

REVIEW

Fasting vs dietary restriction in cellular protection and cancer treatment: from model organisms to patients

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The dietary recommendation for cancer patients receiving chemotherapy, as described by the American Cancer Society, is to increase calorie and protein intake. Yet, in simple organisms, mice, and humans, fasting—no calorie intake—induces a wide range of changes associated with cellular protection, which would be difficult to achieve even with a cocktail of potent drugs. In mammals, the protective effect of fasting is mediated, in part, by an over 50% reduction in glucose and insulin-like growth factor 1 (IGF-I) levels. Because proto-oncogenes function as key negative regulators of the protective changes induced by fasting, cells expressing oncogenes, and therefore the great majority of cancer cells, should not respond to the protective signals generated by fasting, promoting the differential protection (differential stress resistance) of normal and cancer cells. Preliminary reports indicate that fasting for up to 5 days followed by a normal diet, may also protect patients against chemotherapy without causing chronic weight loss. By contrast, the long-term 20 to 40% restriction in calorie intake (dietary restriction, DR), whose effects on cancer progression have been studied extensively for decades, requires weeks–months to be effective, causes much more modest changes in glucose and/or IGF-I levels, and promotes chronic weight loss in both rodents and humans. In this study, we review the basic as well as clinical studies on fasting, cellular protection and chemotherapy resistance, and compare them to those on DR and cancer treatment. Although additional pre-clinical and clinical studies are necessary, fasting has the potential to be translated into effective clinical interventions for the protection of patients and the improvement of therapeutic index.

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Introduction

For the past 50 years, chemotherapy has been a major medical treatment for a wide range of malignancies (Chabner and Roberts, 2005). Its main strategy has been largely based on targeting malignant cells, by means of genotoxicity caused in part by the production of reactive oxygen species (Sangeetha *et al.*, 1990; Look and Musch, 1994; Faber *et al.*, 1995; Conklin, 2004). In fact, the US Food and Drug Administration has approved 132 cancer chemotherapy drugs, of which 56 have been reported to cause oxidative stress (Chen *et al.*, 2007). Although these drugs were first believed to be quite selective for tumor cells, we now know that normal cells also experience severe chemotherapy-dependent damage, leading to dose-limiting side effects including myelosuppression, fatigue, vomiting, diarrhea and in some cases even death. Chemoprotectants such as amifostine, glutathione, mesna and dexrazoxane have been investigated and shown to provide drug-dependent protection to certain tissues, but these drugs have not been shown to increase disease-free or overall survival, in part because they may also protect cancer cells (Links and Lewis, 1999). Despite the focused efforts on the development of drugs designed to specifically target certain cancer cells, side-effects will continue to accompany cytotoxic drugs as well as a wide range of antibody-based drugs until fundamentally novel strategies to selectively eliminate malignant cells are discovered.

Fasting has been demonstrated to selectively protect normal cells and mice, but not cancerous cells against oxidants and common chemotherapeutic agents (Longo *et al.*, 1997; Raffaghello *et al.*, 2008). In cancer patients, preliminary data suggest that fasting is not only safe and feasible, but may also be effective in reducing common side-effects associated with chemotherapy (Safdie *et al.*, 2009). In this review, we discuss how short-term fasting can selectively protect normal cells and organisms from chemotherapy toxicity without interfering with its therapeutic effect (Figure 1), and also compare its effects with those of the much better studied dietary restriction (DR) (Table 1). We also discuss the evolutionarily conserved role of the growth hormone (GH) and insulin-like growth factor 1 (IGF-I) signaling pathways in stress resistance, and how they act as major mediators of fasting-dependent differential protection against chemotherapy and oxidative toxicity (Fontana *et al.*, 2010). Finally, we discuss how these studies may provide grounds for future investigations

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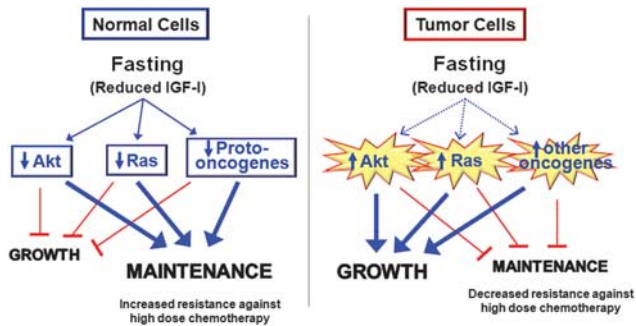


Figure 1 Fasting provides differential stress resistance (DSR) to chemotherapy. The investment of the finite energy available in a cell or an organism is efficiently balanced between growth/reproduction and maintenance/repair. However, challenging conditions, such as fasting and its consequent reduction of IGF-I, withdraws energy from growth/reproduction and reinvests it in maintenance/repair, thereby increasing cellular protection. This switch in cellular metabolic policy is mediated by negatively regulating mitotic pathways such as those major effectors downstream of IGF-I (PI3K/Akt and Ras/ERK). By contrast, oncogenic mutations, which often regulate cellular metabolism and growth, render tumor cells less responsive to fasting due to their independence from external cues. Therefore, cancer cells fail to or only partially respond to fasting and continue to promote growth, leaving them vulnerable to chemotherapy drugs.

on the role of various dietary interventions in enhancing cancer treatment.

Starvation and stress resistance

Most organisms live in environments of fluctuating food availability, where starvation is a commonly encountered condition. Thus, nutrient depletion has been an evolutionary driving force, selecting for organisms able to withstand starvation. Because during periods of food scarcity organisms are exposed to a wide variety of insults including UV radiation, heat, cold and so on, adaptation to starvation requires the organism to invest energy into multiple protective systems to minimize the damage that would reduce fitness (Madia *et al.*, 2008, 2009). For example, yeast switched from glucose and ethanol medium to water becomes resistant to multiple types of stress and long-lived (Longo *et al.*, 1997; Fabrizio *et al.*, 2005; Wei *et al.*, 2008). As discussed later, pro-growth pathways, including those activated by nutrients or growth factors, have central roles in the prevention of the entry into this protected mode.

DR is a term commonly used to describe a 20–40% reduction in calorie intake, although it can also refer to more or less severe restrictions or to reduced or lack of daily intake of particular components of the diet, such as amino acids, protein or fats. Although DR could also describe the complete lack of food or calorie intake, we will use the terms fasting or starvation to avoid confusion with the more common use of DR. Both DR and fasting have been shown to promote stress resistance as well as longevity in model organisms

ranging from unicellular yeast to mammals in part, by downregulating conserved nutrient-signaling proteins, or by activating stress resistance transcription factors negatively regulated by these pro-aging pathways (Figure 2) (Fontana *et al.*, 2010). These pathways have many regulatory effects including those on cellular growth, metabolism and protection against oxidants and other toxins.

The reactive oxygen species generated in response to many chemotherapy drugs can cause a wide range of genetic aberrations including DNA strand breaks and base/nucleotide modifications (Burney *et al.*, 1999). Notably, a functioning immune system is required to delay cancer growth or promote cancer regression in response to several chemotherapy drugs, indicating that oxidative damage may, in many cases, impair the cells sufficiently to be detected and destroyed by the immune system, thereby reducing chemotherapy efficacy (Locher *et al.*, 2010; Ma *et al.*, 2010). For example, natural killer T cells are required for the toxic effect of oxaliplatin and irradiation on cancer cells, which also target rapidly dividing normal immune cells (Locher *et al.*, 2010).

One of the most common reactive oxygen species-mediated DNA modification is the conversion of guanine to 7, 8-dihydro-8-oxoguanine (also known as 8-oxoguanine; 8-oxoG) (David *et al.*, 2007). This damage can lead to mutations in nuclear and/or mitochondrial DNA, but can also cause epigenetic alterations, leading to cellular dysregulation and malignant transformation. Simple organisms such as yeast and bacteria, in part because of their inability to escape harsh environments, have evolved systems that can increase resistance to many insults, in some cases by several orders of magnitude. This protected mode appears to be at least partially conserved in many species. In the prokaryote *E. coli*, lack of glucose or nitrogen (comparable to protein restriction in mammals) increase resistance to high levels of H₂O₂ (15 mM) (Jenkins *et al.*, 1988). In many bacterial species, starvation induces the stationary-phase-specific σ factor RpoS expression, which in turn controls a large set of stress defense genes, in particular oxidative stress (McDougald *et al.*, 2002). In yeast, glucose reduction (2–0.5%) increases protection against oxidative stress, whereas complete starvation by switching the population of cells to water, promotes protection to oxidative insults and heat shock and a major life span increase (Longo *et al.*, 1997; Wei *et al.*, 2008). Although the precise molecular mechanisms of increased protection by fasting have yet to be described in detail, in yeast they involve the reduced activity of the Ras/cAMP/PKA and the Tor/S6K nutrient signaling pathways and the activation of transcription factors downregulated by these pathways (Thevelein and de Winder, 1999; Wei *et al.*, 2008). In fact, deletion of transcription factors Msn2/Msn4 and Gis1 reverses the protection caused by glucose restriction or starvation conditions, suggesting an important role for genes involved in metabolism, cellular protection and repair regulated by these factors in starvation-dependent stress resistance (Longo *et al.*, 1997; Wei *et al.*, 2008).

Table 1 Fasting and dietary restriction (DR) induce similar, yet distinct physiological response in laboratory rodents

	Fasting		DR	
	Effect	Reference	Effect	Reference
<i>Nutrient utilization</i>				
Glucose	~50% ↓ (Blood glucose)	(Wang <i>et al.</i> , 2006; Lee <i>et al.</i> , 2010)	~43%; 25% ↓ (Blood glucose)	(Spindler <i>et al.</i> , 1990; Al-Regaiey <i>et al.</i> , 2007)
Protein	~50% ↑ (Nitrogen excretion)	(Wang <i>et al.</i> , 2006)	~70% ↓ (Nitrogen balance); ~35% ↑ (Plasma ammonia), ~63% ↑ (Uric acid)	(Felgines <i>et al.</i> , 1999; Filaire <i>et al.</i> , 2004)
Lipids	100% ↑ (FFA), 50% ↓ (TG)	(Menahan and Sobocinski, 1983)	~13% ↑ (FFA), 96% ↓ (TG)	(Filaire <i>et al.</i> , 2004)
<i>GH/IGF-I and Insulin</i>				
GH	~90% ↓	(Frystyk <i>et al.</i> , 1999; Wang <i>et al.</i> , 2006)	~13%; ~50-fold ↑	(Shimokawa <i>et al.</i> , 2003; Zhao <i>et al.</i> , 2010)
IGF-I	~40%; 70% ↓	(Frystyk <i>et al.</i> , 1999; Lee <i>et al.</i> , 2010)	~15%; 25%; 40% ↓	(Breese <i>et al.</i> , 1991; Dunn <i>et al.</i> , 1997; Al-Regaiey <i>et al.</i> , 2007)
IGFBP-1	~7-fold; 11-fold ↑	(Frystyk <i>et al.</i> , 1999; Lee <i>et al.</i> , 2010)	~20-fold	(Buschemeyer <i>et al.</i> , 2010)
IGFBP-3	~50%; 40% ↓	(Frystyk <i>et al.</i> , 1999; Lee <i>et al.</i> , 2010)	~20% ↓	(Buschemeyer <i>et al.</i> , 2010)
Insulin	~90% ↓	(Frystyk <i>et al.</i> , 1999)	~40% ↓	(Bonkowski <i>et al.</i> , 2009)
Insulin-sensitivity	~3-fold ↑	(Heijboer <i>et al.</i> , 2005)	~7/1.2-fold (younger/older) ↑	(Escriva <i>et al.</i> , 2007)
Corticosteroid	~2.5-fold ↑	(Shen <i>et al.</i> , 2009)	~2-fold ↑	(Sabatino <i>et al.</i> , 1991)
Body temperature	~50% ↓	(Shen <i>et al.</i> , 2009)	~12% ↓	(Koizumi <i>et al.</i> , 1992)
Reproduction	No fertility (a 48-h fast at 2nd day of estrous cycle)	(Wang <i>et al.</i> , 2006)	Extended reproductive span with reduced litter number (~15%) and size (~50%)	(Longo and Finch 2003a, b; Holehan and Merry, 1985)
Required time	2–3 days	(Raffaghelto <i>et al.</i> , 2008; Lee <i>et al.</i> , 2010; Mitchell <i>et al.</i> , 2009)	Weeks–months	(Longo and Finch 2003a, b; Fontana <i>et al.</i> , 2010)

Abbreviations: DR, dietary restriction; FFA, free fatty acid; GH, growth hormone; IGF-I, insulin-like growth factor 1; TG, triglycerides. Reduction in glucose and increased utilization of protein and lipid occur following both fasting and DR, but more pronounced in the former (Al-Regaiey *et al.*, 2007; Felgines *et al.*, 1999; Filaire *et al.*, 2004; Lee *et al.*, 2010; Menahan and Sobocinski 1983; Spindler *et al.*, 1990; Wang *et al.*, 2006). The GH/IGF-I and insulin system are also significantly altered following fasting and DR. However, differences in GH response and also in the degree of reduction in circulating IGF-I, IGFBP-3, insulin and in the increase of IGFBP-1 and insulin-sensitivity are evitable (Bartke and Turyn 2001; Bonkowski *et al.*, 2009; Breese *et al.*, 1991; Buschemeyer *et al.*, 2010; Dunn *et al.*, 1997; Escriva *et al.*, 2007; Frystyk *et al.*, 1999; Heijboer *et al.*, 2005; Lee *et al.*, 2010; Shimokawa *et al.*, 2003; Wang *et al.*, 2006; Zhao *et al.*, 2010). Corticosteroids tend to increase more following fasting compared with DR (Sabatino *et al.*, 1991; Shen *et al.*, 2009); body temperature showed a steeper decrease following fasting compared with DR (Koizumi *et al.*, 1992; Longo and Finch 2003a; Shen *et al.*, 2009). Fertility was decreased dramatically by fasting for 48-h and reached zero level when done on the 4th day of the estrous cycle (McClure 1959). DR in rats, maintains body weight at 50% of control, reduced both litter number and size (Holehan and Merry 1985). It should be noted that one of the major differences between fasting and DR is the time required to reach the desired stress-resistant state, which makes fasting a much more applicable dietary intervention (Fontana *et al.*, 2010; Lee *et al.*, 2010; Longo and Finch 2003a; Mitchell *et al.*, 2009; Raffaghelto *et al.*, 2008).

Worms also increase their defenses against oxidative insults during fasting. Every 2 days fasting increases the resistance to oxidative stress, as well as lifespan of worms up to 56% via modulation of the RHEB-1 and TOR signaling pathway, both of which are linked to the FOXO transcriptional factor homolog DAF-16 (Weinkove *et al.*, 2006; Honjoh *et al.*, 2009). Conversely, excessive glucose shortens the lifespan of worms, in part, by decreasing DAF-16 activity (Lee *et al.*, 2009).

In flies, fasting-dependent protection against oxidative stress is mediated by d4E-BP, which acts downstream of the PI3K/Akt/dFOXO3 pathway (Tettweiler *et al.*, 2005). d4E-BP binds to eIF4E and represses translation. During fasting, flies increase the expression

of d4E-BP, thereby suppressing eIF4B-dependent translation, a mechanism consistent with the necessity to divert energy from growth to protection. Notably, flies under moderate DR are not protected against oxidative damage following anoxia/reoxygenation injury, whereas a severe DR, that is near starvation, is able to reduce damage (Vigne *et al.*, 2009).

As discussed in more detail later, in mice, fasting for 48–60 h increases protection from oxidative stress and protects three different strains of mice to etoposide, a drug known to promote oxidative stress, with remarkable improvement in survival compared with its normally fed counterparts (Raffaghelto *et al.*, 2008). In addition, 72-h of fasting protects the outbred CD-1

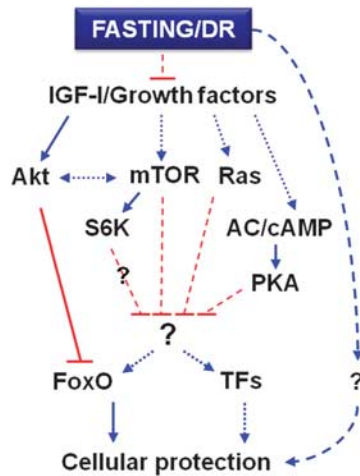


Figure 2 Fasting leads to a significant reduction in circulating IGF-I levels. The partially conserved IGF-I signaling pathways negatively regulate the FoxO family of transcription factors through Akt. Ras and Tor also function downstream of IGF-I, although their roles in the regulation of stress resistance and aging are poorly understood. Mice deficient in type 5 adenylyl cyclase (AC) are stress resistant (Yan *et al.*, 2007). Notably, oncogenic mutations that cause the hyperactivation of IGF-I, Akt, Ras, mTOR and PKA are among the most common in human cancers (Hanahan and Weinberg, 2000).

mice from lethal doses of doxorubicin, a drug also known to cause death by oxidative stress-induced cardiotoxicity (Lee *et al.*, 2010). Studies also show that fasting protects against ischemia injury in rat brain (Go *et al.*, 1988; Marie *et al.*, 1990), mouse kidney and liver (Mitchell *et al.*, 2009; van Ginhoven *et al.*, 2009), and human liver (van Ginhoven *et al.*, 2009). Also, fasting following traumatic brain injury proved to be neuroprotective, resulting in reduced oxidative damage and improved cognitive function (Davis *et al.*, 2008).

Conserved role of nutrient signaling pathways in stress resistance

Nutrient signaling pathways controlled by insulin and/or the GH/IGF-I axis are major regulators of both lifespan and stress response as demonstrated by studies in *S. cerevisiae*, *C. elegans*, *D. melanogaster* and mice (Longo, 1999; Guarente and Kenyon, 2000; Kenyon, 2001; Longo and Finch, 2003b). In yeast, decreased Ras/adenylyl cyclase and/or Tor/S6K (SCH9) increases lifespan by more than 200%, while providing increased stress resistance against oxidants, genotoxins, and heat-shock (Fabrizio *et al.*, 2001, 2003; Longo and Finch, 2003b; Wei *et al.*, 2009). Similarly, in *C. elegans*, age-1 (PI3K homolog) and daf-2 (insulin/IGF-I receptor homolog) mutations extend the life-span in adult organisms by 65–100% by decreasing AKT-1/AKT-2 signaling, and by activating the transcription factor DAF-16 (FOXO family member), while promoting resistance to oxidative and ER stress (Johnson, 1990; Paradis *et al.*, 1999; Hsu *et al.*, 2003; Henis-Korenblit *et al.*, 2010). Also, DAF-16 is thought to be negatively

regulated by PtdInsP₃, which is regulated by the PI3K homolog AGE-1; AGE-1 mutants have been shown to be resistant to oxidative stress (Larsen, 1993; Morris *et al.*, 1996). These longevity mutations are largely associated with the induction of stress resistance transcription factors, superoxide dismutase (MnSOD), and heat shock proteins (HSPs) in both yeast and worms (Longo and Finch, 2003a; McColl *et al.*, 2010). In *D. melanogaster*, mutations in the insulin receptor substrate (chico) extend lifespan, but its role in stress resistance is not clear (Clancy *et al.*, 2001; Giannakou and Partridge, 2007). In mice, stress resistance and lifespan are controlled by the GH/IGF-I axis and the downstream orthologs of the genes that regulate stress resistance and/or life span in yeast and worms (Brown-Borg *et al.*, 1996; Migliaccio *et al.*, 1999; Coschigano *et al.*, 2000; Holzenberger *et al.*, 2003; Yan *et al.*, 2007; Bonkowski *et al.*, 2009; Selman *et al.*, 2009). In agreement with the findings in lower eukaryotes, the activities of anti-oxidant enzymes superoxide dismutases and catalase are decreased in murine hepatocytes exposed to GH or IGF-I and in transgenic mice overexpressing GH (Brown-Borg and Rakoczy, 2000; Brown-Borg *et al.*, 2002). In addition, cultured cells derived from long-lived mice with deficiencies in the GH/IGF-I axis are resistant to oxidative stress (H₂O₂, paraquat), UV, genotoxins (methylmethanesulfonate; MMS), heat and cadmium (Salmon *et al.*, 2005; Murakami, 2006), even though they are cultured in standard medium, suggesting that some of the protective effects observed in long-lived organisms may be also due to epigenetic changes acquired during chronic exposure to reduced growth factors.

In rats, IGF-I attenuates cellular stress response and the expression of stress response proteins HSP72 and heme-oxygenase (Sharma *et al.*, 2000). In primary neurons IGF-I sensitizes cells to oxidative stress by a Ras/Erk-dependent mechanisms (Li *et al.*, 2008), and experiments in rat primary glia and mouse fibroblasts suggest that IGF-I sensitizes against oxidative damage and chemotherapy drugs (Lee *et al.*, 2010).

Together, the results from the range of studies and organisms described above indicate that enhanced stress resistance is a highly conserved phenotype of starved and long-lived organisms, which is partially mediated by downregulation of GH and IGF-I signaling.

DR and cancer treatment

Chronic moderate DR (20–40%) has been shown to promote protective effects in a range of organisms. Tumors rise from a combination of DNA damage and mutations and changes in the environment surrounding pre-cancerous cells (Hanahan and Weinberg, 2000). Not surprisingly, aging, which promotes all the components listed above, is the major risk factor for cancer (DePinho, 2000; Wedding *et al.*, 2007; Campisi and Vijg, 2009; Jemal *et al.*, 2010). It has been 100 years since the first report on the direct relationship between the amount of food intake and the growth or transplanted

tumors in mice showing that severe DR prevented tumor transplantation (Moreschi, 1909). Since then, a large body of work has established that DR reduces the progression of tumors in various animal models (Tannenbaum, 1945; Hursting *et al.*, 1994). However, recently it has been shown that specific oncogenes render tumors unresponsive to DR, suggesting that the efficacy of a reduction in food intake may be limited to a subset of cancers (Kalaany and Sabatini, 2009).

DR has also been shown to reduce cancer incidence. For instance, an 'adult-initiated' DR ('undernutrition without malnutrition') beginning at 12 months of age not only increases life-span, but also reduces spontaneous cancer incidence by more than 50% (Weindruch and Walford, 1982; Weindruch *et al.*, 1982). Furthermore, DR initiated at the time of weaning increased lifespan and reduced tumor incidence in mice (Weindruch *et al.*, 1986), confirming the DR studies in rats. These studies have been extended to primates with a 20-year longitudinal study in rhesus monkeys showing that DR (30%) delays disease onset and mortality, in part by causing a 50% reduction in cancer incidence (Colman *et al.*, 2009). Whether or not DR will reduce cancer incidence in humans is still unclear. However, DR can reduce clinical markers associated with cancer, analogously to its effect in rodents (Longo and Fontana, 2010).

The absence of DR in clinical cancer treatment after so many decades of laboratory findings reflects the limited potential of a chronic reduction in calorie intake in cancer treatment. Among the problems associated with the translation of DR into clinical applications is that chronic DR delays but does not stop the progression of the disease (Mukherjee *et al.*, 2004; Bonorden *et al.*, 2009; Shelton *et al.*, 2010), and that this delay will occur for only a subset of malignancies (Kalaany and Sabatini, 2009). Although weight loss and cachexia in the early stages of cancer progression are not as common as thought (Tisdale, 2002; Fearon *et al.*, 2003; Fox *et al.*, 2009), the ~15% loss of body mass index caused by a moderate (20%) calorie restriction (Racette *et al.*, 2006) would prevent its use in the great majority of cancer treatment scenarios. In addition, the effect of long-term restriction in delaying wound healing and impairing immune function (Fontana *et al.*, 2010; Kristan, 2008; Reed *et al.*, 1996) may impose a significant risk to cancer patients receiving chemotherapy, surgery or immunity-based treatments (Kim and Demetri, 1996).

Intermittent fasting (IF), where fasting is applied every other day, has been also shown to extend healthy lifespan and reduce cancer incidence without causing weight loss (Goodrick *et al.*, 1983; Varady and Hellerstein, 2007). However, the role of IF in combination with chemotherapy is not known. More importantly, because IF requires weeks–months to be effective, it may be more useful for cancer prevention than for cancer treatment. Nonetheless, it will be important to compare the efficacy of IF with that of prolonged fasting (short-term starvation), described in the following section.

Fasting vs DR in cancer treatment

Even if the side effects and limitations of chronic DR and IF were eliminated or reduced to a tolerable level, initial data suggest that prolonged starvation in the 2–3 days range for mice and 4–5 days range for humans has the potential to cause a much stronger protection of the host as well as changes which may retard the growth of cancer cells, without unwanted side effects. For example, the decrease in blood glucose caused by short-term fasting in mice is 75% vs the 15% caused by long-term DR or IF (Heilbronn *et al.*, 2005; Holloszy and Fontana, 2007). Similarly, the 75% reduction in blood IGF-I caused by a 2–5-day fast in mice and humans (Clemmons and Underwood, 1991; Lee *et al.*, 2010) is not matched by DR, which causes a 25% IGF-I reduction in mice (Barger *et al.*, 2008) and does not reduce IGF-I levels in humans unless protein intake is also restricted (Fontana *et al.*, 2008). Even together with protein restriction, chronic DR only causes a ~30% reduction in IGF-I (Fontana *et al.*, 2008).

The physiology of fasting

Although the effects of DR are often viewed as responses that have evolved to adapt to a relatively small (10–40%) reduction in food intake below the normal laboratory food intake established for a particular organism, it is more likely that they have evolved to adapt to long periods of complete starvation. Clearly, all organisms ranging from bacteria to humans have undergone periods of starvation alternated with periods of food availability. This is not only true for bacteria or yeast that can spend weeks or months under starvation conditions, but is also true for mammals. In fact, humans, for example, have encountered many and long periods of famine in their history and can survive for over 1 month on water only. For example, populations in Europe and Asia experienced frequent and severe famines, which in some cases caused hundreds of thousands of deaths (Scrimshaw, 1987).

The physiological response to fasting is quite different from that to DR (Table 1). In mammals, there are three metabolic stages during food deprivation (Wang *et al.*, 2006). First, the post-absorptive phase, which can last for 10 or more hours following food ingestion and involves the use of glycogen as the main stored energy source. When the liver glycogen storage has been depleted, it is followed by the second phase in which amino acids serve as the substrate for gluconeogenesis. Eventually glycerol and fatty acids released from adipose tissues become the major source of energy. The remaining glucose is mostly consumed by the brain, and the newly fat-derived ketone bodies (that is acetoacetate, β -hydroxybutyrate and acetone) become the major carbon sources in a matter of days (Cahill, 1970; Cahill *et al.*, 1970; Cahill, 2006). Following prolonged fasting lasting a week or longer, fat-derived β -hydroxybutyrate becomes the most abundant ketone body, accounting for roughly two thirds of brain fuel,

and glucose production reaches a very modest level (Cahill, 2006). During the last phase of prolonged food deprivation, fat reserves are eventually exhausted and rapid muscle degradation occurs to fuel gluconeogenesis. In rats, this occurs after 4–5 days but does not result in a major increase in glucose levels (Wang *et al.*, 2006). Weight loss caused by fasting is initially rapid but subsequently tapers off as shown in a human study, in which an average of 0.9 kg/day was lost during the first week of fasting, followed by a 0.3 kg/day weight loss by the third week of fasting. This study also reported an approximately 20% body weight loss caused by 30–35 days of fasting (Kerndt *et al.*, 1982). It is estimated that a 70 kg person can obtain basal caloric requirements from fat reserves during up to 2–3 months of fasting (Cahill and Owen, 1968; Cahill *et al.*, 1968; Saudek and Felig, 1976). Thus, prolonged fasting is feasible and generally well tolerated in humans, but may be accompanied by relatively minor side-effects, such as headaches, light-headedness, nausea, weakness, edema, anemia and amenorrhea (Bloom, 1959; Drenick *et al.*, 1964; Thomson *et al.*, 1966). In some rare cases, extreme fasting for periods of several weeks or longer in obese subjects has been reported to cause fatal complications such as renal failure, heart failure and lactic acidosis (Cubberley *et al.*, 1965; Spencer, 1968; Garnett *et al.*, 1969; Runcie and Thomson, 1970). Prolonged fasting can also cause severe problems and possibly death, as it occurred in some prisoners of concentration camps after they were liberated, if the re-feeding period is not gradual. Further, fasting should be limited to subjects that do not have deficiencies in metabolic pathways. For instance, patients with deficiencies in gluconeogenesis (for example, mutation in fructose-1,6-bisphosphatase), are known to experience hypoglycemia, acidosis, as well as failure to produce ketone bodies during fasting, rendering them highly susceptible to this severe restriction (van den Berghe, 1996).

Adaptation to starvation also occurs at the cellular level. A key survival mechanism a cell employs is self-eating, or autophagy, which is also activated in response to cellular stress (for example, chemotherapy) in various eukaryotes. For example, starving mammalian cells of essential nutrients (for example, glucose) and growth factors (for example, serum) induces autophagy, and starving mice for 12–24 h triggers autophagy in various tissues (Kroemer *et al.*, 2010; Marino and Kroemer, 2010). Interestingly, autophagy is regulated by several genes, such as *AMPK*, *mTOR*, and sirtuins, which are also known to regulate aging and stress resistance (Morselli *et al.*, 2009; Kroemer *et al.*, 2010; Marino and Kroemer, 2010). Many oncogenic signals (for example, PI3K and Akt) inhibit autophagy, whereas tumor suppressors (for example, PTEN and TSC2) trigger autophagy. Surprisingly many cancer cells display elevated autophagy, an increase which was recently suggested to be mediated by ammonia, a by-product of glutaminolysis that preferentially occurs in the mitochondria of malignant cells undergoing the Warburg effect (Vander Heiden *et al.*, 2009; Marino and Kroemer, 2010). Under the Warburg effect, mitochon-

dria takes on a different role to supply biosynthetic precursors that are required for the rapid proliferation rate set by oncogenic mutations (Vander Heiden *et al.*, 2009). Therefore, autophagy triggered by fasting may be beneficial to normal cells but detrimental to malignant cells. In fact, considering that autophagy increases the resistance of cancer cells to chemotherapy (Marino and Kroemer, 2010), it will be important to determine whether fasting promotes cancer sensitization alone or in combination with fasting.

Fasting, glucose and growth factors

Glucose levels undergo remarkable changes during food restriction, and can reach 50–60 mg/dl in a healthy person after only 72 h of fasting, but return to normal levels within 30 min of administering 100 g of glucose orally (Unger *et al.*, 1963). Fasting also reduces a plethora of extrinsic and intrinsic growth factors, particularly the insulin-like growth factor 1 (IGF-I), which acts as the major growth effector of growth hormone. If both the reduction and the rate of decrease are considered, the effect of fasting on the GH/IGF-I axis is unlikely to be surpassed by any other intervention including Food and Drug Administration approved drugs such as pegvisomant, a GH receptor antagonist. As described earlier, the GH/IGF-I axis is also a major regulator of metabolism and activates evolutionarily conserved pathways that promote stress sensitivity and aging (Fontana *et al.*, 2010). In humans, IGF-I levels decrease dramatically in response to a short-term starvation (36–120 h) despite increased GH secretion, which is highly lipolytic (Merimee *et al.*, 1982; Isley *et al.*, 1983; Thissen *et al.*, 1999; Maccario *et al.*, 2001; Norrelund, 2005; Moller and Jorgensen, 2009). This effect may be the result of a major increase in the IGF-I inhibitory protein IGFBP-1 (Zapf, 1995), which decreases further IGF-I bioavailability and prevents the feedback inhibition of GH secretion by IGF-I (Muller *et al.*, 1999). Notably, the 65% reduction in IGF-I levels induced by fasting for 5 days persisted 24 h after re-feeding, and only reached 70% of normal levels even 5 days after normal feeding (Isley *et al.*, 1983). In non-obese subjects GH levels eventually level off and drop between 3–10 days of fasting (Cahill *et al.*, 1966; Merimee and Fineberg, 1974; Palmblad *et al.*, 1977). In mice, a short-term starvation (24–72 h) decreases IGF-I production by 70% and causes an 11-fold increase in its inhibitory partner IGF-binding protein 1 (IGFBP-1) (Tannenbaum *et al.*, 1979; Frystyk *et al.*, 1999; Lee *et al.*, 2010). In response to fasting, mice also show reduced protein synthesis, reduced AKT activity via TRB3, a mammalian homolog of *Drosophila tribbles* (Du *et al.*, 2003), reduced mTOR/S6K, increased 4E-BP1 activity (Sans *et al.*, 2004) and increases FOXO-1, -3, -4 (Imae *et al.*, 2003).

Differential stress resistance (DSR) by fasting

Whereas the inactivation of partially conserved pro-aging pathways increases resistance to oxidative and

Table 2 Fasting protects against chemotherapy toxicity, in part by reducing IGF-I levels

Drug	Mode of action	Effect of fasting and/or reduced IGF-I
Doxorubicin	DNA intercalation	Protection by fasting and reduced IGF-I (mice)
Cyclophosphamide	DNA alkylation	Protection by reduced IGF-I (mice)
5-Fluorouracil	Anti-metabolite	Protection by reduced IGF-I (mice)
Etoposide	Topoisomerase II inhibition	Protection by fasting, but sensitized by reduced IGF-I (mice)
Docetaxel	Microtubule stabilization	
Carboplatin	DNA alkylation	Reduced side-effects by fasting (human)
Paclitaxel	Microtubule stabilization	
Gemcitabine	Anti-metabolite	

Abbreviation: IGF-I, insulin-like growth factor 1.

As tested in mice, fasting and the reduction of circulating IGF-I protect against doxorubicin, cyclophosphamide and 5-fluorouracil (5-FU) (Lee *et al.*, 2010; Raffaghello *et al.*, 2008). Notably, fasting protected against etoposide, but the reduction of IGF-I alone showed opposite results, suggesting a more complex role of fasting beyond IGF-I in the protection against certain drug categories. In a recent case series, patients that voluntarily fasted during chemotherapy reported significantly reduced side-effects (Safdie *et al.*, 2009). In several cases, chemotherapy was limited by toxic side-effects, but upon fasting, patients were able to resume normal treatment schedule. The chemotherapeutics administered to these patients include a combination of docetaxel, carboplatin, paclitaxel and gemcitabine.

other stresses in lower eukaryotes, the constitutive activation of analogous pathways is known to promote cancer. In fact, both Ras and Akt, whose orthologs are central in stress sensitization in yeast and worms, function in signal transduction pathways that are among the most frequently found in a constitutively activated form in human cancers (Medema *et al.*, 1993; Kinzler and Vogelstein, 1996; Hanahan and Weinberg, 2000). This connection between cancer and stress resistance provides the theoretical foundation for the differential killing of cancer cells not by the identification of drugs that specifically kill malignant cells, but by the activation of stress resistance in normal cells (differential stress resistance section, DSR) and the treatment with chemotherapy (Table 2). DSR is based on the hypothesis that in response to fasting, normal cells, independently of the cell type, will enter an alternate state characterized by reduced or lack of cell division, a switch to the utilization of the metabolites generated from the breakdown of fats, proteins and organelles (autophagy) described earlier, and resistance to multiple stresses (Longo *et al.*, 1997; Longo and Finch, 2003a; Raffaghello *et al.*, 2008). The inability of cancer cells to respond to anti-growth signals or to grow in the absence of growth factors is a well-established hallmark of cancer (Hanahan and Weinberg, 2000; Luo *et al.*, 2009). More specifically, self sufficiency in growth signals is enabled by gain-of-function mutations in oncogenes (for

example, *Ras*, *Akt*, *mTOR* and so on) that grant constitutive activation of proliferation pathways regardless of conditions. On the contrary, insensitivity to growth inhibitory signals is due to loss-of-function mutations in tumor-suppressor genes (for example, *Rb*, *p53*, *PTEN* and so on), allowing cancer cells to disregard anti-proliferation signals (Hanahan and Weinberg, 2000; Vogelstein and Kinzler, 2004) and therefore also disregard the fasting-induced signals.

Thus an effect of fasting on the growth of normal but not of cancer cells alone provides a method to preferentially target malignant cells with chemotherapy (Raffaghello *et al.*, 2008). However, this effect could be greatly enhanced by increasing stress resistance and activating a number of cell detoxification systems in both dividing and non-dividing cells. In fact, protection against stress in yeast mutants lacking *RAS2* and *SCH9* that are also starved increases 1000-fold or more compared with the approximately 10-fold increase in protection provided by fasting alone (Raffaghello *et al.*, 2008). Because, the inactivation of pro-growth pathways, particularly multiple pathways activated by glucose and amino acids, can increase stress resistance in both yeast and mammalian cells, the expression of constitutively or partially active oncoproteins present in the great majority of tumors is expected to prevent the protective effect of fasting regardless of the type of cancer or oncogene. Furthermore, cancer cells have been known for decades to rely more on glycolysis than on oxidative phosphorylation (Warburg effect), raising the possibility that the fasting-based enrichment of ketone bodies may increase their sensitivity.

The Warburg effect does not only apply to cancer cells, but also to rapidly dividing normal cells such as lymphocytes, thymocytes and enterocytes (Newsholme *et al.*, 1985). Although oxidative phosphorylation is far superior to glycolysis in terms of ATP production, glycolysis provides biosynthetic precursors that are essential to rapidly dividing cells. For instance, during glycolysis, glucose-6-phosphate can be directed to the pentose shunt pathway, providing reducing power and substrate for nucleotide synthesis, and glycerol can be processed into phospholipids that contribute to the cell wall (Alberts *et al.*, 2002). Increased glucose metabolism also allows cancer cells to restrict cytochrome c-mediated apoptosis, further favoring tumor cell survival (Vaughn and Deshmukh, 2008). The Warburg effect may encourage apoptosis evasion by also decreased respiration. It has recently been shown, using yeast as a model organism, that respiration during seeding and development of a cell population triggers apoptosis, suggesting that decreased respiration may contribute to tumorigenesis (Ruckenstuhl *et al.*, 2009).

A major concern regarding the effect of fasting in improving cancer treatment is the possibility that it may also protect malignant cells. However, a number of studies in simple model organisms, mammalian cells and mice indicate that oncogenes can render malignant cells unresponsive to fasting, therefore allowing fasting to not interfere with therapeutic efficacy in the great majority of cancer types. As mentioned above, in yeast, the

combination of starvation (switch from glucose medium to water at day 1) with the deletion of *SCH9/S6K* or both *SCH9/S6K* and *RAS2* increased resistance to treatment with hydrogen peroxide or menadione a 1000-fold, compared with that of cells expressing the constitutively active oncogene homolog *RAS2^{val19}* or cells lacking *SCH9/S6K* (*sch9/s6kΔ*) but expressing *RAS2^{val19}* (*sch9/s6kΔRAS2^{val19}*) (Raffaghello *et al.*, 2008). In fact, the expression of the oncogene-like *RAS2^{val19}* can not only reverse the protective effects of starvation and mutations, but also reduce the resistance of cells compared with wild-type cells, raising the possibility that fasting may have a sensitizing effect on cancer cells. In mice, using a metastatic mouse model of neuroblastoma, induced by intravenous injection of NXS2 cells, fasting effectively protected the mouse without interfering with chemotherapy efficacy, suggesting that it did not protect these cancer cells (Raffaghello *et al.*, 2008). In this study, tumor-bearing mice were fasted for 48 h followed by a single administration of high-dose chemotherapy, which successfully improved survival limited by both drug toxicity and metastases.

Analogously to the activation of yeast Sch9 and Ras by amino acids and glucose, the mammalian IGF-I receptor activates PI3K/Akt/PKB and Ras, and regulates glucose metabolism and cellular proliferation (Kandel and Hay, 1999). PI3K, which is frequently found mutated in human cancers (Yuan and Cantley, 2008), regulates growth and glucose metabolism in part via its downstream effector AKT (Vander Heiden *et al.*, 2009). Notably, in normal cells, fasting can reduce PI3K signaling (Xie *et al.*, 2007), leading to the protection of the cell. mTOR is another well-established master regulator, which integrates three major components—growth factors, nutrients and cellular energy level (ATP)—to regulate cellular growth and metabolism. Therefore, whereas reduced glucose can protect normal cells (Raffaghello *et al.*, 2008) it may be detrimental to the survival of malignant cells.

Reduced IGF-I levels mediate in part fasting-dependent DSR

As mentioned above, fasting causes a 70% decrease in circulating IGF-I levels, and restoring its levels during fasting reverses the protection against doxorubicin in mice (Lee *et al.*, 2010). The effect of reduced IGF-I on protection against chemotherapy toxicity was also studied by using transgenic mice with a conditional liver-specific *igf-1* gene deletion (LID), which results in a 70–80% reduction in circulating IGF-I levels, analogously to that caused by fasting. LID mice showed enhanced protection to commonly used chemotherapy drugs cyclophosphamide, doxorubicin and 5-FU, although they were not protected against the topoisomerase inhibitor etoposide. Reducing IGF-I signaling may provide dual benefits by protecting the organism and reducing tumor progression. In fact a recent study demonstrated the central role of IGF-IR in tumor progression and regression (Jones *et al.*, 2010) and at

least 12 different IGF-IR targeting compounds, including small antagonistic molecules and antibodies, have entered clinical trials (Gualberto and Pollak, 2009).

Fasting in clinical applications

Historically, fasting was performed for both medical and religious purposes (Kerndt *et al.*, 1982; Michalsen *et al.*, 2005; Johnstone, 2007). Much has also been learned about the effects of fasting from data on the victims of famine and war (Scrimshaw, 1987; Kalm and Semba, 2005), and also data from subjects fasting voluntarily for 40 days or longer (Kerndt *et al.*, 1982). Beyond its traditional practice, fasting has been demonstrated to have clinical benefits. Notably, clinical studies have shown that water-only fasting for 10–14 days significantly improved hypertension by reducing systolic blood pressure points more than two-fold compared with that of a combined vegan, low-fat, low-salt diet and exercise (Goldhamer *et al.*, 2001; Goldhamer, 2002). Moreover, the safety of fasting in patients with chronic disease has been studied in a large cohort study with over 2000 participants (Michalsen *et al.*, 2005). In this study, the authors determined that fasting (350kcal/day) was safe and considered by many of the participating subjects to be beneficial to their chronic disease (Michalsen *et al.*, 2005).

In a recent report, 10 patients with a variety of malignancies voluntarily fasted for up to 180 h in combination with chemotherapy, and reported a reduction in common chemotherapy-associated side-effects such as vomiting, diarrhea, fatigue and weakness (Safdie *et al.*, 2009) (Table 2). Notably, in the cases where cancer progression could be followed, there was no evidence that fasting protected tumors or interfered with chemotherapy efficacy. A controlled randomized clinical trial testing the effect of fasting on cancer treatment underway at the University of Southern California Norris Cancer Center is expected to provide more conclusive clinical data on the effect of fasting on chemotherapy toxicity and efficacy.

Conclusion and discussions

According to the American Cancer Society, cancer patients receiving chemotherapy should increase calorie and protein intake (Doyle *et al.*, 2006). Contrary to this dogma, many studies suggest that a 20–40% reduction in calorie intake protects the host against toxins and retards the growth of tumors (Moreschi, 1909; Tannenbaum, 1945; Hursting *et al.*, 1994, 2001, 2003; Fontana *et al.*, 2001, 2006; Harper *et al.*, 2006; Bonorden *et al.*, 2009; Colman *et al.*, 2009; Longo and Fontana, 2010). However, this has never been translated into clinical applications because the effects are limited, as reduced calorie intake unavoidably causes weight loss, and because it was not clear that DR could not also protect cancer cells from chemotherapy. By contrast, based on the DSR studies and on clinical data, fasting for a

limited period can have a potent protective effect on the host and possibly detrimental effects on a variety of tumors. However, fasting still poses a challenge to patients, which calls for an urgent need of substitution diets or targeted drugs that can mimic fasting. Experimental evidence from aging research suggests that diets with specific deficiencies, especially essential amino acids, may provide increased stress resistance and lifespan (Richie *et al.*, 1994; Takenaka *et al.*, 2000; Harper *et al.*, 2006). Although, it is unlikely that the vast effects caused by fasting can be obtained with specific deficiencies, it will be important to determine whether limitations of specific nutrients will be sufficient to promote some of the effects of fasting. Furthermore, because cancer depends on certain nutrients, there is a large potential for the combination of dietary interven-

tions and chemotherapy or other non-toxic cancer therapies for the enhancement of cancer treatment. In addition, as discussed in this study, exploring drug targets based on the IGF-I and related systems may also identify fasting-mimetics that provide DSR. Interventions that could provide a differential protection of host and cancer cells in the 1000-fold differential protection range observed in yeast cell lacking or expressing an oncogene analog, could have paradigm shifting effects in cancer treatment.

Conflict of interest

Valter Longo founded L-Nutra, which develops diets for cancer patients.

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