

Nutrition, hormetic stress and health

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Hormesis defines an effect where exposure to a low dose of a toxic agent results in a beneficial response. It has been described in organisms exposed to low-dose radiation, heat stress, and chemicals. The effect is characterised by a J-shaped dose–response as opposed to a linear dose–response. Confirmation of the general phenomena of hormesis has proved difficult due to the lack of appropriate methodology and the absence of well-defined mechanisms to support the experimental observations. In the nutritional field there are few reports of its existence. The clearest illustration of the effect is seen in animals that are energy restricted when there is a clear benefit in the reduction of age-related disease, and an extension of maximum lifespan. DNA microarray experiments have shown that there is a down regulation of the stress-response genes that are up regulated through the ageing process. Electrophilic phytochemicals, that have been shown to have beneficial health effects at low doses, up regulate the antioxidant–electrophile response element. This probably occurs through an alteration in the redox state of the target cells which causes activation of protein kinases, the activation of the Nrf2 transcription factor and the up regulation of the phase II enzymes, similar to responses that occur under mild chemical stress. This situation might enable organisms to adapt to stress such that the effects of a subsequent exposure to a harmful challenge are reduced. There may be a permanent alteration in cellular homeostasis, or redox state, if the low level exposure is maintained. It remains to be proven if such a situation occurs in response to chronic low-dose exposure to dietary phytochemicals such that the target cells are better able to respond to a subsequent stress challenge.

Hormesis: Stress responses: Phytochemicals: Radiation: Energy restriction: Antioxidant response element

Introduction

The concept that the exposure of cells or whole organisms to toxic agents, at doses far below those for which the threshold of toxicity can be measured, can provide benefits to the organism has been termed hormesis. The phenomenon was described by Schultz (1888) who observed that small doses of poisons seemed to stimulate the growth of yeast. Previous work by Arndt claimed that low doses of drugs were also beneficial. These observations developed into the Arndt–Schulz Law which stated that all poisons are stimulatory in low doses, i.e. doses below which any toxic effects are probable. The concept attracted hostility due to its adoption by homeopaths, even though evidence continued to be produced which claimed to support the proposition.

It is only in the last decade or so that the debate has opened up again (Calabrese & Baldwin, 2001). Hormesis in its modern form is described as the capacity of an organism to display a stimulatory response to a compound, or physical

agent, at low doses, but to show an inhibitory response to the same agent at higher doses. The focus of toxicology has been on the potential of chemicals to cause toxicity, depending on dose; the experimental study of dose–response relationships is heavily weighted towards the use of high doses that cause toxicity. Within the field of food regulatory toxicology the concept prevails that low doses of a compound shown to be toxic must pose some degree of risk, no matter what the dose. The purpose of regulatory toxicology is to derive levels of intake that appear to be without ‘measurable risk’ and refers to these as ‘acceptable’ or ‘tolerable’ intakes. The idea that a toxic agent might cause benefits at low-dose exposure is still regarded as unproven (Dybing *et al.* 2002; Rodricks, 2003).

Detection of a hormetic effect

Toxic substances are normally assumed to show a dose–response relationship that shows an increased risk in relation

Abbreviations: CSR, cellular stress response; ER, energy restriction, PKG, cGMP-dependent protein kinase; GST-P, glutathione-S-transferase placental form; MnSOD, Mn superoxide dismutase.

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to dose. A hormetic response is one where the dose–response shows a decreased risk at certain low doses; in other words, the nature of the dose–response is J-shaped as shown in Fig. 1.

As an example, if exposure to a substance up regulated DNA repair, and if the induced repair also repaired background DNA damage, a J-shaped dose–response, rather than a linear dose–response, would be expected.

Hormetic responses have also been described as U-shaped but this implies that the adverse effects at very low doses are the same as the adverse effects at high doses and there is no evidence to confirm this. Not all J-shaped dose–response relationships are necessarily hormetic, however. It is probably a characteristic shown by essential nutrients. Insufficient intake of essential nutrients results in a loss of function, for example, homeostasis, or overt disease, whereas an optimal intake of these nutrients maximises the functional ability of cells. Intake of nutrients above the optimal intake results in no further benefit and may even lead to toxicity.

Experimental obstacles

Studies on hormetic effects have mostly been confined to empirical observations rather than exploring the molecular basis of any effect. Experimental studies are difficult to undertake since the statistical proof of any effect requires the use of an enormous number of animals. Because hormetic responses are generally modest responses, more treatment groups are needed to define the nature of the dose–response relationship in the low-dose region, which is rarely undertaken in toxicological bioassays. To have a reasonable chance of finding evidence of hormesis in classical toxicological studies it would be necessary to use at least six dose levels, three of which would need to be below the no-observed adverse effect level (Calabrese *et al.* 1999). Very few studies have been undertaken that satisfy these criteria. Similar arguments have been adduced for the lack of detection of hormetic effects in developmental toxicity protocols (Razzaghi & Lummis, 2001).

To identify modest hormetic effects in human epidemiological studies an enormous input of resources is needed to quantify the effect with a useful degree of precision. The effects of exposure to low-dose radiation have been studied

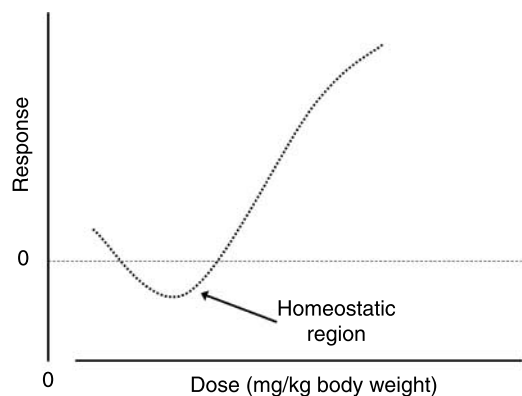


Fig. 1. Hypothetical dose–response curve for essential nutrients.

in large populations. However, the studies are ecological studies, which are well known to be susceptible to various types of bias, and this makes their interpretation open to challenge.

Examples of hormetic effects

Xenobiotic environmental agents

Toxicological studies indicate that a diverse range of toxic chemical compounds can show hormetic effects. Low-dose carcinogenesis studies have been undertaken on a number of chlorinated, lipophilic chemicals that are persistent and result in human low-dose exposure through their accumulation in the food chain.

Studies of the effects of p,p-dichlorodiphenyltrichloroethane on the formation of liver tumours in the F344 rat have shown that, like classic liver carcinogens, it initially produces pre-neoplastic lesions. These lesions are associated with the induction of glutathione-S-transferase placental form (GST-P) which is hardly detectable in the normal liver, and which can be used as a surrogate biomarker for tumour formation in chemical hepatocarcinogenesis. GST-P induction by p,p-dichlorodiphenyltrichloroethane only occurs at high doses, however. At low-dose exposure, consistent with the levels that are found in the diet (≤ 2 parts per million), GST-P induction does not occur. Indeed there is evidence for a protective effect since the GST-P levels were below those of control animals, albeit not significantly (Sukata *et al.* 2002).

Similar effects have been observed with phenobarbitone and α -benzene hexachloride at low-dose exposure. All these chemicals also induce cytochrome P-450 3A2 in a dose-dependent manner similar to GST-P. Phenobarbitone shows a significant inhibition of cytochrome P-450 3A2 expression at low doses (Kitano *et al.* 1998) whereas α -benzene hexachloride also shows reduction of expression below the baseline in controls but the effect was not statistically significant (Masuda *et al.* 2001; Kinoshita *et al.* 2003). mRNA for IL-1 receptor type 1 and TNF- α receptor type 1, whose ligands have roles not only in down regulating cytochrome P-450 3A2 expression but also in inducing anti-proliferative effects or apoptosis in hepatocytes, have also been studied (Sukata *et al.* 2002). An increase in mRNA expression was seen at low doses of p,p-dichlorodiphenyltrichloroethane exposure. Oxidative stress in liver DNA was also decreased, as assessed by a reduction in 8-hydroxyguanosine production, suggesting that the possible hormetic effects seen may be linked to the levels of oxidative stress and pro-inflammatory cytokines.

One of the best examples of a hormetic effect is from a long-term carcinogenicity study on 2,3,7,8-tetrachlorodibenzo dioxin. The data obtained for female rats are shown in Table 1.

Females show a higher incidence of tumours in controls than males. The data show that for total tumours there is a hormetic effect. The principal decrease in tumour incidence was accounted for by changes in tumour incidence of the uterus, mammary glands, and pituitary. For males, a clear hormetic effect was also seen for all tumours, with tumours of the adrenals and pancreas showing the biggest decrease. The liver is regarded as the most sensitive organ to the toxic

Table 1. Tumour frequency in female rats exposed to 2,3,7,8-tetrachlorodibenzo dioxin (Cook, 1994)

Dose ($\mu\text{g/kg per d}$)...	0		0.001		0.01		0.1	
	No. of tumours	Rate*	No. of tumours	Rate*	No. of tumours	Rate*	No. of tumours	Rate*
Rats (<i>n</i>)	86		50		50		49	
Total tumours	230	267.4	96	192.0	102	204.0	120	244.9
Liver	9	10.5	3	6.0	20	40.0	34	69.4
Pulmonary	0	0.0	0	0.0	1	2.0	7	14.3
Ovary	3	3.5	1	2.0	1	2.0	0	0.0
Uterus	36	41.9	14	28.0	14	28.0	11	22.4
Cervix and vagina	2	2.3	0	0.0	1	2.0	0	0.0
Mammary	81	94.2	39	78.0	40	80.0	24	49.0
Pituitary	49	57.0	18	36.0	14	28.0	14	28.6
Pancreas	5	5.8	4	8.0	1	2.0	1	2.0
Adrenal	16	18.6	8	16.0	3	6.0	8	16.3

* Number of tumours per 100 animals.

effects of 2,3,7,8-tetrachlorodibenzo dioxin. Although there are few liver tumours produced at these low doses, a trend in their reduction was seen. However, it has been argued that the hormetic effects are most strongly seen when total tumours are combined, and that the effect is not statistically significant in the case of the 'critical' organ, the liver (Rodricks, 2003).

It has been suggested that the beneficial effects of low doses of toxic chemicals derive from the stimulation of a beneficial stress response that enhances normal function protecting the organism against subsequent stress. The mechanism is perceived as the disruption of cellular homeostasis, followed by a modest overcompensation on the part of the cell, and the re-establishment of homeostasis, but in an adapted state (Calabrese & Baldwin, 2001).

Some of the arguments that have been advanced to counter the hormesis concept are that the effects seen, for example, in studies on low-dose reduction of cancer incidence in animals, have failed to take into account whether or not there are benefits to other toxic end-points, other than cancer end-points, or whether the effect is reproducible across animal species.

It is likely that any protocol testing for hormetic effects could be criticised because of the experimental difficulties of studies where an effect may be modest compared with controls. Hormesis cannot be observed when the control group in a standard long-term carcinogenicity study has a low background incidence of tumours (<0.5%). It is statistically impossible to demonstrate a decrease in tumour formation when the response is low to begin with. Solid mechanistic data supporting the idea that mild stress occurs, and is of benefit to a clinically relevant end-point, is critical to the confirmation of a hormetic effect.

Low-dose exposure to radiation

Exposure to radiation is known to induce the formation of free radicals. Although as an experimental system it might reveal information of value in determining the potential of oxidative stress to up regulate protective responses, there are limitations in its relevance, given the relative paucity of nutritional-related data. In the case of UV radiation it has

been shown that the wavelength influences the gene expression, with UVB radiation triggering the broadest response (Koch-Paiz *et al.* 2004). Much more energetic radiation, even at low doses, could trigger a stress response that differs from the more controlled process that occurs from the uptake of low levels of a chemical into the cell.

Background average radiation is 3 mSv annually but individuals are exposed to up to a ten times higher dose in certain regions of the world where there is no evidence for increased cancer rates, for example, Western China and Colorado, USA. Since radiation has always been a normal part of the evolutionary environment, it follows that there are effective compensatory mechanisms to deal with it. As a fundamental tenet of evolutionary biology organisms should be less fit to deal with zero levels of radiation than at normal background exposures. Thus on evolutionary grounds, radiation hormesis might be expected. The existence of radiation hormesis has been reviewed. Whilst some authors propose that the data support a threshold dose below which there are no adverse effects of radiation (Luckey, 1999), others argue that the evidence is inconclusive (Brenner *et al.* 2003). Like so much research on the topic of hormesis, the evidence that low-dose radiation exposures may be of benefit is far from being universally accepted.

Epidemiological evidence with the highest statistical power for evaluating low-dose risks to radiation is that obtained from the cancer incidence of the cohort of the atomic bomb survivors (Preston *et al.* 2003). This exposure was acute, but analysis of the data suggests that there is a linear, not a hormetic, dose-response over the range of 5 to <500 mSv. Additional review of low-dose radiation exposure by a US expert group has concluded that for X- or γ -ray exposure there is good evidence for an increased cancer risk at acute doses >50 mSv, and reasonable evidence for an increase in cancer risks above about 5 mSv (Brenner *et al.* 2003). There was also reasonable evidence for an increase in cancer risks for protracted exposures above about 50 mSv. But, because of the limitations of the epidemiological method, it is unlikely that it will be possible to estimate risks below about 5 mSv, which is the dose when hormetic effects might have been seen.

Some, but not all, experiments in animals have suggested that low and intermediate doses of radiation can produce a hormetic response by enhancing longevity (reviewed by Upton, 2001). This analysis concluded that in those studies where there was an increase in lifespan, the reduction in cancer incidence was less significant than the reduction in mortality from infections and other non-malignant diseases (Maisin *et al.* 1996; Upton, 2001). If real, then this suggests that the probable mechanism is unlikely to be associated with a radiation-related stimulation of DNA repair and more likely to be due to a radiation-induced response from the immune system (Xu *et al.* 1996).

More recently, radiation-sensitive and cancer-prone female mice (Trp53 heterozygous) have been subjected to a single dose of 10 mGy 24 h before a 4 Gy dose of ^{60}Co γ radiation (Mitchel *et al.* 2004). A small protective response was detected in lifetime studies, reducing the carcinogenic effects of a subsequent large, high-dose-rate exposure by increasing tumour latency, but not frequency, for all tumours taken together. The upper dose threshold at which low-dose protective effects gave way to detrimental effects was tumour type-specific. Given the sensitivity of these animals to radiation effects, the finding suggests that radiation hormesis is a real phenomenon that is able to increase the time to tumour formation albeit with modest protective effects.

Hormesis in the nutritional context

The problem in the nutritional field is that there is a paucity of relevant data. The optimal intake of a nutrient may place the target cells in a better response mode to external stress than if the nutrient supply is sub-optimal, but there are few data available, either to define the optimal intake in terms of well-characterised biomarkers, or that have measured stress responses at various doses above or below optimal intakes.

It is possible that hormesis is important in relation to nutrition, especially in terms of maximising the health benefits arising from components of the diet. The wide range of phytochemicals that are present in plant foods, and for which there is no clear biological function, act through mechanisms that operate in response to other xenobiotic substances normally classified as toxic. Many of the naturally occurring food phytochemicals are metabolised and excreted using the same system of phase I and phase II enzymes that metabolise toxic compounds. Thus they are likely to have many features in common with low-level exposure to other environmental xenobiotics, the only difference being that they are generally not very toxic compounds, and that the exposure is at a chronic low level, rather than acute and transitory, as is the case for many other environmental chemicals.

Plant phytochemicals

A remarkable aspect of the epidemiological studies that have defined the nutritional benefits of consuming a diet rich in fruit, vegetables, and whole grains, is that they are consistent in showing a reduction of risk of cancer and CVD across populations (World Cancer Research Fund, 1997; Van't Veer & Kok, 2000). Considering the wide variety of

plant foods that are consumed, it would suggest that the observed health benefits are unlikely to be attributable to individual phytochemicals, but are attributable to:

- a class of phytochemicals that is widespread throughout the entire plant kingdom; or
- metabolite(s) produced that are the breakdown product(s) of many phytochemicals; or
- effects resulting from the up regulation of pathways that are influenced by many classes of phytochemicals.

As far as the first explanation is concerned, a class of phytochemicals that is quite widely present in most plant-rich diets is the flavonoids. Those found most commonly in plant foods vary widely in their bioavailability, but nearly all have a low bioavailability (Arts *et al.* 2004). In addition, much of the data obtained on their bioactivity have been from *in vitro* studies or *in vivo* studies where the dose levels used are not of nutritional relevance. Neither has their metabolic behaviour always been taken into account in studying their effects at the cellular level (Kroon *et al.* 2004). It remains to be proven if this class of compounds is the most likely to result in health benefits.

It has been proposed that plant phytochemicals, that are of benefit to health, should be systematically studied by selecting those that are bioactive by virtue of a reactive chemical group in their structure, and to determine whether or not they are bioavailable. If so, then the compound should be further investigated *in vitro* to see if it shows a hormetic-like dose-response effect in relation to carcinogenesis at a dose range corresponding with the plasma levels found (Brandt *et al.* 2004). If positive effects are found, then further biological studies should be undertaken. Obviously there are other health end-points that need to be considered as well in similar studies, but this approach is yet to be systematically adopted by research groups.

At present, the evidence suggests that there is a diverse range of chemicals in plant foods with certain characteristics in common that lead to a beneficial response. Dietary phytochemicals that have been shown to be protective against age-related disease are all chemically reactive, and nearly all are electrophilic. Most are substrates for glutathione transferase and are capable of modifying thiol groups in proteins by alkylation, oxidation, or reduction. The probability is that they influence the redox potential of their target cells and set in motion a series of gene transcriptions that result in the activation of the phase I and phase II metabolism genes.

Energy restriction

Energy restriction (ER) is the only stress condition that results in a uniform prolongation of ageing and the time of onset of age-related disease across all of the organisms tested to date. For more than 60 years now it has been known that the lifespan of laboratory animals could be increased if they were restricted in their food intake and were not allowed to feed *ad libitum* (Tannenbaum, 1942). The principal observation was that there was a reduced incidence of spontaneous tumours. Subsequent work has confirmed that this observation is not explainable on the basis of a reduced intake of exogenous carcinogens (Roe *et al.* 1995).

It suggests that spontaneous tumours arise from endogenous processes, such as oxidative damage, that increase with age, and whose incidence can be influenced by energy intake. Animals put onto ER later in life can also show the benefits seen in younger animals (Dhabi *et al.* 2004). There is only one report that there is an increase in lifespan after short periods of ER early in life (Yu *et al.* 1985) and more research is required to know whether the effects are observed only if ER is continuous.

For obvious reasons the only species where the effects of ER are unknown are in the long-lived primates and man. However, studies in rhesus monkeys have been underway for some time now. The indications are that they are showing a decrease in the rate of ageing (Mattison *et al.* 2003).

ER is defined as a 30–60% reduction in energy intake over the normal *ad libitum* intake, whilst maintaining the nutritional balance. In practice, this means that the concentrations of essential nutrients are increased in the experimental diets, thus increasing the dose/kg body weight. But whilst the animal is under mild stress through hunger, the response is to cause changes in gene expression, and this results in a maximal response on the part of the animal to environmental or intrinsic ageing challenges. Thus the model may well offer a good experimental system to provide for a better understanding between stress, nutrition, health and ageing.

Miscellaneous dietary constituents

Caffeic acid

Caffeic acid is present in fruit, vegetables and coffee. Classical toxicological studies show that high doses of caffeic acid (2% in the diet) produce cancers of the forestomach and kidney in F344 rats and B6C3F1 mice (Lutz *et al.* 1997). Tumour formation was preceded by hyperplasia in both organs, which could be the reason for the carcinogenicity of the compound. The dose–response relationship was studied at four dose levels (0.05, 0.14, 0.40 and 1.64%). As shown in Fig. 2, increased cell proliferation was detected in the forestomach in the two high-dose groups. However, at the 0.14% dose level, cell proliferation was decreased by about 30%. The effects in the proximal tubular cells of the kidney showed a similar J-shaped dose–response.

Falcarinol

Falcarinol, a polyacetylene present in carrots, had been thought of as a potential hazard as it is capable of producing allergic dermatitis due to skin reactions. However, like a number of other components of vegetables that were originally thought to be toxins (glucosinolates, isothiocyanates), falcarinol can show beneficial effects at low doses. A possible hormetic effect has been seen in testing the compound in relation to the proliferation of mammary epithelial cells (Hansen *et al.* 2003). Proliferation is stimulated at concentrations that are equivalent to those that can be found in plasma after ingestion of carrot juice,

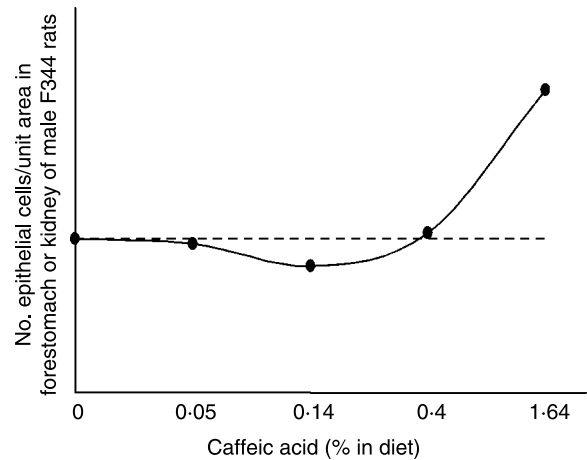


Fig. 2. Dose–response relationship showing the relationship of epithelial tumours in the forestomach and kidney of the rat fed caffeic acid.

whereas at higher doses there is an inhibition of proliferation, possibly due to toxicity.

Ethanol consumption

A good example of where hormetic effects can be demonstrated is the relationship between ethanol consumption and CHD.

In the middle-aged and elderly a meta-analysis of fifty epidemiological studies of all causes of mortality showed that there was an inverse association between moderate drinking and mortality (Gmel *et al.* 2003). The data from individual studies show a dose–response curve that is J-shaped with the lowest mortality corresponding with the consumption of one or two alcoholic drinks per d. The protective effects of moderate alcohol consumption are seen both in CHD and ischaemic stroke (Marmot, 2001; Mukamel, 2003; Reynolds *et al.* 2003). The beneficial effects of low-dose intakes of alcohol have been recognised in the recent US Health and Human Services–US Department of Agriculture Dietary Guidelines Report (United States Department of Agriculture, 2005).

Mechanisms of hormesis

The adaptation of an organism to an external stress is an essential component of living systems. The cellular homeostatic response to ‘environmental insult’ results in the facilitated removal of the potentially ‘harmful’ compound. Thermoregulation, detoxification, cell proliferation and apoptosis, DNA repair, heat-shock protein synthesis, and up regulation of antioxidant responses are all mechanisms that cells utilise to adapt to their temporal changes in their environment. Whilst some of these processes result in cell death, followed by regeneration, others detect a change in cellular homeostasis and bring the cell back to the ‘normal’ state when the exposure to the agent is removed. It may be that the ‘normal’ state may differ from the previous one in that the cell may have become more able to resist similar challenges in the future.

Enzymes may have been induced and, in the process, the cell becomes adapted. This may be especially relevant in the context of nutrients, or other food chemicals, where constant, low level, exposure to the chemicals keeps the cells in a state of alert. With the advent of genomics it is now feasible to study the effects of low-dose exposure on responses at the molecular level.

Stress responses

The cellular stress response (CSR) is a universal mechanism of providing a defence reaction of cells to damage by environmental factors. All organisms have a minimal set of stress proteins that are universally conserved. Stress initiates a very complex set of responses with many interacting factors determining the outcome. Many aspects of the CSR are not stressor specific because cells sense stress based on macromolecular damage without regard to the type of stress that causes such damage. Other cellular responses directed at re-establishing homeostasis are stressor specific and are often activated in parallel to the CSR. Key aspects of the CSR include: the sensing of membrane lipid, protein, and DNA damage; redox sensing and regulation; cell-cycle control; macromolecular stabilisation and repair; control of energy metabolism (Kültz, 2005). In addition, cells can quantify stress and cause cell death (apoptosis) when tolerance limits are exceeded.

In 1992 it was proposed that a common pathway through which the hormetic response arises is through the stress response (Smith-Sonneborn, 1992). The argument was advanced that certain stressors result in a stress response that acts as a buffer against what would otherwise be a harmful agent. Low levels of stress result in: expression of protein repair proteins, for example, the heat-shock proteins; the elimination of damaged proteins that cannot otherwise be repaired; the induction of DNA repair and replication molecules; alteration of chromatin structure to facilitate repair, or to alter gene expression to accelerate function; the induction of tolerance towards the same, or to other unrelated environmental toxins.

The rapid progress that has been made since in understanding the stress response provides some support for the argument. Increased tolerance towards a stress factor after the organism has been exposed to low doses of that stress (stress hardening) and increased tolerance to a stress after pre-conditioning by another stress (cross-tolerance) are known (Kültz, 2005). Both types of stress are hormetic responses. Whilst many examples of this phenomenon have been observed in simple organisms (Koga *et al.* 1999; Alexieva *et al.* 2001; Mary *et al.* 2003), ischaemic pre-conditioning and mild hyperthermia have been shown to decrease reperfusion injury of human muscle and kidney through the induction of Hsp70 (Lepore *et al.* 2001). Hsp70 induction is also associated with stress hardening and cross-tolerance in *Drosophila melanogaster* towards heat and cold stress (Sejerkilde *et al.* 2003). Temperature salinity, ionising radiation, pH, and chemical stressors are reported to reduce a variety of stress proteins with hormetic responses (Koga *et al.* 1999; Alexieva *et al.* 2001; Mary *et al.* 2003; Alsbury *et al.* 2004).

Exercise is known to result in mild stress and results in the production of potentially harmful products, such as free radicals and aldehydes (McArdle *et al.* 2002; Singh, 2002). Apart from the benefit on muscle function, it has other benefits in improving function on the immune system, the cardiovascular system, libido and mood (Venkatraman & Fernandes, 1997; Singh, 2002). However, little is known about the biological processes involved.

In the nutritional context cells are likely to be constantly challenged at a low level, rather than the situation that arises when a pharmaceutical preparation is consumed. Food phytochemicals associated with potential health benefits can induce the phase II metabolising enzymes. Many phase II enzyme genes are regulated by upstream antioxidant–electrophile response element, present in their genomes, that are targets of the activation of the leucine zipper Nrf2 transcription factor. The response element is found in many of the genes coding for cellular defensive enzymes, such as the thioredoxins, γ -glutamylcysteine synthetase and haem oxygenase, with subsequent gene induction following phytochemical exposure.

Under basal conditions, Nrf2 resides mainly in the cytoplasm bound to its cysteine-rich, Kelch domain-containing partner Keap1, which is itself anchored to the actin cytoskeleton and represses Nrf2 activity. Inducers disrupt the Keap1–Nrf2 complex by modifying two of the many cysteine residues of Keap1, resulting in the translocation of Nrf2 to the nucleus where transcriptional activity is induced.

The mechanism that has been proposed is shown in Fig. 3, although the detailed mechanisms whereby Nrf2 levels are

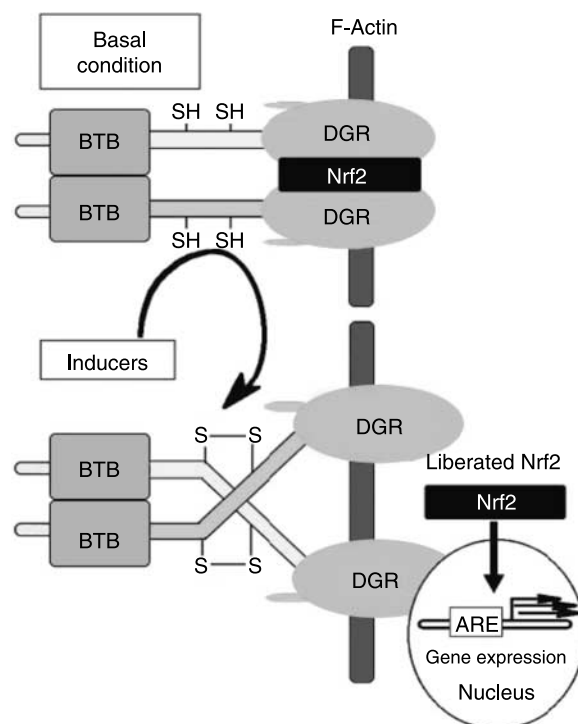


Fig. 3. Proposed mechanism for the activation of the antioxidant response element (ARE) by phytochemical inducers. BTB, DGR, specific domains of the Keap1 protein. (From Wakabayashi *et al.* 2004, with permission. Copyright 2004 National Academy of Sciences, USA.)

increased in the nucleus are still unclear (Nguyen *et al.* 2004).

There is some evidence that compounds such as dithiolethione, present in allium vegetables, are able to cause an inhibition of the proteasome degradation complex (Kwak *et al.* 2003). Phosphorylation is also thought to play a role in regulating the stability of the Nrf2 protein. The identity of the protein kinase(s) involved is not known (Nguyen *et al.* 2004). It remains to be seen whether the phosphorylation step occurs through a number of protein kinases in response to inducers of the antioxidant response element, or whether Nrf2 phosphorylation is mediated only by a specific kinase in a specific cell type. Compounds such as green tea polyphenol, (-)-epicatechin-3-gallate and the isothiocyanates can activate mitogen-activated protein kinase pathway via the electrophile-mediated stress response (Loo, 2003).

The Keap1–Nrf2 complex has been proposed as the cellular redox sensor through which the activation of the antioxidant response element-dependent genes are regulated in response to oxidative stress (Itoh *et al.* 1999). Under physiological conditions Keap1 plays an important role in the degradation of Nrf2 (Nguyen *et al.* 2004). Because of the rich content of thiol groups in the Keap1 protein it is probable that many of the phytochemical protectants are able to cause direct, or indirect, modifications through subtle alterations in the redox potential of cells. It may be that hormetic-type effects are created if this subtle alteration in the redox state is maintained, which will result in a permanent up regulation of phase II enzymes unlike the situation in the unchallenged cell.

Ageing results in a decrease in adaptation due to a progressive failure in homeostatic regulation and maintenance. It has been proposed that any approach that could result in producing conditions of mild stress, so that stress response-induced gene expression occurs, as well as effects on related pathways of maintenance and repair, might prolong the ageing process (Rattan, 2004). In *Drosophila melanogaster* it has recently been shown that ageing and oxidative stress responses showed similar gene expression patterns. The genes responsible for purine biosynthesis, heat-shock protein, antioxidant defences and immune response genes were commonly expressed (Landis *et al.* 2004). The expression of numerous genes involved with stress responses, hormone, cytokine, and growth-factor signalling, and control of the cell cycle and apoptosis appeared to be affected by ageing (Welle *et al.* 2003).

Ageing also results in the up regulation of genes that encode for the inflammatory response. The inflammatory responses are tissue dependent, whereas the stress response has been observed in all tissues examined to date (Lee *et al.* 1999, 2000, 2002; Cao *et al.* 2001). ER opposes the age-related induction of stress response genes in mouse muscle and the stress response and inflammatory genes in the liver, neocortex and cerebellum (Lee *et al.* 2000; Cao *et al.* 2001).

Adaptive responses to stress

It has been clearly shown that exposure of cells to ionising radiation induces an adaptive response that results in an enhanced tolerance to the subsequent cytotoxicity of

radiation. Thus at least in this context hormesis is a real phenomenon. Many stress response genes are induced in response to radiation (Fornace *et al.* 1999), but it is believed that only a fraction of the radiation-inducible genes play a role in the stress-tolerance phenotype. These appear to be genes involved in the arrest of cell-cycle progression, apoptosis and DNA repair.

Selected radio-resistant variants (MCF-FIR), isolated from human breast adenocarcinoma cells (MCF-7) and subjected to successive doses of radiation, as well as MCF-7 cells exposed to a single dose of radiation, strongly induced Mn superoxide dismutase (MnSOD) expression (Guo *et al.* 2003). Radio resistance in both the MCF-FIR cells, and in MCF-7 cells that overexpressed MnSOD (MCF + SOD), was reduced following the expression of antisense MnSOD. Genes coding for p21, Myc, 14-3-3 zeta, cyclin A, cyclin B1 and GADD 153 were over-expressed in the MCF-FIR and MCF-SOD lines, which were suppressed by antisense MnSOD treatment. If NF- κ B was inhibited in MTR-FIR cells, radio resistance was inhibited as well as reducing steady-state levels of MnSOD, and the signalling proteins. In contrast, mutant I κ B α was unable to inhibit radio resistance or reduce the production of the signalling proteins in MCF + SOD cells where MnSOD overexpression was independent of NF- κ B. These results support the view that NF- κ B can regulate the expression of MnSOD, which in turn is capable of expressing the genes that participate in radiation-induced adaptive responses (see Fig. 4).

A number of reports have documented that eukaryotic cells acquire tolerance to lethal doses of H₂O₂ by prior exposure to a sub-lethal dose of H₂O₂ (Spitz *et al.* 1987; Davies, 1995; Wiese *et al.* 1995; Lee & Um, 1999). Moreover, conditioning cells of various origins with H₂O₂ induced adaptive tolerance to other oxidative insults, for

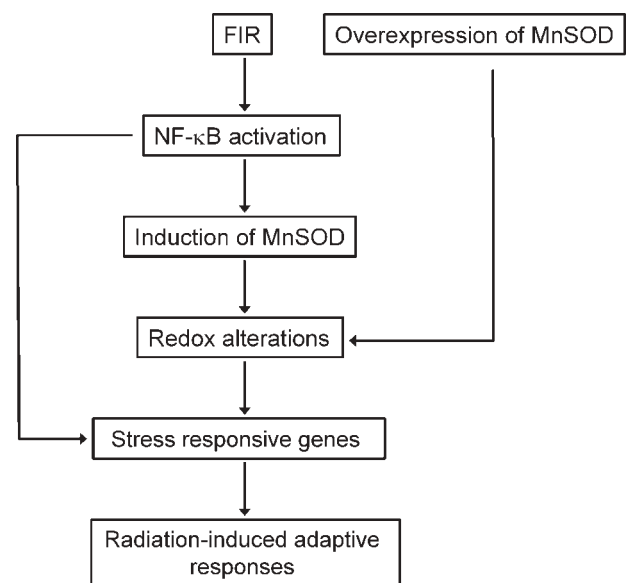


Fig. 4. Model of stress-responsive gene regulation in MCF-FIR cells. The radiation treatment up regulates Mn superoxide dismutase (MnSOD) production which in turn up regulates gene resistance associated with the acquisition of resistance. (From Guo *et al.* 2003.)

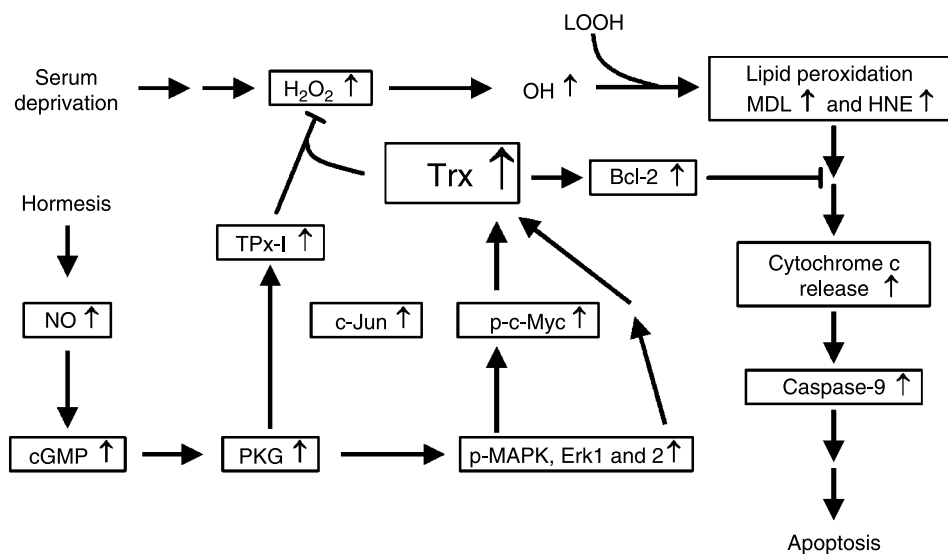


Fig. 5. Proposed pathways for serum-deprived induced apoptosis and NO-mediated hormesis in SH-SY5Y cells. ↑, Increase; LOOH, organic peroxides; MDL, malondialdehyde; HNE, 4-hydroxy-2-nonenal; Trx, thioredoxin; TPx-1, thioredoxin peroxidase-1; PKG, cGMP-dependent protein kinase; MAPK, mitogen-activated protein kinase. (From Andoh *et al.* 2003, with permission)

example, radiation injury (Flores *et al.* 1996; Laval, 1998). The acquisition of tolerance to lethal doses of H_2O_2 was accompanied by the induction of glutathione peroxidase (Lee & Um, 1999), but this was not the major contributing factor to the overall resistance. It has been reported that the content of glutathione can also be regulated by oxidative stimuli, via increased synthesis by γ -glutamylcysteine synthetase activation (Tian *et al.* 1997; Yamane *et al.* 1998). This has been confirmed in recent work which demonstrated that both the glutathione system and catalase could be enhanced by H_2O_2 stimulation. However, it was the increase in glutathione content through an increase in the activity of γ -glutamylcysteine synthetase, rather than catalase activity, that ensured cytoprotection against lethal H_2O_2 stress (Seo *et al.* 2004).

Experiments using human neuroblastoma cells, which contain relatively low levels of thioredoxin and therefore exhibit high sensitivity to oxidative stress, were subjected to non-lethal serum deprivation which puts the cells under oxidative stress. Free radicals were produced, as well as the products of lipid oxidation. Neuronal NO synthase and thioredoxin were up regulated. The cells became more tolerant to subsequent oxidative stress showing a clear hormetic effect. Evidence suggests that NO, elevated by the preconditioning stress-induced synthesis, protects cells from serum-deprivation-induced apoptosis through a cGMP-dependent protein kinase (PKG) pathway by activating guanylate cyclase (Andoh *et al.* 2003). Activated PKG protects cells from lipid peroxidation and apoptosis. It was also shown that the protective effect required the biosynthesis of thioredoxin. Apart from an increased synthesis of Bcl-2, it results in the increased biosynthesis of Mn(II)-SOD, a reduction in cytochrome c, and the inhibition of the activation of the procaspases 3 and 9. Inhibition of any one of the enzymes neuronal NO synthase, guanylate cyclase and PKG results in an inhibition of the hormetic effect and indicates that the PKG-mediated

pathway is involved in the neuroprotective effect. The probable mechanism is shown in Fig. 5.

Conclusions

Hormesis, the probability that low-level exposure to agents that are toxic at higher exposures, possibly resulting in benefits to health, rather than risks, has its proponents and opponents. In part this stems from the difficulty in demonstrating any effect by epidemiology. Nonetheless, there is a wide range of toxic substances and physical agents that have shown hormetic-like effects in experimental systems.

There is good evidence that exposure to dietary phytochemicals, as well as energy restriction, produces a mild stress response, which might result in adaptation to that response if the exposure is prolonged. It remains to be proven if such situations cause a permanent alteration in cellular homeostasis, or redox state, such that the cells are better able to respond to subsequent stress challenges.

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