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Abstract	<p>Hormesis in aging is represented by stress-induced stimulation of protective mechanisms and strengthened homeostasis/homeodynamics. Since calorie restriction (CR) generally stimulates various maintenance and repair systems, hormesis has been considered as one of the possible mechanisms. The evidence in favour of the hormetic explanation for the beneficial effects of CR includes the induction of various stress responses including heat shock response, unfolded protein response, autophagy, DNA repair response, sirtuin response, and antioxidant responses. In addition, chronically increased levels of corticosterones and an accompanying decrease in body weight in CR animals is indicative of CR being a stressor and a hormetic agent. Although the exact nature of the low level damage-inducing aspect of CR is yet to be elucidated, a metabolic shift from glycolytic pathways to mitochondrial pathways, which results in enhanced oxidative stress and increased damage to macromolecules and organelles, appears to be the initiator of stress-induced hormetic effects of CR. Periodic modulation of dietary input as a hormetic intervention may be applicable to human beings for slowing down aging.</p>	
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## Chapter 13

# Hormesis as a Mechanism for the Anti-aging Effects of Calorie Restriction

Suresh I.S. Rattan and Dino Demirovic

### 13.1 Introduction

Chronic, intermittent or periodic calorie restriction (CR) brings about a wide range of biological effects in various organisms, including slowing down of aging and extension of lifespan. Several mechanisms have been suggested to explain the multiple biological effects of CR, which include a reduction in the levels of molecular damage due to reduced metabolism, a reduction in body temperature, alteration in the extent cell proliferation and cell death, a decline in responsiveness to hormones, and changes in oncogenic expression leading to reduced carcinogenesis (Masoro 2006; Cavallini et al. 2008). Another mechanism that has been invoked in order to understand and explain the beneficial effects of CR is that of mild stressed-induced hormesis, which stimulates maintenance and repair systems (MARS). Here we describe the phenomenon of hormesis in the context of aging and anti-aging, and review the data with respect to the view that CR is a hormetic anti-aging intervention.

### 13.2 Homeostasis, Homeodynamics and Aging

A fundamental characteristic of animate systems is their intrinsic ability to respond, to counteract, and to adapt to the external and internal sources of disturbance. The traditional conceptual model to describe this characteristic is *homeostasis*, which has now been shown to be incomplete and insufficient to fully describe the complexities of the living systems. The main reason for the incompleteness of the homeostasis model is its defining principle of “stability through constancy”, which does not take into account the new themes, such as cybernetics, control theory, catastrophe theory, chaos theory, information and interaction networks, that comprise and

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46 underlie the modern biology of complexity. Therefore, since 1990s, the term *home-*  
47 *odynamics* (Yates 1994), is being increasingly used, which accounts for the fact that  
48 the internal milieu of complex biological systems is not permanently fixed, is not at  
49 equilibrium, and is in a dynamic regulation and interaction among various levels of  
50 organization.

51 Various molecular, cellular and physiological MARS are well known, and these  
52 include nuclear and mitochondrial DNA repair, free radical quenching and coun-  
53 teraction, protein turnover and repair, detoxification, immune response and stress  
54 responses. All these processes involve numerous genes whose products and their  
55 interactions give rise to a “homeodynamic space” or the “buffering capacity”, which  
56 is the ultimate determinant of an individual’s chance and ability to survive and main-  
57 tain a healthy state (Rattan 2006, 2008b). Aging is a progressive shrinkage of the  
58 homeodynamic space due to the occurrence and accumulation of molecular damage,  
59 which makes an individual more vulnerable and prone to physiological imbalance.  
60 Thus, aging, age-related diseases, and eventual death are the final manifestations of  
61 unsuccessful homeodynamics and of failure of MARS (Holliday 2007; Rattan 2006,  
62 2008b).

63 A critical component of the homeodynamic property of living systems is their  
64 capacity to respond to stress. In this context, the term “stress” is defined as a signal  
65 generated by any physical, chemical or biological factor (stressor), which in a living  
66 system initiates a series of biological events that enable it to counteract, adapt and  
67 survive. Table 13.1 gives a list of main molecular stress responses (SR), and are  
68 integral to the organismic property of homeodynamics. Based on the involvement  
69 of one or more molecular SR, higher order (cellular, organ level and body level) SR  
70 are manifested, which include apoptosis, inflammation, and hyperadrenocorticism  
71 leading to increased levels of circulating corticosterones in the body.

72 Not all pathways of the SR respond to every stressor, and although there may  
73 be some overlap, generally SR pathways are quite specific. The specificity of the  
74 response is mostly determined by the nature of the damage induced by the stressor  
75 and the variety of downstream effectors involved. For example, cytoplasmic induc-  
76 tion of protein denaturation by heat, heavy metals and antibiotics will initiate the  
77 so-called heat shock response (HSR) by inducing the synthesis of heat shock pro-  
78 teins (HSP) followed by the activation of proteasome-mediated protein degradation  
79 (Verbeke et al. 2001; Liberek et al. 2008). But, unfolded proteins in the endoplasmic  
80 reticulum (ER) will induce unfolded protein response (UPR), and will initiate  
81 the induction of synthesis of a totally different set of proteins and their downstream  
82 effectors (Banhegyi et al. 2007; Yoshida 2007).

83 However, often the source of activation (stressor) cannot be easily identi-  
84 fied, and may involve more than one stressor and their effectors. Examples of  
85 such SR include early inflammatory SR and neuroendocrinal SR, which lead to  
86 the synthesis and release of interleukins and corticoid hormones, respectively.  
87 Similarly, pathways involving NF- $\kappa$ B, Nrf2, FOXO, sirtuins and heme-oxygenase  
88 (HO) activation may involve more than one type of stressors and stress signals,  
89 including pro-oxidants, free radicals, reactive oxygen species (ROS), and nutritional  
90 components. Yet, an optimal SR is an essential aspect of successful homeodynamics  
and continued survival.

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**Table 13.1** Major molecular level stress responses

Response	Stressors	Effectors	Reference
Heat shock response	Heat, heavy metals, antibiotics, protein denaturation	Heat shock proteins, proteasome and other proteases	(Liberek et al. 2008)
Unfolded protein response	Unfolded and misfolded proteins in endoplasmic reticulum	Chaperones, co-chaperones	(Yoshida, 2007)
Autophagic response	Food starvation, hypoxia, damaged organelles	Lysosomes	(Yen and Klionsky 2008)
DNA-repair response	Radiation, oxidants, free radicals	DNA-repair enzymes	(Hakem 2008)
Antioxidant response	Free radicals, reactive oxygen species, pro-oxidants	Nrf-2, heme-oxygenase, FOXO	(Ishii et al. 2002)
Sirtuin response	Energy depletion	Sirtuins	(Longo 2009)
NF- $\kappa$ B inflammatory response	Pathogens, allergens, damaged macromolecules	cytokines, nitric oxide synthase	(Medzhitov 2008)

### 13.3 Stress and Hormesis

The consequences of SR can be both harmful and beneficial depending both on the intensity, duration and frequency of the stress, and on the price paid in terms of energy utilisation and other metabolic disturbances. But the most important aspect of SR is that it is not monotonic with respect to the dose of the stressor, rather it is almost always characterized by a nonlinear biphasic relationship. Several meta-analyses performed on a large number of papers published in the fields of toxicology, pharmacology, medicine, and radiation biology have led to the conclusion that the most fundamental shape of the dose response is neither threshold nor linear, but is U- or inverted U-shaped, depending on the endpoint being measured (Calabrese et al. 2007; Calabrese 2008). This phenomenon of biphasic dose response was termed as hormesis (Southam and Ehrlich 1943).

Since several terms such as, autoprotection, heteroprotection, adaptive response, preconditioning, hormesis, xenohormesis and others, have been used to describe the biological responses to various stressors, recommendations have been made for the use of a common terminology which is consistent with the quantitative features of the dose-response and underlying molecular foundations (for historical development of the term, see (Calabrese 2002)). It has been proposed that a common terminology should include the operational term hormesis, which would be preceded by the type

of inducing agent and whether or not conditioning was present (Calabrese et al. 2007). Three main categories of such terms are:

- (1) physiological conditioning hormesis, in which an exposure to a stressful condition, such as hypoxia, ischemia, radiation or a toxic chemical, conditions the system to tolerate much higher doses of the same condition subsequently
- (2) physiological/chemical/radiation hormesis: when hormesis occurs without prior conditioning; and
- (3) post-exposure conditioning hormesis: The post-conditioning hormesis in which exposure to high doses of a stressor, such as radiation for cancer therapy, is followed by repeated low dose radiation exposure.

The key conceptual features of hormesis are the disruption of homeodynamics, the modest overcompensation, the reestablishment of homeodynamics and the adaptive nature of the process. An example of stress-induced hormesis is the well documented beneficial effects of moderate exercise as a hormetic agent, which initially increases the production of free radicals, acids and aldehydes (Alessio and Hagerman 2006; Ji et al. 2006; Radak et al. 2008). Another frequent observation in studies of hormesis is that a single hormetic agent, such as heat shock or physical activity, can improve the overall homeodynamics of cells and enhance other activities such as tolerance to other stresses, by initiating a cascade of processes resulting in a biological amplification and eventual beneficial effects (Mattson 2008b; Rattan 2008a). Hormesis in aging research and anti-aging interventions is represented by mild stress-induced stimulation of protective mechanisms in cells and organisms resulting in biologically beneficial effects (Le Bourg and Rattan 2008; Rattan 2008a).

### 13.4 Calorie Restriction as a Hormetic Agent

Based on the observations that diurnal maxima of corticosterone stress hormone concentration was higher in dietary restricted rats, it was proposed that CR acted through hormesis (Masoro and Austad 1996). Daily periods of moderate hyperadrenocorticism were considered to be protective against damage-inducing agents by restricting the energy intake. CR as a hormetic agent was further supported by the observations that CR resulted in a decrease in the body weight of animals, and this had multiple metabolic and physiological beneficial effects (Turturro et al. 1998, 2000). Additionally, corticosterone treatment was shown to modulate the level of oxidative stress in proteins and mitochondrial DNA in male Wistar rat livers, which was similar to the effects of CR (Caro et al. 2007). In the case of human beings too, an increase in the levels of corticosteroids and a loss of body weight have been observed during short-term and long-term dietary restriction (Walford et al. 2002; Yu 2006; Fontana 2009). Thus, at the whole body physiological level, CR is a sustained low intensity stressor (Masoro 2007).

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181 However, it is important to know what other hormetic pathways may be involved  
182 in the causation of anti-aging and other health beneficial effects of CR. What follows  
183 is the review of published data on the effects of CR on various SR pathways and their  
184 effectors, as listed in the Table 13.1.

### 187 **13.4.1 Heat Shock Response (HSR)**

189 It is a universal and primordial SR achieved by the activation of the HS transcription  
190 factor(s), followed by the preferential synthesis of several HSPs, and is considered  
191 to be one of the important markers of ensuing hormesis. HS-induced anti-aging  
192 and life prolonging hormetic effects are well documented in various model systems  
193 including insects, nematodes, rodents, and human cells in culture (Rattan 2008a).  
194 Therefore, in order to consider CR to be hormetic, it is important to know if CR  
195 induces HSR. Indeed, basal levels of HSP70 have been reported to be higher in the  
196 brains of old male Wistar rats under CR than those in freely fed animals (Unno et al.  
197 2000). Similarly, life long CR of male Fischer rats resulted in a 4-fold higher level  
198 of HSP90 and an accompanying increase in the activity of its effector, proteasome,  
199 in the soleus muscle (Selsby et al. 2005). Increased proteasomal activities have also  
200 been reported in CR Fischer rats (Shibatani et al. 1996; Shibatani and Ward 1996),  
201 and in CR yeast (Hahn et al. 2004).

202 Levels of HSP70 are also reported to increase in hippocampal neurones in CR  
203 Sprague-Dawley rats (Guo et al. 2000; Arumugum et al. 2006). It was also reported  
204 that hepatocytes isolated from old CR Fischer 344 rats maintained HSR in terms of  
205 transcription and translation of HSP70 after exposure to HS, as compared with the  
206 loss of HSR in freely fed old animals (Heydari and Richardson 1992). Activation  
207 of HSR was also reported for glucose-starved yeast with increased longevity (Hahn  
208 et al. 2004). Thus, activation and maintenance of HSR by CR is a strong evidence  
209 in favour of the view that CR is hormetic. However, it is not clear how exactly  
210 CR initiates HSR, and what is the exact nature of the CR-induced damage, such as  
211 protein denaturation and aggregation, which are the normally required intracellular  
212 signals HSR.

### 214 **13.4.2 Unfolded Protein Response (UPR)**

218 Accumulation of misfolded proteins in the ER leads to the so-called ER stress  
219 response, also known as UPR, resulting in the synthesis, activation and translo-  
220 cation of several chaperones. UPR-induced stress proteins include GRP78/BiP,  
221 GRP94/gp96, GRP170/ORP150, GRP58/ERp57, PDI, ERp72, calnexin, calretic-  
222 ulin, EDEM, Herp and other co-chaperones (Lin et al. 2007; Ni and Lee 2007;  
223 Yoshida 2007; Banhegyi et al. 2007). In addition, there are mitochondrial-specific  
224 SR in mammalian cells, and involves the induction and activation of various other  
225 chaperones, such as chaperonin-10 (Cpn10/Hsp10), chaperonin-60 (Cpn60/Hsp60),

226 and mortalin (Zhao et al. 2002; Kaul et al. 2007). An age-related decline in UPR-  
227 induced proteins and other chaperones has been reported in the neural retina of  
228 Brown Norway rats, which is prevented by CR, and the high levels of ER chaperones  
229 are maintained (Li et al. 2004). Furthermore, transient methylglyoxal production  
230 during periods of glycolysis in dietary restricted animals induces modification of the  
231 chaperone and anti-apoptotic protein HSP27, and increases its protective functions,  
232 suggesting a possible hormetic response (Hipkiss 2007).  
233  
234

### 235 **13.4.3 Autophagy Response**

236  
237  
238 Autophagy response is the lysosome-mediated and chaperone-mediated sequestering  
239 of damaged membranes and organelles, which is a SR induced during nutritional  
240 limitation, starvation, and hypoxia (Martinez-Vicente et al. 2005; Terman et al.  
241 2007; Yen and Klionsky 2008). Several studies have shown that intermittent or  
242 chronic CR is a inducer of autophagy in the nematode *Caenorhabditis elegans*, fruit-  
243 flies, and rats and mice (Bergamini et al. 2003; Cavallini et al. 2008; Cuervo 2008;  
244 Dröge 2004; Meléndez et al. 2003; Hansen et al. 2008; Ravikumar et al. 2006).  
245 Furthermore, down regulation of the nutrient sensor TOR (target of rapamycin) also  
246 elicits autophagy and extends lifespan of *C. elegans*, while inhibition of autophagy  
247 genes (ATG) eliminates the life extending effects of CR (Hansen et al. 2008).  
248 Similarly, feeding-defective *eat-2* mutants of *C. elegans* have increased autophagy  
249 and a longer lifespan, while abolishing autophagy in ATG mutants eliminates the  
250 life extending effects of reduced dietary intake (Morck and Pilon 2006). Promoting  
251 the expression of an autophagy gene, *Atg8a*, in the brains of adult *Drosophila*  
252 extended their average lifespan (Simonsen et al. 2008). Enhanced autophagy was  
253 also observed in periodic low serum-related anti-aging effects in human skin  
254 fibroblasts in culture (Rattan 2007).  
255  
256  
257

### 258 **13.4.4 Antioxidant Response**

259  
260 An important mechanism by which cells responds to oxidative stress is through the  
261 regulation of transcription of several antioxidant genes. The main regulator of this  
262 specific antioxidant phenotype is the nuclear factor-erythroid-2 (Nrf2) transcription  
263 factor, which regulates the basal and inducible expression of numerous detoxifying  
264 and antioxidant genes (Ishii et al. 2002). Under normal conditions, Nrf2 is held in  
265 the cytoplasm by the specific inhibitory protein KEAP1. Oxidative modification of  
266 cysteine residues of KEAP1 induces conformational changes and a loss of Nrf-2  
267 binding, allowing Nrf2 to translocate to the nucleus where it heterodimerizes with  
268 specific co-factors, and leads to the transcription of various genes through the reg-  
269 ulatory regions of antioxidant response elements (Motohashi and Yamamoto 2004;  
270 Yamamoto et al. 2004). Some of the genes activated by stress-induced activation of

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271 Nrf2 are heme-oxygenase 1 (HO-1), NAD(P)H-quinone oxidoreductase-1 (NQO1),  
272 and glutathione S-transferases (GSTs).

273 CR has been shown to increase the levels of Nrf2-regulated HO-1, NQO1 and  
274 GST mRNA and proteins in rodent tissues (Calabrese et al. 2008; Pearson et al.  
275 2008). The so-called mimetics of CR, including resveratrol and curcumin, also act  
276 through Nrf2-dependent pathways (Calabrese et al. 2008; Pearson et al. 2008). In  
277 the case of *C. elegans*, transcription factor SKN-1, which is a homologue of Nrf2,  
278 gets induced by CR and activates the gene *skn-1* in a pair of nutrition sensing neu-  
279 rons, considered to be responsible for CR-induced extension in lifespan (Bishop and  
280 Guarente 2007).

281

282

283

#### 284 **13.4.5 DNA Repair Response**

285

286 An activation of DNA repair enzymes in response to DNA damage is a SR, which is  
287 essential for the maintenance of genomic stability (Hakem 2008; Vijg 2008). Earlier  
288 studies had shown that unscheduled DNA synthesis (UDS) induced by UV irradiation  
289 was significantly higher both in the hepatocytes and kidney cells isolated from  
290 CR Fischer F344 rats (Weraarchakul et al. 1989), and in the skin cells isolated from  
291 Brown Norway x Fischer 344 F1 hybrid BNF rats (Lipman et al. 1989). Similarly,  
292 several other reports showed that DNA repair activity was maintained at higher lev-  
293 els in CR organisms, including yeast, *C. elegans*, rodents and humans (Haley-Zitlin  
294 and Richardson 1993; Guo et al. 1998; Raji et al. 1998; Lee et al. 1999; Howitz  
295 et al. 2003; Um et al. 2003; Cohen et al. 2004). However, these studies provide only  
296 indirect evidence that CR may be hormetic in terms of increased DNA repair ability  
297 at all ages.

298 Direct evidence that CR upregulates DNA repair enzyme activities at transcrip-  
299 tional and translation level comes from studies on young male Fischer 344 rats in  
300 which a significant increase in base excision repair, and a corresponding increase in  
301  $\beta$ -pol protein and mRNA was observed after a short term CR (Cabelof et al. 2003).  
302 Furthermore, there was also an increase in the levels of DNA damage check point  
303 protein p53, which is also a sign of effective SR (Cabelof et al. 2003). However, it  
304 is not clear what kind of DNA damage signal is initiated by CR that leads to the  
305 stimulation of DNA repair activities.

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#### 309 **13.4.6 Sirtuin Response**

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311 Activation of sirtuins causing deacetylation of histones and other proteins is consid-  
312 ered to be a kind of SR to reduced levels of metabolic energy (North and Sinclair  
313 2007; Longo 2009). Since an independent chapter in this book deals in detail  
314 with the role of sirtuins in CR, here we only emphasize the fact that CR-induced  
315 activation of sirtuins, and its consequential beneficial effects, including the down

316 regulation of insulin and IGF-1 pathways, can be considered as hormetic (North  
317 and Sinclair 2007; Longo 2009).

#### 320 **13.4.7 NF- $\kappa$ B Inflammatory Response**

322 Both acute and chronic inflammations are protective SR mechanisms in the wake  
323 of cell and tissue injury (Njemini et al. 2004; Medzhitov 2008; Vasto et al. 2009).  
324 One of the main inflammatory mediators is the transcription factor NF- $\kappa$ B, which  
325 together with other downstream mediators, such as TNF- $\alpha$ , cytokines, nitric oxide  
326 synthase (NOS), and prostaglandins, have important homeostatic functions, includ-  
327 ing repair of tissues, control of metabolism, and regulation of the hypothalamus-  
328 pituitary axis (Medzhitov 2008). However, this inflammatory SR, over a longer time  
329 scale becomes chronic inflammation, which is considered to be one of the major  
330 causes of aging and age-related diseases (Franceschi et al. 2000).

331 Activation of NF- $\kappa$ B during acute bout of exercise and leading to low level  
332 inflammation has been considered as a hormetic response (Ji et al. 2006). Other  
333 hormetic agent which stimulate NF- $\kappa$ B include various phytochemicals, such as cur-  
334 cumin and resveratrol (Calabrese et al. 2008; Mattson 2008a). However, as regards  
335 CR being a hormetic agent working through inflammatory response, there is no evi-  
336 dence so far that CR is pro-inflammatory even for a short while. In contrast to this,  
337 there are several reports that show the anti-inflammatory effects of CR in terms  
338 of various molecular markers of inflammation, including inactivation of NF- $\kappa$ B  
339 (Chung et al. 2002; Kim et al. 2002; Witte et al. 2009).

### 342 **13.5 So, Is CR Hormetic?**

345 In order to justify that hormesis is a mode in bringing about the anti-aging and life  
346 prolonging effects of CR, it is important to show that CR is a mild stressor that initi-  
347 ates one or more stress responses. Various lines of evidence reviewed above clearly  
348 show that CR induces several, if not all, stress responses; and that the observed bio-  
349 logical effects in terms of enhanced maintenance and repair systems fit perfectly  
350 well with the hormetic theory of the mode of action of CR.

351 One of the requirements for any agent to be called hormetic is that it should  
352 act as a stressor by causing a disturbance in homeodynamics at the physiologi-  
353 cal, cellular or molecular levels. At the whole body physiological level, CR via  
354 glucose-sensing neurons and pancreatic cells initiates a series of neuroendocrine  
355 processes which lead to the reduction of IGF-1 and an increase of corticosterones  
356 (Klebanov et al. 1995; Koubova and Guarante 2003). At the tissue and cell level,  
357 CR appears to elicit SR by transiently increasing the production of ROS, which then  
358 lead to increased oxidative damage to proteins and DNA. There is some evidence  
359 that CR increases oxygen consumption and mitochondrial respiration, for example  
360 in yeast (Lin et al. 2002), and in *C. elegans* accompanied by a 3-fold increase in ROS

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361 formation (Schulz et al. 2007). Increased production of free radicals during short-  
 362 term fasting has also been reported for Wistar rat liver mitochondria (Marczuk-  
 363 Krynicka et al. 2003; Sorensen et al. 2006). There is also some evidence that the  
 364 protein carbonyl content, a sign of oxidative damage to proteins, was significantly  
 365 increased in Fischer F344 rats exposed to CR for 2 months (Judge et al. 2004).  
 366 However, when measured in long-term CR animals, ROS levels and oxidatively  
 367 damaged protein levels are either decreased or not different from freely fed ani-  
 368 mals (López-Torres et al. 2002; Gomez et al. 2007; Caro et al. 2008). Therefore,  
 369 further studies are needed to establish the effects of other regimens of CR, such as  
 370 intermittent total fasting, intermittent partial fasting, every-other-day feeding and  
 371 so on.

372 Finally, it is clear that there are two phases of CR: an immediate adaptive  
 373 response through hormesis, and a steady state and life long response in terms of  
 374 improved MARS (Koubova and Guarante 2003). So far, most of the data on the  
 375 phenomenological and mechanistic aspects have been collected after longer period  
 376 of CR. However, it is the immediate responses to CR which best qualify to be  
 377 considered as hormetic. For example, an exposure to CR or nutritional deprivation  
 378 induces a metabolic shift from the production of ATP through glycolytic pathways  
 379 to mitochondrial pathways (Schulz et al. 2007), which then leads to increased respi-  
 380 ration, increased production of ROS, and consequent damage to mitochondria, other  
 381 organelles, and to macromolecules. This sequence of events then leads to compen-  
 382 satory hormetic responses including HSR, autophagy, DNA repair response, and  
 383 antioxidant responses.

384 CR-induced hormesis results in an overall improvement in homeodynamics by  
 385 strengthening the networks of MARS, and by reducing the rate of occurrence and  
 386 accumulation of macromolecular damage. Therefore, periodic bouts of complete or  
 387 partial CR, which initiate a series of protective stress responses and induce hormesis,  
 388 may be also applicable to human beings as an effective anti-aging intervention.  
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