Neuroendocrine and Pharmacological Manipulations to Assess How Caloric Restriction Increases Life Span

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As part of an effort to review current understanding of the mechanisms by which caloric restriction (CR) extends maximum life span, the authors of the present review were requested to develop a list of key issues concerning the potential role of neuroendocrine systems in mediating these effects. It has long been hypothesized that failure of specific neuroendocrine functions during aging leads to key age-related systemic and physiological failures, and more recently it has been postulated that physiological neuroendocrine responses to CR may increase life span. However, although the acute neuroendocrine responses to fasting have been well studied, it is not clear that these responses are necessarily identical to those observed in response to the chronic moderate (30% to 50% reduction) CR that increases maximum life span. Therefore the recommendations of this panel fall into two categories. First, further characterization of neuroendocrine responses to CR over the entire life span is needed. Second, rigorous interventional studies are needed to test the extent to which neuroendocrine responses to CR mediate the effects of CR on life span, or alternatively if CR protects the function of essential neuroendocrine cells whose impairment reduces life span. Complementary studies using rodent models, nonhuman primates, and humans will be essential to assess the generality of elucidated mechanisms, and to determine if such mechanisms might apply to humans.

A recent view of the role of the neuroendocrine system in mediating age-related pathologies and mortality was based on the observation that cellular functionality in many tissues is generally preserved during aging (1). Age-related pathologies were therefore hypothesized to be due to age-related impairments in the regulatory or hormonal milieu impinging upon these cells (2). On the other hand, based in part on the precedent that life span is limited in spawning salmon by the activation of the hypothalamic–pituitary–adrenal axis (3), several investigators have proposed that specific neuroendocrine systems may actively drive distinct senescent processes (4–6).

Role of Neuroendocrine Functions

A more general hypothesis that neuroendocrine systems drive a fundamental process of senescence was supported by the observation that hypophysectomy may delay many aspects of senescence and extend life span (7). Consistent with, and perhaps even more striking than, these observations, loss of function of single genes in two possibly related neuroendocrine pathways also increases maximum life span in mice. First, single gene defects, Pit 1 (8) and Prop1 (9), that produce dwarfism in mice by ablating development of cells that express growth hormone, prolactin, and thyroid hormone (8), extend maximum life span of mice (9). Similarly, ablation of the growth hormone receptor also increases life span in mice (10). Furthermore, ablation of p66Shc, which appears to mediate some effects of growth factors including insulin (11), also extends maximum life span in mice (12). Similarly, loss of function of several individual genes in the insulin-like signaling pathway of C. elegans extends maximum life span in this species (13). Finally, loss of function of a hormone/GTP-binding receptor-like protein also extends maximum life span in fruit flies (14). Taken together, these genetic studies suggest that the action of hormones may drive important processes in senescence, including the
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limitation of life span. Since CR reduces the level of many hormones [except glucocorticoids, which are elevated by CR (15,16)], thereby producing in some respects a “functional hypophysectomy,” these genetic studies support the hypothesis that neuroendocrine responses to CR may mediate effects of CR to increase life span.

An alternate mechanism by which the neuroendocrine system may mediate effects of CR on senescence is suggested by a confluence of data indicating that the cumulative exposure to glucose may drive key aspects of the aging process (17–19). Glucose economy is fundamentally regulated by, and in turn regulates, neuroendocrine function; indeed, neuroendocrine responses to fasting and CR (especially the elevation of glucocorticoids, as indicated by the name) generally have the effect of mobilizing and channeling glucose metabolism. Together with a wide variety of convergent data, these observations led to the hypothesis that glucose metabolism in glucose-sensitive neuroendocrine cells, especially in the hypothalamus, cumulatively compromises the function of these key neuroendocrine cells and leads to age-associated metabolic impairments (20,21). This hypothesis has been supported by evidence that a key subset of glucose-sensitive neurons in the hypothalamus produces propiomelancortin [POMC; (22,23)], impairments in which cause metabolic syndromes similar to those observed during aging (24). POMC-producing neurons are among the most vulnerable to early and robust age-related impairments in mice (25), rats (26), and humans (27–29).

The hypothesis that glucose-sensitive neuroendocrine cells regulate life span has also received surprising support from two rather unexpected lines of evidence. First, a series of studies has demonstrated that life span in C. elegans is regulated by the action of an insulin-like pathway specifically in neurons (30). Several lines of evidence suggest a convergence of insulin and glucose signaling in glucose-sensitive neuroendocrine cells (31–35) and, indeed, that many effects of insulin are mediated through glucose metabolism (36). Another series of studies has indicated that an essential metabolic step mediating effects of caloric restriction on yeast life span is the conversion of NAD (nicotinamide adenine dinucleotide) to NADH (the reduced form of NAD) (and not, for example, ATP [adenosine triphosphate]) (37). Several lines of evidence have now indicated that an essential metabolic step by which neuroendocrine cells sense glucose is also the conversion of NAD to NADH, rather than production of ATP (38–40). Thus, these data suggest that conversion of NAD+ to NADH in glucose-sensitive neuroendocrine cells may drive key aspects of the aging process.

Nevertheless, the results from C. elegans and yeast have not resolved the critical question of whether it is the neuroendocrine responses to CR that lead to increased life span, or if CR acts by protecting nutrition-sensitive neuroendocrine cells from metabolism-induced damage. Results in C. elegans tend to support the latter mechanism (30), whereas results from yeast tend to support the former mechanism (37); however, none of these results is conclusive. The role of the neuroendocrine system in mammals remains similarly unresolved. Although it has been vigorously argued that elevated glucocorticoid secretion during aging drives many aspects of senescence (6), nevertheless CR leads to an elevation of glucocorticoid secretion while increasing life span (15,16). Similarly, several lines of evidence support the hypothesis that the age-related decline in growth hormone causes a variety of age-related impairments (41). On the other hand, CR causes a reduction in growth hormone (at least in young rodents), and mice deficient in secretion or sensitivity to growth hormone actually live longer than wild-type controls (9,10). These considerations have led the panel to suggest the studies outlined below.

EFFECTS OF CALORIE RESTRICTION ON NEUROENDOCRINE FUNCTION

Considerable data exist on the neuroendocrine responses to acute caloric deprivation (42–45). However, such data are based almost entirely on the effects of completely depriving subjects of food for 24–72 hours (i.e., fasting). On the other hand, the manipulation relevant to life span, referred to as CR, entails much more moderate restriction of access to food (generally around 30%) over the life span (extending over months or even years). Given the tremendous difference in both degree and time domain of acute fasting compared with lifelong CR, it is likely that many neuroendocrine responses to CR are quite different from those of fasting. Surprisingly, however, other than changes in peripheral hormone levels (15,16,46–48), little is known about the neuroendocrine pathways that are altered by chronic CR, some of which may be critical to orchestrating the anti-aging effects of CR.

One example serves to explain why more baseline knowledge of the effects of CR on neuroendocrine function is needed at the hypothalamic and pituitary level. An enhancement of the diurnal elevation of corticosterone appears to be a common feature of chronic CR in several strains of rats and mice (15,16). However, this elevation is associated with increased adrenal sensitivity to adrenocorticotropic hormone (ACTH)—not to increased release of ACTH and uniform activation of the hypothalamo–pituitary–adrenal axis (49). Consistent with this observation, corticotrophin releasing hormone (CRH) mRNA levels in the paraventricular nucleus are reduced in young mice subjected to chronic CR (50). Interestingly, chronic CR reduces hypothalamic pre-proenkephalin mRNA, but acute (24- or 48-hour) caloric restriction does not (51); this is consistent with the general observation that effects of chronic CR on hypothalamic gene expression are greater than effects of acute fasting (50). Therefore, mimicking the hyperadrenocortical state of CR by activating the CRH–POMC–ACTH pathway would not be an accurate model and might have effects other than those achieved by a more accurate model that elevated serum corticosterone or adrenal sensitivity to ACTH.

Our panel therefore recommends a major effort to understand the alterations that are induced by chronic CR on hypothalamic and hypophysial function and the impact of these changes on peripheral hormone secretion and on the autonomic nervous system. This knowledge will serve two main functions. First, it will address current hypotheses concerning the anti-aging role of the neuroendocrine system in CR. Second, it will likely lead to new points of entry for manipulation and testing of hypotheses relating to the action of CR.
Effects of CR on Neuroendocrine Function in Rodents

Research Question. What is the effect of CR on the TRF-TSH-T4/T3 axis from the inception of CR throughout the life span?

Hypothesis.—CR suppresses circulating levels and cellular actions of T3 through central and/or peripheral mechanisms throughout the life span.

Rationale.—Acute fasting reduces T3 levels (52), and chronic CR in one rat strain reduces T3 throughout the life span (47). The potential impact of altered thyroid function on metabolic and other cellular activities in the CR rodent warrants research to assess more thoroughly the effect of CR on this system and on the hypothalamic–pituitary axis regulating T4/T3 synthesis, release, action, and metabolism.

Examples of questions and measurements.—Comprehensive diurnal measurements of the effect of CR on plasma levels of TSH-T4/T3, as well as expression of thyroid hormone receptor subtypes in different organs, are needed. Assessment of TRF/TSH regulation and secretion in the CR animal is advised. Studies to determine whether CR influences target tissue sensitivity to thyroid hormones are recommended.

Research Question. What is the effect of CR on the CRH-POMC/ACTH-Corticosterone axis from the inception of CR throughout the life span?

Hypothesis.—CR elevates circulating levels and cellular actions of corticosterone through central and/or peripheral mechanisms throughout the life span.

Rationale.—Acute CR elevates corticosterone; chronic CR, in one rat strain and one mouse strain, elevates corticosterone throughout the life span (15,16). Moreover, in one rat strain, chronic CR is associated with increased sensitivity of the adrenal to ACTH with no evidence of central activation of the hypothalamic–pituitary axis regulating corticosterone synthesis and release (49). The potential impact of altered glucocorticoid activity on metabolic and cytoprotective/stress resistant mechanisms warrants research to assess more thoroughly the effect of CR on this system and on the hypothalamic–pituitary axis regulating corticosterone synthesis, release, action, and metabolism.

Examples of questions and measurements.—Measurement of plasma levels of ACTH-corticosterone (or cortisol, depending on species) diurnally, as well as expression of glucocorticoid hormone receptors in different organs, is recommended. Assessing whether CR influences target tissue sensitivity to glucocorticoids should also be incorporated into studies of this axis during CR.

Research Question. What is the effect of CR on levels and actions of hypothalamic and peripheral peptides that regulate food intake and body composition?

Hypothesis.—Effects of CR will not precisely mimic effects of fasting on peptides that regulate food intake and body weight, and systems that are not influenced by CR do not mediate effects of CR on life span.

Rationale.—Acute food deprivation has marked effects on orexigenic and anorectic peptides (23,53,54). These peptides, in addition to influencing eating behavior, have marked influences on other neuroendocrine systems, such as those involved in regulating glucocorticoids and insulin (55). On the other hand, acute food deprivation produces metabolic effects distinct from those produced by CR on the regulation of glucocorticoid secretion, as well as on hypothalamic gene expression (50,51). It is therefore possible that not all of the neuroendocrine effects of acute food deprivation will be produced by CR; neuroendocrine systems not influenced by CR, even if influenced by acute food deprivation, are unlikely to mediate life-prolonging effects of CR. Therefore, such studies may usefully lead to the exclusion of some plausible hypothetical mechanisms mediating effects of CR, as well as provide new points of entry for interventions and mimetics of CR.

Examples of questions and measurements.—Measurement of plasma levels of leptin, cholecystokinin, as well as hypothalamic expression of proopiomelanocortin, agouti-related peptide, and neuropeptide Y and other peptides during the life history of animals subject to CR is recommended. Studies to identify additional neural and endocrine factors involved in regulating metabolic responses to CR and measurement of the synthesis and secretion of candidate regulatory peptides under caloric restriction are also needed. Studies to identify the roles and relative importance of regulatory peptides in physiological responses to CR actions are considered to be of particular importance.

Research Question. What is the effect of CR on the autonomic nervous system?

Hypothesis.—CR has marked effects to reduce the activity of the sympathetic nervous system relative to the parasympathetic nervous system.

Rationale.—There is very little information on the effect of chronic CR on this critical integrative component in any animal model. However, the evidence that CR reduces sympathetic tone (56,57) and the correlating evidence that this reduction is associated with reduction in blood pressure (58,59) is of potential importance to elucidate the prophylactic actions of CR against chronic disease. We therefore urge that studies be designed to examine the effects of CR on the autonomic nervous system.

Examples of questions and measurements.—We urge studies to further characterize the effect of CR on sympathetic activity and to identify the underlying mechanisms and physiological significance and role in the anti-aging actions of CR.
Effects of CR on Neuroendocrine Function in Nonhuman Primates

Research question. What are the effects of CR on neuroendocrine functions in young and aging nonhuman primates?

Hypothesis.—Neuroendocrine responses to CR will be largely similar, but not identical, in rodents and primates, and in young and aging individuals.

Rationale.—Far less is known about neuroendocrine responses to CR in nonhuman primates than in rodents or humans. Furthermore, assessment of effects of CR over the life span in nonhuman primates is quite problematic, and questions that can be addressed in such studies are highly constrained. Our panel strongly encourages research to identify those changes induced by CR in neuroendocrine function that are common to and different between rodents and primates. Thus, where feasible, we recommend that the same measurements be carried out in nonhuman primates as were identified as important targets for study in nonprimates. Such studies will set the stage for testing if mechanisms mediating effects of CR in rodents are likely to extend to primates.

Examples of questions and measurements.—We urge studies to characterize the effects of CR on growth hormone, insulin-like growth factor (IGF-1), thyroid hormone, adrenal hormones, and plasma catecholamine levels, both in young nonhuman primates and in nonhuman primates in which CR was initiated later in life.

Effects of CR on Neuroendocrine Function in Humans

Research question. What are the effects of CR on neuroendocrine functions in young and aging nonobese humans?

Hypothesis.—Neuroendocrine responses to CR will be largely similar, but not identical, in humans compared with rodents and primates, and in young and aging individuals.

Rationale.—The vast majority of studies that have examined effects of CR in humans have examined these effects in humans that were obese before CR. Because obese individuals may react differently to CR than nonobese individuals, our panel strongly encourages examination of neuroendocrine responses to CR in normal nonobese young and older individuals. Such studies will set the stage for testing if mechanisms mediating effects of CR in rodents are likely to extend to humans.

Examples of questions and measurements.—We urge studies to characterize the effects of CR on growth hormone, IGF-1, thyroid hormone, adrenal hormones, and plasma catecholamine levels, both in young humans and in humans in which CR was initiated later in life. It is noted that there may be practical obstacles to recruiting and maintaining humans on CR for extended periods of time, so developing methods to address these obstacles, such as minimizing the effective level of CR or recruiting individuals who for other reasons have voluntarily practiced CR, should also be considered.

Do Neuroendocrine Systems Mediate Effects of CR on Life Span?

As described earlier, several lines of evidence suggest that neuroendocrine responses to CR may mediate some of the effects of CR to extend life span. For example, at least in young individuals, CR reduces levels of growth hormone (48) and thyroid hormone (47), and hypophysectomy also delays many age-related impairments and extends life span (7). Similarly, mutations that reduce production or sensitivity to growth hormone increase life span (9,10), whereas transgenic mice that overexpress growth hormone exhibit shorter life spans than wild-type controls (60). Some of the same mutations that increase life span (9) also decrease thyroid secretion (81), and hyperthyroid rats are reported to have decreased life span relative to euthyroid controls (61). Therefore, it is plausible to hypothesize that reduction of activity in either the growth hormone or the thyroid hormone axes, or both, might mediate some effects of CR to extend life span. Conversely, it has been proposed that the elevation of glucocorticoids, whose function is to mobilize and channel glucose metabolism in response to the low glucose associated with CR, may also mediate some effects of CR to extend life span (15,16). The hypothesis that neuroendocrine responses to caloric restriction lead to increased life span would be consistent with the hypothesis that specific mechanisms have evolved to prolong life span when access to adequate nutrition is reduced for prolonged periods of time (62). Such mechanisms would also be consistent with molecular mechanisms in yeast, in which effects of CR on life span are mediated by the activation of specific nuclear factors by NAD+ (37), because glucose-sensitive hypothalamic and pancreatic cells sense glucose through the conversion of NAD+ to NADH (38–40). Such a mechanism would imply that manipulations that produce the neuroendocrine responses to CR without reducing caloric intake would nevertheless extend life span. Conversely, such a mechanism would also imply that manipulations blocking neuroendocrine responses to CR would also block the effects of CR on life span.

On the other hand, several lines of evidence also suggest that CR might delay age-related impairments not by producing normal neuroendocrine responses, but instead by preventing metabolism-driven, age-related impairments of neuroendocrine function (20,21). Specifically, it has been proposed that a key process that limits life span is the erosion of glucose-sensitive neuroendocrine cells by repetitive (postprandial) cycles of glucose metabolism, and that dietary restriction increases life span by reducing exposure of these sensitive cells to glucose (20,21). Such a mechanism is consistent with recent results suggesting that stimulation of glucose metabolism through an insulin-like pathway specifically in neurons produces oxidative damage in these neurons that limits life span in C. elegans (30). Although the identity of these neurons is not yet known in C. elegans, in the mammalian nervous systems the primary direct target of insulin is probably the same set of hypothalamic neurons...
that are regulated by glucose and leptin (63,64). If the effects of CR are due to the sparing of essential neuroendocrine cells whose function would otherwise decline with age, then manipulations that protect those specific cells (or their functions) from age-related decline should increase life span regardless of caloric intake. Alternatively, manipulations that impair those cells should shorten life span and block the effects of CR on life span.

Nevertheless, there has been no direct assessment of the mechanisms by which dietary restriction extends life span (except for the recent study in yeast, described above), and many other non-neuroendocrine, physicochemical mechanisms have been proposed, including that glucose-induced glycation drives many aspects of the aging process and may mediate effects of caloric restriction (18). Such non-neuroendocrine physicochemical mechanisms imply that purely neuroendocrine manipulations will not dissociate the effects of CR on life span from caloric intake, in contrast to the predictions implied by neuroendocrine mechanisms. Thus, for example, if glycation drives senescence independent of neuroendocrine mechanisms, blocking neuroendocrine responses to CR without reducing caloric intake should have no effect on life span. Our panel suggests that direct assessment of the role of neuroendocrine responses to CR in mediating effects of CR on life span should be given the highest priority.

**Genetic Manipulations in Rodents**

**Research Question.** Do genetic manipulations that block or mimic neuroendocrine responses to CR block or mimic effects of CR on life span?

**Hypothesis.**—Genetic manipulations that block neuroendocrine responses to CR will block effects of CR on life span, independent of effects on caloric intake. Conversely, genetic manipulations that mimic neuroendocrine effects of CR will mimic effects of CR on life span.

**Rationale.**—As described above, hypophysectomy increases life span (7), as do mutations that prevent the secretion of growth hormone, thyroid hormone, and prolactin (9). Conversely, other evidence suggests that elevated glucocorticoids may attenuate some age-related impairments (16). Because CR in young animals reduces secretion of growth hormone (48), thyroid hormones (65), and thyroid-stimulating hormone (66) and increases glucocorticoid secretion (16), it is plausible to hypothesize that neuroendocrine responses to CR mediate some effects of CR on life span. On the other hand, many mechanisms might plausibly mediate effects of CR on life span, and whether any or all of the neuroendocrine responses to CR mediate any of the effects of CR on life span has not been directly tested.

**Examples of questions and measurements.**—The role of reduced growth hormone in mediating effects of CR on life span could be tested by assessing whether CR extends life span in transgenic mice that overexpress growth hormone. If CR fails to reduce growth hormone in these transgenic mice and nevertheless extends life span, then it would be reasonable to conclude that decreased growth hormone does not play an obligatory role in mediating effects of CR on life span. Conversely, if CR fails to increase life span in such transgenic mice, this result would support (though not prove) the hypothesis that a fall in growth hormone may mediate some effects of CR on life span. Similarly, the role of increased glucocorticoids in mediating effects of CR on life span could be tested by assessing if CR extends life span in mice in which the corticotropin-releasing factor (CRF) gene has been ablated (67). If CR fails to stimulate glucocorticoid secretion in such CRF knockout mice and nevertheless extends life span, then it would be reasonable to conclude that elevated glucocorticoids do not play an obligatory role in mediating effects of CR on life span. Conversely, if CR fails to increase life span in such transgenic mice, this result would support the hypothesis that an increase in glucocorticoids may mediate some effects of CR on life span. By similar logic, it would be of great interest to examine effects of manipulating expression of hypothalamic genes that probably mediate many neuroendocrine effects of CR. For example, fasting elevates hypothalamic melanin concentrating hormone [MCH; (68)], whereas ablation of the MCH gene leads to voluntary reduction of food intake and reduction in body weight without reducing fertility (69). It would therefore be of interest to assess if ablation of the MCH gene, by leading to reduced voluntary food intake, reproduces the effect of CR; if not, this would suggest that the elevation of MCH under conditions of nutritional deficit, like other neuroendocrine responses, might itself mediate effects of dietary restriction on life span.

On the other hand, the effect of CR might be mediated by the combined effect of several neuroendocrine or hypothalamic responses. To address this possibility, it would be of interest to assess the life span of ad libitum (AL)-fed and calorically restricted transgenic mice overexpressing leptin (70), as leptin administration blocks several neuroendocrine responses to fasting (71). If CR fails to reduce growth hormone and thyroid hormone and increase glucocorticoids in such leptin transgenic mice (as expected from studies in acutely fasted mice) and nevertheless extends life span, then it would be reasonable to conclude that none of the leptin-regulated neuroendocrine responses to CR play an obligatory role in mediating effects of CR on life span. Conversely, if CR fails to extend life span in such transgenic mice, such a result would suggest that the neuroendocrine responses play an obligatory role in mediating effects of CR on life span. In such a study it would be of particular interest to simultaneously assess (a) if, as expected, CR nevertheless reduced glycation; and (b) if glycation can be dissociated from the effects of CR to increase life span. Leptin transgenic mice voluntarily reduce food intake and exhibit reduced body weight (70), while presumably failing to exhibit neuroendocrine responses to the reduced food intake and body weight [e.g., exhibiting early puberty even though fat stores are almost nonexistent (72)]. Thus, the life span of AL-fed leptin transgenic mice would be of considerable interest because if the neuroendocrine responses play a role in mediating effects of CR, leptin transgenic mice would not be expected to exhibit increased life span despite voluntary caloric restriction, whereas if neuroendocrine responses are not important in mediating effects of CR on life span, AL-
fed leptin transgenic mice would be expected to exhibit increased life span compared to AL-fed wild-type mice. It similarly would be of interest to assess longevity and responses to CR in mice homozygous for the mahogany gene [which, though resisting diet-induced obesity, are nevertheless hyperphagic and hypermetabolic (73)]. Assessment of longevity and response to CR in other genetic variants that are hypermetabolic and resist obesity (74,75) also would be of interest.

From these few examples, the value of developing genetically manipulated mouse models that block or mimic the effects of CR on neuroendocrine function is clear. Nevertheless, there are limits to the interpretation of such studies, including the possibility that genetic manipulation could have developmental effects. As described in the final section, such concerns can be addressed by the application and development of improved methods of genetic manipulation, including methods allowing conditional expression of genes. Similarly, designing these experiments and accounting for secondary effects of the manipulations will require careful consideration. For example, models that block glucocorticoid secretion or elevate growth hormone, without influencing food intake, should be matched with models that increase glucocorticoid secretion or reduce growth hormone expression to demonstrate that reversal of outcome can be achieved by reversing the direction of change in the model. By the same token, gene ablation models would be usefully compared with transgenic models (e.g., leptin-deficient ob/ob mice to leptin transgenic mice).

Epidemiology and Voluntary Weight Loss in Humans

Intervention studies of CR in humans are very difficult, and there are concerns about instituting them because of potential deleterious physical and psychological consequences of anorexia. Nevertheless, as noted by other panels, there are ongoing and planned research programs such as the Study of Health Outcomes of Weight Loss Trial that implement weight reduction interventions. This panel recommends, where possible, characterizing the impact of such weight reduction regimens on the neuroendocrine measures described. Such characterizations would most likely be restricted to those that could be accomplished with peripheral blood, urine, or salivary samples.

As noted earlier, reductions in plasma concentrations of growth hormone, IGF-1, and triiodothyronine and elevations in plasma levels of corticosterone have been documented in CR rodents. It is conceivable that genetic and/or environmental influences that alter T3, growth hormone, and glucocorticoids to similar degrees, through mechanisms other than CR, might also have anti-aging actions. Thus, high normal T3 and growth hormone or low normal cortisol might be “risk factors” for more rapid rates of aging of selected physiological systems/parameters or pathologies, or even all-cause mortality. This possibility provides motivation for studying mimetics of CR in humans.

We suggest that this hypothesis can be tested in at least two ways in humans. In the first approach, epidemiologic studies in middle-aged and older individuals could be undertaken to assess prospectively or retrospectively whether variation in the aforementioned hormone levels or other neuroendocrine parameters affects age-specific mortality or age-related changes (e.g., forced expiratory volume) that are clinically important or potentially predictive of longevity. A second approach would be to measure hormones or other neuroendocrine parameters implicated in the anti-aging action of CR in ongoing weight reduction studies. Thus, in new studies, one could determine whether weight reduction increased glucocorticoid “tone” (e.g., levels or lymphocyte sensitivity to cortisol) and concomitantly retarded the rate of decline in forced expiratory volume, which has already been found to correlate with high normal morning cortisol concentrations (76). These studies could be coordinated with twin studies and other genetic mapping and epidemiologic approaches to establish hereditability of these variables.

Research Question. What is the correlation between plasma concentrations of T3, growth hormone, IGF-1 and cortisol and age-specific disease and mortality?

Hypothesis.—Individuals in the lower tertile of T3, growth hormone, and IGF-1 levels and the higher tertile of cortisol levels will have reduced age-specific mortality and morbidity, or at least reduced age-related change in a subset of parameters.

Rationale.—It has been hypothesized that neuroendocrine responses to fasting (elevated glucocorticoids and reduced T3 and growth hormone) mediate some effects of CR on the development of age-related pathologies. If these hormonal changes are important, humans exhibiting the hormonal phenotype of CR may also show retardation of age-related changes.

Examples of questions and measurements.—We urge large-scale epidemiological studies to assess the relationship between T3, growth hormone, IGF-1, and cortisol with age-related pathologies including cardiovascular disease and cancer, as well as life span.

Research Question. What is the effect of voluntary weight reduction on plasma concentrations of T3, growth hormone, IGF-1, and cortisol and the relationship of these changes to changes in risk factors for specific diseases?

Hypothesis.—Chronic weight reduction in humans will alter the aforementioned hormone levels and other neuroendocrine parameters in the same way they are altered by chronic CR in rodents. Moreover, those individuals exhibiting the most marked alterations will exhibit the greatest change in associated risk factors and other measures of disease outcome, or, prospectively, age-related morbidity and mortality.

Rationale.—As described above, it has been hypothesized that the neuroendocrine responses to CR mediate some of the effects of CR to increase life span. If such a mechanism is operative in humans, humans undergoing weight reduction regimens should exhibit similar changes.
Specifically, individuals who show the greatest neuroendocrine responses to voluntary weight loss should show the greatest benefit in terms of effects on risk factors for age-specific disease and reduction in age-specific morbidity and mortality.

Examples of questions and measurements.—We urge support to study T3, growth hormone, IGF-1, and cortisol, as well as cardiovascular function and other pathologies, in patients that have voluntarily lost weight.

Anti-obesity Drugs in Rodents

Research Question. Do drugs that reduce food intake without producing neuroendocrine responses to CR increase longevity?

Hypothesis.—Voluntary reduction of food intake under the influence of appetite-inhibiting drugs will produce the same effects on life span as involuntary reduction of food intake only if the neuroendocrine responses to the reduced food intake are preserved.

Rationale.—The neuroendocrine responses to voluntary reduction of food intake probably differ in a number of ways from the neuroendocrine responses to involuntary intake. For example, leptin administration reduces food intake but prevents the elevation of glucocorticoids that would normally accompany this reduction in food intake (71). Therefore, if neuroendocrine responses to CR mediate the life-prolonging effects of CR, administration of leptin will produce voluntary CR without prolonging life. On the other hand, although many new drugs have been developed that may reduce voluntary food intake (77), it is not yet known if these drugs block neuroendocrine responses to caloric intake. We hypothesize that administration of drugs that reduce food intake will extend life span only if these drugs do not block neuroendocrine responses to caloric restriction, or indeed that such drugs actually produce a neuroendocrine profile, such as elevated glucocorticoids and reduced IGF-1, similar to responses to CR.

Examples of questions and measurements.—We urge the assessment of the effects of chronic administration of satiety agents such as cholecystokinin or leptin, or possibly even the use of an unpalatable diet or production of conditioned taste aversion, on age-related pathologies including cancer, as well as longevity.

Research Question. Do drugs that decrease food intake and/or body weight but increase metabolic rate increase life span?

Hypothesis.—Anti-obesity drugs that increase metabolic rate will shorten life span.

Rationale.—The role of metabolic activity in mediating effects of CR remains unclear. Since acute fasting reduces metabolic rate (78), and many lines of evidence suggest that metabolism drives the aging process [e.g., cooling poikilo-therms increases life span (79)], it was widely hypothesized that CR would increase life span at least in part by reducing metabolic activity. However, direct measurements failed to confirm this hypothesis (80), suggesting that in chronic CR metabolic adaptation occurs that allows overall metabolic activity to remain, relative to the lean body mass, similar to metabolic activity of AL-fed individuals. A drug that simultaneously decreased food intake while increasing metabolic rate would directly test the role of metabolic rate in the life-extending effects of CR.

Examples of questions and measurements.—Agents that might reduce food intake while increasing metabolic rate might include cytokines such as TNF-alpha or interleukin 1-beta, or possibly an amphetamine-like drug. It would be of interest to assess if chronic administration of these agents would or would not mimic effects of CR on thyroid hormones, the growth hormone axis, glucocorticoids, and age-associated pathologies and life span.

Anti-obesity Drugs in Nonhuman Primates

Research question. Do anti-obesity drugs delay age-related pathologies in nonhuman primates?

Hypothesis.—Anti-obesity drugs that reduce energy intake result in delay in the biomarkers of aging if these drugs produce the appropriate neuroendocrine responses to reduced energy intake.

Rationale.—Although it is not feasible to test anti-obesity drugs on life span, it is possible to assess the impact of these drugs on variables that are risk factors for age-related morbidity and mortality. Although there is debate about the strength of the associations between such risk factors and aging, this panel believes it is an area worthy of investigation.

Examples of questions and measurements.—Effects of anti-obesity drugs on neuroendocrine functions, as well as biomarkers such as cataract formation, cardiac function, and tumor incidence, would be of considerable interest.

Anti-obesity Drugs in Humans

Research question. Do anti-obesity drugs reduce the severity of complications of obesity that are usually seen with aging?

Hypothesis.—Anti-obesity drugs produce long-term improvement or disappearance of complications of obesity and age-correlated pathologies.

Rationale.—Although it is well established that voluntary weight loss in overweight humans produces clear benefits to health, it is not known if anti-obesity drugs produce the same long-term benefits. For example, it is possible that the use of such drugs might prevent the neuroendocrine responses that normally occur during voluntary weight loss (in fact, precisely this result would be expected if leptin...
were ever approved for clinical application), and thus the neuroendocrine benefits of such a regime (if such benefits normally exist) might be attenuated. Therefore, it is necessary to directly assess the long-term neuroendocrine responses and health consequences of anti-obesity drugs in both young and older humans.

Examples of questions and measurements.—It would be of considerable interest to assess effects of current anti-obesity drug treatment on neuroendocrine function and age-related pathologies, including cardiovascular disease and cancer, in both young and older individuals.

Anti-obesity Surgery in Rodents

Research Question. Does anti-obesity surgery mimic effects of CR on neuroendocrine function and life span?

Hypothesis.—Bariatric surgery, involving either gastric banding or gastric bypass, will increase life span only if accompanied by appropriate neuroendocrine responses to the reduced food intake.

Rationale.—Bariatric surgery is currently the most effective long-term method to reduce body weight in obese humans (81). Much remains to be determined concerning the mechanisms by which gastric bypass surgery leads to reduced body weight, but some evidence exists that individuals who have undergone this procedure are actually more easily satiated than before the surgery. This raises the possibility that the body weight setpoint is altered after the surgery, possibly due to increased satiety sensory input into central circuits regulating appetite. However, it remains to be determined if weight loss after this surgery is accompanied by the neuroendocrine responses that are appropriate for the reduced food intake. In view of the current prevalence of gastric bypass surgery (81), it is of interest to assess if the reduction in food intake associated with gastric bypass will lead to increased life span.

Examples of questions and measurements.—It would be of considerable interest to assess effects of gastric bypass surgery on food intake and body weight, plasma hormone levels, reproductive function, hypothalamic gene expression, and metabolic rate in young rodents and older (obese) rodents, and to assess if gastric bypass surgery mimics effects of CR on life span.

Anti-obesity Surgery in Humans

Research question. Does anti-obesity surgery produce the full neuroendocrine profile expected from reduced caloric intake and alter the biomarkers of aging or ameliorate age-related pathologies?

Hypothesis.—Gastric bypass surgery will produce expected neuroendocrine responses to caloric restriction and reverse and delay age-related metabolic pathologies in humans.

Rationale.—The large number of individuals that have, for clinical reasons, undergone anti-obesity surgery, including gastric bypass surgery, constitute a valuable resource for ascertaining the long-term consequences of caloric restriction in humans. While some clinical information is available on these individuals, numerous complexities, including lack of appropriate controls, have hindered exploitation of this resource to clarify biological mechanisms mediating effects of CR in humans. It is possible that obese patients have a relatively higher body weight set point, so that bariatric surgery, by reducing body weight to normal levels, will nevertheless produce neuroendocrine responses to caloric restriction. The panel therefore urges support for a systematic analysis of age-related metabolic pathologies in patients who have undergone anti-obesity surgery.

Examples of questions and measurements.—It would be of considerable interest to assess cortisol, growth hormone, IGF-1, thyroid hormone, testosterone, and insulin sensitivity in obese patients before and after gastric bypass surgery, and compare these parameters to humans matched for age and adiposity.

Methodological Issues

As with all fields in biomedical research, the recent advent of large-scale genomic and expression library sequencing has set the stage for a revolution in methodological approaches to gerontological research in general, and studies of the mechanisms of CR in particular. One application of this abundance of sequence information is the development of DNA array technology. For example, use of such arrays has indicated that CR can delay age-related changes in metabolic and stress-induced genes (82,83). Although DNA arrays exist for up to 50,000 genes, application of such large arrays is extremely expensive and can indeed be an inappropriate utilization of array technology, depending on the type of questions under consideration. We therefore urge the development of arrays that are particularly appropriate for the study of metabolic and neuroendocrine functions. Such arrays would include cDNAs for metabolic enzymes (e.g., enzymes responsible for the metabolism of glucose and other nutrients) as well as for genes that are responsive to glucose, insulin, thyroid hormone, glucocorticoids, and growth hormone. Of particular interest would be genes expressed in the hypothalamus that are involved in the neuroendocrine responses to CR, including neuropeptide Y, proopiomelanocortin, and agouti-related peptide. Because far more genes are currently available on arrays representing mouse sequences than rat sequences, particular effort should be made to support the development of appropriate arrays for both mice and rats.

On the other hand, because of (a) the impending availability of the complete genomic sequences of four strains of mice, (b) the vast and growing database of natural mouse genetic variations (both induced), and perhaps above all because of (c) the availability of transgenic and gene ablation technology in mice, it is clear that the importance of studying mice in gerontological research will continue to increase. While the availability of transgenic and gene ablation technology holds great promise for gerontological
research, it will be particularly important to manipulate gene expression at various times in the life span. Therefore, we urge support for the development of methods that will allow induction or attenuation of expression of specific genes in specific tissues at any time in the life span. For the same reasons, we urge support for the development of protocols to assess the full range of neuroendocrine, metabolic, and physiological functions in mice.

**RESOURCES**

Given the growing numbers of potentially valuable mouse models for assessing life span and other age-related traits, it is critical to develop resources where these studies can be most efficiently and economically conducted. We envision the need for assessing longevity in tens if not hundreds of models (e.g., induced and spontaneous mutants, pharmacologically treated models). One or more central animal facilities capable of undertaking these studies and distributing animals for cross-sectional studies may be the most appropriate means of accomplishing this task.

**CONCLUSION**

While neuroendocrine systems have long been hypothesized to mediate effects of CR on life span, recent studies demonstrating that single genes acting through neuroendocrine pathways can regulate maximum life span have dramatically supported the plausibility of these hypotheses. On the other hand, it is still unclear if normal responses to CR mediate effects of CR on life span, or if CR prevents damage to key neuroendocrine cells whose damage in turn limits its life span, or even if any neuroendocrine systems play an obligatory role in mediating effects of CR on life span. Nevertheless, given the tremendous progress that has been made in the last decade in understanding the mechanisms mediating effects of nutrition on neuroendocrine function, together with the development of improved methods to genetically manipulate rodents as well as new anti-obesity drugs and treatments, the panel believes it is now possible to directly assess whether neuroendocrine responses to CR mediate effects of CR on age-related pathologies and life span and/or if cumulative nutritional stimulation (especially by glucose) drives age-related impairments of essential neuroendocrine functions over the life span. The panel therefore suggests that such studies be given the highest priority.

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