondrial DNA does not care about its effects on males has occasionally a silver lining for them. For example, a type of mitochondrial DNA found in fruit flies renders females fertile but short-lived and males sterile but long-lasting (Camus et al 2015).

5   Guarding the Gate

We are not alone in our bodies; we house some 38 trillion microbes (Sender et al 2016) belonging to at least 2,172 known species (Hugon et al 2015). Our health depends also on theirs (Gilbert et al 2016; Kramer and Bressan 2015; Rogers et al 2016) and so does the health of our mitochondria. Most of these microbes live in our gut and are heavily affected by the food we eat. For example, protein is mostly digested by enzymes in our nearly sterile small intestine. The rest progresses to the colon, where it is fermented by bacteria that expel, as waste products, toxins like hydrogen sulfide (Linden 2014; Pal et al 2018; Yao et al 2016)—the gas that gives rotten eggs their foul smell. Some vegetables, like cauliflower and broccoli, have evolved an abundance of sulphur compounds to ward off their own parasites (Björkman et al 2011). Such compounds harm the bacteria in our gut that disgorge hydrogen sulfide (Kellingray et al 2017), opening up opportunities for rival, beneficial ones; which

might be part of the reason why a regular consumption of this family of vegetables is associated with a lower probability of developing diabetes, cardiovascular diseases, and cancer (Björkman et al 2011).

Hydrogen sulfide is not entirely bad; some of our cells assemble it in small amounts from cysteine and use it, for example, to send important messages to other cells (Banerjee et al. 2015). Still, the more protein we eat, the more hydrogen sulfide we get; eventually, it may become too much. If so, the gas can tear open the mucus layer that protects the gut wall, breach into the wall's cells, and harm their mitochondria (Blachier et al 2007; Mottawea et al 2016). This chain of events raises the mitochondria's free radical production—which in turn increases the permeability of the barrier that separates the gut from the bloodstream (Mottawea et al 2016), granting entry into the body to those microbes, viruses (Pal et al 2018), and potentially immunoreactive food particles (Bressan and Kramer 2016) that are normally held back inside the gut. It is no wonder that specific assortments of gut microbes have been associated with disorders of every description (Bastiaanssen et al 2018; Gilbert et al 2016; Kramer and Bressan 2015; Rogers et al 2016).

The task of keeping pathogens confined within the gut is delegated to the immune cells that line its inside. Curiously, these cells get their energy from mitochondria whose electron transport chains are embedded in an unusual variant of the inner membrane—one that is purposely fashioned to *limit* energy production (Konjar et al 2018). In response to the inflammatory signals triggered by an infection, the properties of this membrane suddenly change: the brakes come off and energy production shoots up to full capacity. Now the immune cells get a jolt of energy and multiply rapidly to attack any pathogens that may be hanging about. The concomitant increase in free radical levels, which is indeed typical of inflammation (Angajala et al 2018; López-Armada et al 2013), helps wrecking the pathogens further. The mitochondria with the unusual membranes are thus effectively gatekeepers: they keep a large number of guard dogs (the immune cells) under control when all is well, but set them loose when evil characters (the pathogens) arrive on the scene. With the gut's immune cells under the control of mitochondria and the immune cells themselves always at beck and call to assail gut microbes, it makes a lot of sense that differences in mitochondrial DNA are associated with differences in gut microbe composition (Clark and Mach 2017; Hirose et al 2017). In an overt attempt to overcome an immune response against them—a response which is invariably assisted by a hefty release of free radicals—several pathogens discharge toxins that interfere with their host's mitochondrial signaling and overactivate its antioxidant mechanisms (Clark and Mach 2017).

Indeed, every so often our frontline troops are not up to the task and we need to destroy the pathogens ourselves. In most societies, few go through life without ever taking antibiotics: globally, just over the period 2000-2015, their consumption has increased by 65%, reaching nearly 35 billion doses per day (Klein et al 2018). Although they can be indispensable occasionally, antibiotics famously wipe out beneficial gut bacteria along with offensive ones—allies and enemies both—and worsen the balance between them after

recovery (Keeney et al 2014). On top of that, antibiotics harm mitochondria very directly, because of course mitochondria resemble bacteria, their forebears. Even at recommended doses for the recommended time, antibiotics injure the outer wall and DNA of mitochondria, upset their quality control activities, and disrupt electron transport chains—making them produce less energy and more free radicals (Kalghatgi et al 2013; Wang et al 2015). Whatever hurts microbes—most prominently, common pesticides and pollutants (Karami-Mohajeri and Abdollahi 2013; Meyer et al 2013; Mostafalou and Abdollahi 2013)—is indeed likely to hurt mitochondria. In our fight against pathogens and pests, we ought to be much more careful about collateral damage to our tenants.

6   A Call to (Some) Action

During the day we eat; digested food is stripped of electrons and these are fed into the transport chain to make fresh ATP. Yet if we do not move, not much ATP is broken down into ADP, and our ATP synthases run out of supplies. When ADP is unavailable, the ATP synthase stops and protons can no longer flow, through it, into the matrix (Wallace 2005). As the protons accumulate inside the intermembrane space, their increasing concentration impedes further pumping of protons into this space. The pumping slows down, and the stream of electrons through the electron transport chain slows down with it. Electrons continue to be fed into the chain, however; as they heap up, more and more of them escape before reaching their final destination (shown *in vitro*: Herrero and Barja 1997), react with oxygen, and form free radicals.

By consuming ATP and thus speeding up the flow of electrons, exercise hence decreases (rather than increases, as one might have thought), the formation of free radicals *inside* mitochondria. But mitochondria are not the only source of free radicals; these are fabricated in various chemical reactions elsewhere too, and during exercise their net level goes up, not down (He et al 2016; Powers and Jackson 2008; Trewin et al 2018). If one exercises regularly, the body puts into effect a suite of countermeasures to be ready to cope with similar stresses in the future (Goto et al 2007; Gradari et al 2016; Hood et al 2011). Such countermeasures include the upregulation of repair mechanisms and the production of both antioxidants and enzymes meant to get more glucose out of the blood and into cells (reducing, incidentally, the risk of diabetes). They also include an upgrade in the number of mitochondria and of the electron transport chains inside them. This has an antioxidant effect because the more chains there are, the fewer electrons can be fed into each—per unit of food burned down—and thus the fewer electrons leak out to form free radicals. Somewhat counterintuitively, then, the beneficial effects of exercise turn out to be due (albeit indirectly) to a *rise* in free radical levels.

Motivated by the wish to do the right thing, many healthy people—athletes prominently included—exercise (which raises free radical levels) and take antioxidant supplements

(which lowers them). Yet antioxidant supplements turn out to *block* the health-promoting effect of exercise (Merry and Ristow 2016; Peternelj and Coombes 2011; Ristow et al 2009; Villanueva and Kross 2012). The harmful effects of the free radicals produced by exercise are compensated—surpassed, in fact—by the countermeasures they trigger, and this is why exercise is healthy after all.

Note that this holds when exercise is moderate. When it is excessive, free radical levels may increase too much for benefits to outweigh costs (in which case taking antioxidants might even be fine: Radak et al 2017, though not wearing oneself out seems like a better plan). As it happens, rats that are forced to swim either 10 or 30 minutes a day for 20 weeks develop fewer mutations in their mitochondrial DNA than rats who remain idle (Cao et al 2012). Yet, whereas rats that swim for 10 or, better, 30 minutes a day end up with more copies of mitochondrial genes (associated with greater resilience), rats that swim for 60 or 90 minutes end up with fewer. Similarly, in humans, overtraining has been associated with considerable free-radical damage to fat tissues, proteins, and DNA (Radak et al 2008; Radak et al 2013).

It stands to reason, then, that the effects of exercise depend on how much one is biologically capable of generating energy and on how easily one produces free radicals. Both traits are determined, in part, by the type of mitochondrial DNA one has inherited from one's mother. For example, carriers of one type manufacture more ATP and free radicals than carriers of another (Martínez-Redondo et al 2010). Interestingly, Olympic endurance athletes (such as swimmers, cyclists, long-distance runners, and cross-country skiers: Maruszak et al 2014) tend to carry the former type.

7 There is a Time for Everything

Unexpectedly, the production of energy and regulation of free radical levels have popped up as crucial in the supervision of a still largely mysterious activity in which we engage about a third of our time: sleep. For the past decades, researchers of biorhythms (recurring cycles in biological activity) have been focusing their attention mostly on a central biological clock sitting in the hypothalamus and on the genes in charge of it (Borbély et al 2016; Rutter et al 2002). To keep track of time, the clock accumulates certain chemicals until a threshold is reached and then it starts afresh. Presumably, this central clock allows organisms to prepare for, rather than merely react to, recurring changes in light and temperature, so that they can anticipate opportunities to eat and engage in fruitful activities. To this effect, it adjusts the release of hormones that are responsible for one's inclination to eat during the day and sleep during the night (Laje et al 2018). Although it induces a sleep/wake cycle regardless of the external, night/day one, the clock manages to keep the two cycles in sync by using a report, issued by our eyes, of the changes in overall illumination.

If people are given full control over artificial lighting but are deprived of natural light, their patterns of eating, moving, and sleeping change—sometimes drastically, with wake periods shorter than 12 hours or longer than 30 (Aschoff et al 1986). Remarkably, these alterations mirror changes in energy intake and expenditure (Borbély et al 2016; Reddy and Rey 2014). People who spontaneously slow down their day from 24 to 48 hours, for example, proportionally slow down their food intake from three meals per 24-hour day to three per 48-hour day (Aschoff et al 1986; Green et al 1987). Their total amount of activity slows down proportionally too (Aschoff 1993). In these conditions, "warmer" people (those whose mean body temperature is higher) tend to shorten their sleep/wake cycle whilst "cooler" people tend to lengthen it (Daan et al 2013). A tiny variation in core temperature (0.5° C) corresponds to hugely different individual day lengths—spanning from 16 to 48 hours. This agrees with the notion that the alternation of sleep and wake strongly depends on energy management.

The central clock regulates other clocks that are stationed throughout the body—in the liver, in the heart and such. Both the central clock and these local ones speed up when the level of an important nutrient-sensing (and growth-promoting) enzyme rises, and slow down when it falls (shown in mice: Ramanathan et al 2018). Exposure to light can reset one's central clock, and interestingly it does so, at least in part, via this same nutrient-sensing enzyme; inhibiting the enzyme blocks this reset (Cao et al 2010; see also Cao et al 2008).

Indeed, energy management may be more important for keeping track of time than any central or local clocks. In red blood cells kept *in vitro* in complete darkness, for example, the activity of antioxidant mechanisms and the level of ATP turn out to wax and wane with a 24-hour circadian rhythm (O'Neill and Reddy 2011). This is an impressive finding, because red blood cells lack a nucleus and with it the genes necessary to maintain a local clock; sitting in a Petri dish, they cannot possibly be affected by a central brain clock either—which suggests that biorhythms are driven by energy production *per se*.

Red blood cells lack not only a nucleus but mitochondria too; they rely on an alternative, less efficient source of energy. In most of our cells, however, it is primarily the mitochondria that produce energy. One may thus suppose that it is primarily the mitochondria that control our biorhythms, including the sleep/wake cycle. Consistent with this idea, rats whose brain is infused with low levels of a free-radical-producing chemical fall asleep more often and sleep longer (Ikeda et al 2005).

The burning of digested food in mitochondria increases and decreases with recurrent ups and downs in food intake and therefore with the alternation of wake and sleep. The cyclic production of energy is accompanied by a cyclic production of free radicals and a cyclic need to neutralize them, and it appears that sleep is part of a larger mechanism to accomplish this feat (Ikeda et al 2005). As a matter of fact, glutathione—an important antioxidant in many organisms—induces sleep (Inoué et al 1995) and melatonin—a well-known inducer of sleep—is not only a hormone but also an antioxidant. Melatonin is plentiful in mitochondria, and it has been argued that the bacterial ancestors of mitochondria might have produced

melatonin as an antioxidant before it ever became a sleep hormone in creatures like us (Reiter et al 2017).

The importance of our biorhythms is well illustrated by what happens when we mess with them. The invention of artificial light has allowed people to be active any time of day or night. Yet, whether it is due to going to bed too late, to jet lag, or most prominently to shift work, being awake or asleep at the wrong time has been associated with a myriad physical and mental disorders, including anxiety, depression, obesity, heart disease, and cancer (Kondratova and Kondratov 2012; Liu and Chung 2015; Roenneberg and Merrow 2016). Reversing the sleep/wake cycle of human volunteers for just two days disrupts the normal levels and daily variation patterns of more than 100 proteins in the blood, including several that are involved in the regulation of blood sugar, weight gain, immune function, and cancer (Depner et al 2018).

8   Coda: Have your cake or eat it

Mitochondria produce nearly all of the energy we animals need. We have evolved to invest this energy to grow, find food and shelter, and leave descendants, and to last long enough to make it all happen. Yet because the continuous manufacturing of energy is inevitably linked to wear-and-tear, mitochondria give us death as well as life. And because energy spent on growing and procreating is energy not spent on recycling and repairing, producing many offspring and living a long life trade off with each other.

What compromises are made in our own special case (how large we become, how early we can reproduce, whether we happen to be born women or men, or for that matter pigeons or rats) is very largely beyond our control. So is the unappetizing truth that hordes of uninvited guests (microbes, viruses, and other selfish entities) are feasting on our resources too, shaping all along how we function and behave (Kramer and Bressan 2015). What we *can* do is treat our mitochondria as allies and adjust our lifestyle so as to take good care of their necessities. By eating properly, exercising regularly, and sleeping at night, we can keep our companions' proton pumps well oiled and their ATP synthases plugging away. We may get in exchange the energy we need to live interestingly.

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