Mechanisms of lifespan extension and preventive effects of calorie restriction on tumor development: Possible link between central neuroendocrine system and peripheral metabolic adaptation

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Abstract Restriction of food intake (calorie restriction [CR]) in laboratory animals extends their lifespan and delays the onset of various age-associated diseases, including cancer development. Recent studies revealed that the molecular mechanisms underlying CR-mediated anti-aging effects may be regulated by a confined number of signal transduction pathways that are triggered by neuropeptide Y (NPY) neurons in the neuroendocrine system. On the other hand, possible peripheral regulators of the beneficial effects of CR involve a transcriptional regulator complex, the hepatocyte nuclear factor 4α/peroxisome proliferator-activated receptor gamma coactivator 1-α (HNF-4α/PGC-1α) complex. This complex could regulate not only glucose and lipid metabolism, but also DNA damage responses in the liver. Therefore, maintenance of optimal glucose and lipid levels to prevent metabolic syndrome, and activation of the DNA damage response for suppression of tumor development, may depend on the HNF-4α/PGC-1α complex. Hence, small molecules modulating the activity of this complex could be an important target for development of CR mimetics (CRM), which mimic the beneficial effects of CR without actual food restriction.

Keywords: aging, calorie restriction, glucose metabolism, neuroendocrine, oxidative stress

Introduction

The beneficial effects of calorie restriction (CR) have been recognized for many decades. However, the precise molecular mechanisms underlying the beneficial effects of CR have not yet been fully identified. Recent advances in molecular biology and genetic engineering of laboratory animals has made it possible to investigate the signaling pathways important for the regulation of lifespan and aging processes upon CR. Although it remains controversial, the beneficial effects of CR may apply to primates, including humans, as well as rodents1,9). However, imposing CR on humans may be difficult to achieve, because they would have to experience unbearable hunger. Hence, many researchers are now investigating CR mimetics (CRM), which are compounds that mimic the beneficial effects of CR without actual food restriction4-7). Development of CR mimetics (CRM) could be a magic bullet not only for age-associated disorders, but also for various rare disorders, such as mitochondrial diseases. Here we review recent findings of the molecular signaling mechanisms of CR-mediated anti-aging effects and the potential therapeutic target molecules of these signaling pathways.

Evolutionary view of beneficial effects of calorie restriction

Moderate restriction of calorie intake without malnutrition reduces morbidity and increases the lifespan of laboratory rodents, with lower plasma glucose and insulin levels8). It has been implied that the effects of CR on endocrine and/or neural regulatory systems are responsible, at least in part, for its anti-aging effects5). The beneficial effects of CR, such as lifespan extension and suppression of the onset of various age-associated diseases, could be explained from an evolutionary point of view (Fig. 1). In ancient life, food shortages, due to the difficulties of hunting or famine, could occur frequently due to environmental reasons. Therefore, organisms needed to adapt to these situations (naturally occurring CR) to avoid extinction of the species. Such adaptation mechanisms may induce the
diversion of limited energy resources. In particular, the maintenance of somatic cells requires activation; whereas activities that consume energy resources, such as growth, reproduction, and thermogenic activities, require suppression, to increase the chance of survival of individuals and the species. These mechanisms may be preserved and intrinsically maintained even in present-day organisms. Indeed, the increased incidence of obesity and diabetes in current industrialized countries may be explained by these phenotypes, although this proposal is still controversial. Therefore, when organisms encounter CR, either naturally or experimentally, they activate this survival mechanism, leading to increased lifespan and activation of anti-aging processes.

**Regulatory center for metabolic shift on CR**

Hypothalamic neurons, especially neuropeptide Y (NPY) neurons, play important roles in appetite regulation and have also been implicated in the various beneficial effects of CR (Fig. 2). In the neuroendocrine adaptation processes that follow upon CR, neurons of the hypothalamic arcuate nucleus could be crucial. These neurons regulate whole body energy balance and express various kinds of hormone receptors for peripheral signaling, such as leptin, insulin, and ghrelin.

Leptin is secreted from adipocytes in fat tissue. Recently, adipose tissue has been recognized as an endocrine organ, and the hormones derived from adipocytes are called adipocytokines. Insulin is also an important neuroendocrine regulator. Both leptin and insulin negatively regulate NPY expression in the hypothalamus, thus decreased blood levels of leptin and insulin increase NPY expression. On the other hand, ghrelin, which is secreted from the gastro-intestinal tract, is a positive regulator of NPY. These hormonal changes favor up-regulation of NPY under CR conditions; namely, CR decreases leptin and insulin levels, but increase ghrelin levels. Signal transduction pathways of these ligands and receptors may play important roles in CR-mediated anti-aging effects, because these mechanisms explain the effects of CR from an evolutionary point of view. For instance, suppression of growth and reproduction, and activation of stress response, could be induced by the activities of NPY neurons. This neuroendocrine adaptation hypothesis of the anti-aging effects of CR was supported, in part, by the findings of NPY neuron activation upon CR in animal models. Considering that NPY activity is crucial for the beneficial effects of CR, NPY-knockout mice should not demonstrate an extended lifespan from CR. Currently, we are elucidating whether a lack of NPY diminishes the beneficial effects of CR.

![Fig. 1](image1.png)

**Fig. 1** The evolutionary point of view: Triggering of the beneficial effects of calorie restriction (CR).

Only those organisms that have adapted to food shortages or periods of famine (naturally occurring CR) may have evolved to still be in existence today. These adaptations activate a shift in the utilization of limited energy resources, viz., suppression of those activities that consume energy, such as growth and reproduction, and activation of mechanisms that maintain somatic cells to increase the chance of survival. Neuroendocrine and peripheral signaling could regulate these energy shifts. This would lead to an increase in lifespan and activate anti-aging effects.

![Fig. 2](image2.png)

**Fig. 2** Neuropeptide Y (NPY) activation via peripheral hormones could induce various calorie restriction (CR) effects.

The activation of NPY/AgRP neurons enhances appetite, whereas the activation of POMC/CART neurons down-regulates appetite. These 2 types of neurons mutually regulate their activities. These signals are further transmitted to down-stream neurons, which regulate processes such as growth, reproduction, stress resistance, and metabolism. Hence, the characteristics of CR animals, such as growth suppression and increased stress-resistance, could be explained by neuroendocrine changes triggered by NPY activation through peripheral hormonal signals.

Molecular regulators of metabolic adaptation in peripheral tissues due to hypothalamic signals

Hormones derived from peripheral tissues could regulate neuroendocrine adaptation upon CR. These signals are further transmitted from the brain to peripheral tissues, possibly through the vagus nerve, to regulate glucose metabolism in the liver. One of the key molecules for CR-mediated metabolic effects in the cell is peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), a transcriptional coactivator. In Fig. 3, we depict the transcriptional regulation of PGC-1α expression and regulation of expression of its target genes under CR conditions. CR could suppress insulin/insulin-like growth factor-I (IGF-I) pathways, thereby activating forkhead transcription factor O1 (FoxO1). FoxO1 is involved in the transcriptional activation of PGC-1α; also, PGC-1α modulates FoxO1 transcriptional activity through the interaction of these proteins.

On the other hand, the activity of PGC-1α is probably directly modulated through pathways that depend on sirtuin 1 (SIRT1), an NAD+-dependent deacetylase. PGC-1α could interact with and activate various kinds of transcription factors, and regulate expression of various metabolism-related genes. This includes regulation of phosphoenolpyruvate carboxykinase (PEPCK), mitochondrial transcription factor A (mTFA) and carnitine palmitoyltransferase I (CPT-I), and nuclear factor erythroid-derived 2-related factor 2 (Nrf2). These proteins are important for the regulation of glucose metabolism, lipid metabolism, and oxidative stress resistance.

Therefore, PGC-1α activation may be critical for the induction of the beneficial effects of CR. Although there is limited information about the direct link between NPY and glucose and lipid metabolism in the liver, leptin plays key roles in the regulation of central nervous system neuro circuits, which affects vagus nerve functions and regulates liver metabolism (see review (30)).

Possible roles of HNF-4α in metabolic adaptation of CR

We previously reported that the putative HNF-4α response element (HNF-4RE) could play a role in the beneficial effects of CR. HNF-4α is an important transcription factor in glucose and lipid metabolism in the liver and forms a complex with PGC-1α. Levels of the HNF-4α/PGC-1α complex are increased in the liver of CR animals, and putative HNF-4RE–containing genes are up-regulated in pro-longevity contexts.

HNF-4α has already been shown to be involved in not only glucose and lipid metabolism, but also in the oxidative stress response, similar to PGC-1α. Interestingly, Yamamoto et al. showed that HNF-4α/PGC-1α complex levels are increased in the pre-prandial phase; whereas their interaction and expression of their target genes are reduced in the post-prandial phase. In CR animals, these pre- and post-prandial phases are dynamically and reciprocally changing, and pre-prandial phases are relatively prolonged compared to ad libitum (AL)-fed animals. Therefore, expression of HNF-4RE–containing pro-longevity genes could be up-regulated in the pre-prandial phase of CR animals to activate CR-mediated anti-aging effects (Fig. 4). This long lasting pre-prandial phase in CR animals would sustain target gene expression and contribute to the cumulative dosage of pro-longevity proteins.

Potential role of HNF-4α in the suppression of tumor development

One of the major features of CR effects is its anti-tumor effect, caused by the reduction of various stress-induced lesions, including oxidative stress. This effect may be regulated by FoxO1 and Nrf2 transcription factors. Interestingly, HNF-4α can interact with FoxO1 and modulate its transcriptional activity. Moreover, levels of Nrf2 may be regulated by HNF-4α. However, the molecular mediators that regulate HNF-4α activity in the presence of DNA damage are not fully understood.

Ataxia telangiectasia mutated (ATM) is important for genome maintenance, to prevent tumorigenesis, via interaction with various molecules important for response to DNA damage. Among these signaling pathways,
the ATM–p53 pathway plays a crucial role in oxidative stress-resistance induced by the genotoxic drug doxorubicin40). Moreover, we have reported that ATM regulates Dbf4-dependent protein kinase (DDK), and the Mre11/Rad50/Nbs1 (MRN) complex, both of which are important for the maintenance of genome integrity, for protection against tumor development41-43). Furthermore, ATM may directly interact with and phosphorylate FoxO1 in the case of DNA damage44).

On the other hand, as described above, we previously demonstrated that HNF-4α/PGC-1α is an important regulator for regulation of gene expression in the liver of CR animals. Recently, it has been shown that HNF-4α may be involved not only in glucose and lipid metabolism, but also in the DNA damage response, because HNF-4α can interact with FoxO1, p53, and Rad50 (a component of the MRN complex), which are the targets of ATM kinase, as described above. These results emphasize the importance of HNF-4α in tumor suppression.

However, the interaction between HNF-4α and FoxO1, p53, or Rad50 may decrease levels of the HNF-4α/PGC-1α complex. This would suppress transcriptional activity of HNF-4α and down-regulate the expression of the metabolism-related genes. This is probably because, in the absence of DNA damage, HNF-4α is activated by PGC-1α; whereas, under DNA damage conditions, HNF-4α would switch its interaction partner for FoxO1, p53, or Rad50 to activate the DNA damage response. Indeed, HNF-4α interaction with FoxO1 has been shown to decrease transcriptional activity for certain target genes45).

ATM is phosphorylated by insulin/IGF-I signaling pathways and oxidative stress even in the absence of double-stranded DNA breaks46). This may suggest that enhanced insulin sensitivity and reactive oxygen species (ROS) sensitivity (responsiveness to hazardous ROS) may be important for tumor prevention. CR may maintain ATM kinase responsiveness, facilitating rapid reaction to DNA damage. Together these results, although limited, imply that HNF-4α plays an important role in DNA damage response pathways under CR conditions (Fig. 5).

Causes of age-related diseases: macromolecular damage induced by higher plasma glucose levels and oxidative stress

Kristal et al. have proposed a convincing theory of aging49), which holds that glycation and ROS accelerate aging processes via macromolecular damage (Fig. 6). In humans, lower glucose and insulin levels (which represent the characteristic phenotype of CR in animals) are potential longevity phenotypes49). Lower glucose levels could prevent potentially harmful modifications, such as glycation and the Maillard reaction49). Recently, higher blood glucose levels have also been considered to increase the risk of development of Alzheimer’s disease in patients with type 2 diabetes50). Hence, lower glucose levels are one of the most important anti-aging features of CR animals.

Free radicals could leak from mitochondrial metabo-
Detoxification mechanisms involved in stress resistance, and xenobiotic and other agents. Therefore, lower glucose levels are likely to be achieved under lower calorie intake conditions. To avoid increases in blood glucose levels in the post-prandial phase, and free radical generation during metabolism, are inevitable during the life activities of an organism. However, excess levels of these potentially hazardous molecules could induce cellular damage. Indirect or direct damage to cellular macromolecules could modulate and affect their functions. This would affect the regulation of gene expression. Moreover, accumulation of such damage could induce age-related diseases and functional deterioration. Under calorie restriction (CR) conditions, regulation of the HNF-4α/PGC-1α pathway may contribute to the suppression of these accelerated aging processes through metabolic adaptation.

Glycation could potentially accelerate aging processes via macromolecular damage; but, if so, the reason for activation of the potential gluconeogenic HNF-4α/PGC-1α pathway in CR animals is not clear. One explanation is that glucose levels are lower in CR than in AL animals, despite activation of gluconeogenic pathways in CR animals. Therefore, lower glucose levels are likely to be achieved under lower calorie intake conditions. To avoid development of hypoglycemic disorders, the HNF-4α/PGC-1α pathway must be activated under CR conditions, which would then lead to activation of other target genes involved in stress resistance, and xenobiotic and other detoxification mechanisms. Hence, activation of gluconeogenic pathways in CR animals could induce various metabolic and protective effects that could contribute to increased lifespan and suppression of age-related disorders.

**Candidates of CR mimetics and target molecules**

Ultimately, CR mimetics may be the drugs that will allow extension of the human lifespan. However, it is almost impossible to test such an effect, due to the extended period of time that would be required. Therefore, as discussed above, it is considered that drugs that improve glucose and lipid metabolism, and increase oxidative stress resistance are good candidate CR mimetics. Among these candidates, statins (HMG-CoA reductase inhibitors, which are used for the treatment of hyperlipidemia), STACs (which activate sirtuin family proteins, such as SIRT1), and rapamycin (a TOR pathway inhibitor) have been shown to increase the lifespan of laboratory organisms. Interestingly, it has been shown that the hypolipidemic drug atorvastatin could suppress the reduction of an age-dependent decrease of HNF-4α activity in rats. Therefore, the CR mimetic effects of statins may be involved in HNF-4α activity modulation. On the other hand, analysis of NPY-knockout mice has shown that NPY is not essential for feeding responses and leptin signaling. This result suggests the presence of the compensatory mechanism for feeding control. However, interestingly, NPY and the hypothalamic arcuate nucleus have been shown to have important anti-tumor effects under CR conditions. Indeed, NPY transgenic rats showed increased lifespan, along with hypotension. Nonetheless, transgenic mice overexpressing NPY in noradrenergic neurons showed impaired glucose tolerance and increased adiposity. Hence, NPY activators, such as ghrelin, would be a candidate CR mimetic; however, NPY activation under AL conditions induces impaired glucose metabolism.

Therefore, these results indicate that both the HNF-4α/PGC-1α pathway and NPY signaling potentially enhance glucose production and obesity. Hence, CR mimetics targeting these pathways should be used in the pre-prandial phase so as not to increase blood glucose and adiposity.

**Conclusion**

HNF-4α/PGC-1α may be an attractive target for the development of CR mimetics. Small molecules that activate this pathway could mimic the beneficial effects of CR. However, this paper mainly focused on the peripheral effects of CR on liver metabolism; whereas the beneficial effects of CR may involve several different signaling pathways in various organs and tissues. Moreover, CR regimens consist of 2 phases, namely, pre-prandial and post-prandial phases. It is important to investigate which phase is responsible for the beneficial effects of CR, and for the metabolic adaptation required to achieve the increased lifespan and anti-aging effects. It is also possible that the transition phases of these 2 prandial phases are important for CR effects. Nonetheless, further investigation of the neuroendocrine system, and its target organs...
and tissues, can elucidate the molecular mechanisms underly-
ing the anti-aging effects of CR. The application of such findings may allow development of new drugs for various age-related disorders.

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