

Review Article

Healthy aging and anti-aging treatments

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Responses to Many Anti-Aging Interventions Are Sexually Dimorphic

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There is increasing appreciation that sex differences are not limited to reproductive organs or traits related to reproduction and that sex is an important biological variable in most characteristics of a living organism. The biological process of aging and aging-related traits are no exception and exhibit numerous, often major, sex differences. This article explores one aspect of these differences, namely sex differences in the responses to anti-aging interventions. Aging can be slowed down and/or postponed by a variety of environmental ("lifestyle"), genetic or pharmacological interventions. Although many, particularly older studies utilized only one sex of experimental animals, there is considerable evidence that responses to these interventions can be very different in females and males. Calorie restriction (CR), that is reducing food intake without malnutrition can extend longevity in both sexes, but specific metabolic alterations and health benefits induced by CR are not the same in women and men. In laboratory mice, several of the genetic alterations that reduce insulin-like growth factor I (IGF-1) signaling extend longevity more effectively in females or in females only. Beneficial effects of rapamycin, an inhibitor of mTOR signaling, on mouse longevity are greater in females. In contrast, several anti-aging compounds, including a weak estrogen, 17 alpha estradiol, extend longevity of male, but not female, mice. Apparently, fundamental mechanisms of aging are not identical in females and males and it is essential to use both sexes in studies aimed at identifying novel anti-aging interventions. Recommendations for lifestyle modifications, drugs, and dietary supplements to maintain good health and functionality into advanced age and to live longer will likely need to be tailored to the sex of the user.

Keywords: Aging; Caloric restriction; Estradiol-17 alpha; IGF-1; Rapamycin; Sex

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INTRODUCTION

In this article we will address sex differences in the responses to interventions that extend longevity by slowing down and/or postponing the process of aging. Why should we be interested in sex differences in this

particular context? Aging obviously affects both females and males, and efforts to develop effective and safe anti-aging interventions are not meant to benefit one sex more than the other. Historically, much of biomedical research was conducted using mainly (or exclusively) male subjects. Both clinical and "basic"

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researchers felt that using females would introduce an additional and unwelcome source of variation, namely the impact of hormonal fluctuations during the menstrual cycle in women and estrous cycle in experimental animals. Implicit in this reasoning was the expectation that results of testing drugs or exploring physiological mechanisms which have been obtained in males will apply to females of the same species. These assumptions are unproven, and, in many cases, are now known to be incorrect [1]. Consequently, there is increasing emphasis on the importance of including both females and males in biomedical research, pharmaceutical development, and evaluation of novel drugs or non-pharmacological interventions. Not surprisingly, these concerns and considerations also apply to the studies of aging, a research field in which many older publications report results obtained in one sex. In this brief article, we will identify interventions shown to extend mammalian longevity, provide examples of major sex differences in responses to various anti-aging interventions, and offer some speculations on the mechanisms that may be responsible for these differences. First, we will describe the main categories of interventions that have anti-aging activity implied by their ability to increase mean, median, and/or maximal lifespan. Extension of longevity is a very useful, objective, and quantitative outcome in studies of the biology of aging, although it may represent prevention of a particular disease rather than alteration in the biological process of aging. Dissociation of various measures of biological aging from changes in lifespan was recently discussed by Xie et al [2].

ANTI-AGING INTERVENTIONS

Studies in laboratory animals (primarily house mice, *Mus musculus*, but also rats and other species) identified multiple interventions that can extend longevity. In this article, we will discuss only interventions that have been shown to increase longevity in normal, “wild type” (as opposed to mutant or genetically altered) animals fed standard laboratory diet. Thus, we will not deal with interventions that increase longevity only in animals with premature or accelerated aging due to a genetic predisposition (such as various types of progeria) or to being fed high fat (or high fat/ high sucrose) diet, often referred to as a “Western diet.” We will also not cover the information on treatments or genetic

alterations that have beneficial effects on metabolic health, body composition, various aging related traits (such as glucose homeostasis), or healthspan, that is the part of life that free of disability and disease, but have no proven ability to extend longevity.

Effective anti-aging interventions can be divided into three broad categories: environmental, genetic, and pharmacological. Environmental (or lifestyle) interventions, include multiple regimens of calorie restriction (CR), changes in diet composition such as reducing the amount of a specific amino acid, and altered ambient temperature or environmental enrichment (EE; such as a more complex housing system). Genetic interventions include primarily spontaneous loss-of-function mutations and targeted deletion of genes related to somatotrophic (growth hormone releasing hormone/growth hormone/insulin-like growth factor-1, GHRH/GH/IGF-1), mechanistic target of rapamycin (mTOR), or insulin signaling. Pharmacological interventions include a rapidly growing list of compounds affecting various signaling pathways and processes related to known or suspected mechanisms of aging. This includes mTOR signaling inhibitors, anti-diabetic drugs, senolytics (drugs depleting senescent cells), precursors of nicotinamide adenine dinucleotide (NAD), and compounds affecting glutathione levels [3-5].

1. Lifestyle Interventions

1) Calorie restriction

Decades of research provided undisputable evidence that CR, which consists of reducing food intake without malnutrition, can improve health, slow the process of aging, and produce impressive extension of longevity [6]. Lifespan extension is generally proportional to the percent reduction of food intake. Although very modest CR can produce measurable health and survival benefits, most of the available data were derived from studies in which laboratory animals were allowed to consume 60%–70 % of what would have been their unrestricted (*ad libitum*) food intake. While a great majority of the studies of CR were conducted in mice and rats, CR was shown to also extend longevity in other species, including domestic dogs [7] and rhesus monkeys [8-10]. Intriguingly similar, although generally somewhat smaller, metabolic and longevity benefits can be obtained by reducing the content of protein, methionine, or other amino acids in the diet (the so-called

dietary restriction, DR) [11], by restricting food access to a particular period of the 24 hour light/dark cycle [12] or by periodic (intermittent) fasting [13].

Detailed discussion of the effects of CR, DR, time restricted feeding, or intermittent fasting and the mechanisms thought to underpin these effects is outside the scope of this brief article. However, we would like to point out that CR affects most, if not all, of the recognized key mechanisms (“pillars” or “hallmarks”) of aging [14]. These mechanisms include targets of many genetic and pharmacologic anti-aging interventions which will be discussed later in this article, namely somatotrophic and insulin signaling, glucose homeostasis, energy and oxidative metabolism. In most studies in which both females and males were included, CR extended longevity in both sexes. Meta-analysis of the results of multiple CR studies revealed that, on the average, the magnitude of this sex effect was species dependent, with better outcomes in female mice and male rats [15,16]. However, these differences were not seen consistently and subsequent studies indicated that the sexual dimorphism in health and longevity benefits of CR in mice depends on the strain employed and also on the severity of CR [17].

While effects of long-term CR, as different from malnutrition or starvation, on human longevity remain to be conclusively demonstrated, there is great amount of evidence for beneficial impact of CR on traits strongly related to health and longevity. These include blood pressure, serum lipids, insulin sensitivity and inflammation markers, as well as the amount and distribution of adipose tissue [18,19]. These benefits are seen in both men and women, but this does not mean that the responses of the two sexes are identical. In non-obese (normal to slightly overweight) adults 12 months of approximately 12% CR produced greater improvements in risk markers for cardiovascular disease, insulin resistance, and diabetes in men than women [20]. In a recent diabetes prevention study in overweight individuals (PREVIEW, ClinicalTrials.gov NCT01777893), low energy diet was used to produce rapid weight loss and this was followed by three years of life-style based weight maintenance. In response to these interventions, women had greater improvements in fasting glucose levels, triacyl glycerol and HDL-cholesterol but smaller improvements in HbA1c and LDL-cholesterol and greater loss of fat-free mass and bone mass [21].

2) Environmental enrichment

There is considerable evidence that housing laboratory animals in an enriched environment can have a variety of beneficial effects including amelioration of changes in age-related traits and extension of longevity [22-24]. EE in most studies consists of adding nesting material, novel objects (“toys”), shelters or running wheels, housing the animals in larger cages and/or increasing the number of cage mates. Beneficial effects of enrichment of the acoustic environment have also been reported [25]. In some of the studies involving both sexes of mice, the extension of longevity by EE was seen in males only or was more pronounced in males [25,26], but in others there was no significant effect of sex on the responses to EE [27].

2. Genetic Interventions

1) Effects of suppression of GH signaling

Disruption of the GH receptor gene, starting at conception, leads to GH resistance with consequent reduction in circulating IGF-1 levels, somatic growth, and adult body size [28]. Suppression of GH signaling in *Ghr*^{-/-} (also known as GHRKO or Laron dwarf) mice is associated with increased adiposity, reduced insulin levels, enhanced insulin sensitivity, and a remarkable extension of mean and maximal lifespan [29,30]. Similar to findings in GH-deficient hypopituitary Snell dwarf, Ames dwarf, and *Ghrh*^{-/-} mice [31-33], both female and male *Ghr*^{-/-} mice are long-lived. Interestingly, when the *Ghr* gene is disrupted later in life or only in specific tissues, the impact on longevity is sex-specific. Thus, global disruption of GH receptor at six weeks or at six months of age increased lifespan of females, but not males [34,35], while disruption of this receptor selectively in adipocytes increased longevity of males only [36]. Extension of longevity in mice with muscle-specific GHRKO was also detected only in males. In addition, this effect was seen only in one of two laboratories participating in this study [37].

A prominent phenotypic characteristic of long-lived GH-deficient and GH-resistant mice is drastic suppression of hepatic *Igf1* gene expression and circulating levels of IGF-1. Interestingly, genes coding for IGF-1 and its receptor in mammals exhibit extensive homology to genes known to control aging and longevity in worms and insects [38,39]. Thus, reduction of IGF-1 levels in Ames dwarf, Snell dwarf, *Ghrhr*^{-/-}, Little, and

GHRKO mice emerged as a key candidate mechanism of extended longevity of these animals. In support of this hypothesis, heterozygous deletion of the *Igf1r* gene, reduction of IGF-1 levels by gene insertion, or heterozygous deletion of *IRS-2*, and deletion of *IRS-1*, key components of IGF-1 signaling were shown to extend murine longevity [40-43]. However, increases in the lifespan in most of these studies were seen only in females or were limited to maximal longevity without altering median lifespan. Moreover, the increase in female lifespan detected in early studies of mice with reduced IGF-1 levels was not consistent in different cohorts of animals and not statistically significant. The authors suggested that this may have been related to the increase in GH secretion caused by reduced IGF-1 negative feedback [40]. Interestingly, the maximum lifespan was significantly extended and age-specific mortality was reduced in both females and males with reduced IGF-1 levels [40]. Interpretation of the effects of IGF-1-related mutations on longevity and comparisons with GH-related mutants are complicated by the critical role of IGF-1 in early development and by severe consequences of complete IGF-1 or IGF-1 receptor deficiency [44,45]. Nevertheless, information available to date implies that GH, rather than IGF-1, is a key regulator of aging and longevity in mammals [46-48].

Extension of longevity by mutations that block or reduce somatotrophic signaling indicates that aging is strongly influenced by trade-offs between nutrient responsive anabolic processes and processes involved in maintenance and repair. Findings in these mutants are also consistent with the role of evolutionarily conserved insulin/insulin-like growth factors signaling (IIS) in the control of aging and longevity [38,49]. Further support for these broad generalizations was provided by the demonstration that deletion of ribosomal protein S6 kinase 1, which is activated by IIS can also extend longevity [50]. Increase in lifespan of *S6K1*^{-/-} mice is also consistent with the role of mTOR, another upstream regulator of *S6K1*, in the control of aging in organisms ranging from yeast to mammals [51,52]. The latter possibility was strongly supported by the subsequent demonstration that pharmacological inhibition of mTOR signaling by incorporating rapamycin into the diet also extends murine longevity [53,54]. Relevant to this article, deletion of *S6K1* gene extends median and maximal lifespan only in females [50] and beneficial impact of rapamycin on longevity is greater in

females than males [53,55].

Transgenic overexpression of the “starvation hormone,” fibroblast growth factor-21 (FGF21) extended longevity in both sexes of mice, but the effect was greater in females [56]. Based on transcriptomic analysis, the authors suggested that the effect of FGF21 on lifespan is due primarily to suppression of hepatic GH/IGF-1 signaling leading to increased fatty acids oxidation, ketogenesis, and insulin sensitivity, while alterations in mTOR signaling did not seem to be involved [56]. The results of this study add to the evidence linking GH actions to the control of mammalian aging.

Hemizyosity for a null mutation in the insulin receptor and associated reduction of insulin sensitivity in the *IRKO*^{+/-} mice led to reduced hazard of mortality in males and an opposite effect in females [57]. However, mean lifespan was not significantly altered in either sex. This may have been related to a small number of normal (wild type) controls (eight females and 11 males) and perhaps also to differential impact of this intervention on late life *vs.* early mortality.

It should also be mentioned that lifespan responses to some genetic anti-aging interventions are not sexually dimorphic and instead are identical or very similar in females and males. This includes extension of longevity in mice with selective deletion of insulin receptor in the adipose tissue [58], transgenic overexpression of *Klotho*, an inhibitor of insulin and IGF-1 signaling [59], and overexpression of the *Atg5* gene or disruption of the *beclin 1* gene, both of which are involved in autophagy regulation [60].

2) Pharmacological interventions

Much of the progress in identifying compounds capable of extending mammalian longevity can be credited to the Interventions Testing Program (ITP), a coordinated effort of three laboratories in the USA supported by the National Institute of Aging. In this program, compounds selected from suggestions provided by the scientific community are tested in each of the participating laboratories for impact on longevity of female and male genetically heterogeneous UM-HET3 mice. Mice used for these studies are generated by crossing four inbred strains that have no recent common ancestors and thus their genetic architecture is roughly comparable to genetic diversity of human populations [61,62]. Studies supported by ITP identified a number of compounds that significantly extend longevity of UM-

Table 1. Anti-aging interventions reported to increase longevity of mice in one sex only

Intervention	Sex of animals that live longer
Genetic interventions	
Heterozygous deletion of IGF-1 receptor	Females [41,45]
Heterozygous deletion of insulin receptor	Males [57]
Heterozygous deletion of mTOR or mlst8	Females [55]
Deletion of S6K1	Females [50]
Deletion of RII β (PKA)	Males [3]
Overexpression of Sirt6	Males [5]
Pharmacological interventions	
17 alpha estradiol (17aE2)	Males [64]
NDGA	Males [63]
Aspirin	Males [63]
Protandim (Nrf2 activator)	Males [66]
78c (CD38 inhibitor)	Males [4]

HET3 mice and this list continues to increase (Miller, personal communication). Most of the identified effects on the lifespan were sexually dimorphic and three compounds, 17 alpha estradiol (17aE2), nordihydroguaiaretic acid (NDGA), and aspirin extended longevity only in males [63,64] (Table 1). Rapamycin and acarbose increased longevity in both sexes, but the effect of acarbose was much greater in males [64], while effect of rapamycin was greater in females [54].

Responses to combinations of different anti-aging compounds can also be sexually dimorphic. Combined treatment with rapamycin and acarbose was more effective than rapamycin alone in extending longevity in male, but not female, mice [65,66]. Interestingly, sex differences in the effects of rapamycin on hepatic and muscle proteostasis and on longevity in mice were minimized when the animals received metformin in addition to rapamycin [67].

Similar to what was mentioned earlier in the discussion of genetic interventions, longevity responses to some anti-aging compounds are not sexually dimorphic. This includes supplementation with glycine and N-acetyl cystine (GlyNAC), which affects glutathione levels, oxidative stress, mitochondrial function, and genome maintenance, and extends longevity by nearly an identical percent in females and males [68].

SEARCH FOR MECHANISMS RESPONSIBLE FOR SEX DIFFERENCES IN RESPONSES TO ANTI-AGING INTERVENTIONS

Evidence that females and males can respond very differently to various anti-aging interventions invites questions about the mechanisms that may be responsible. From the practically endless list of sexually dimorphic physiological characteristics, differences in the levels of gonadal steroids and GH-driven differences in hepatic gene expression emerge as likely contributors to the sex-specific responses.

Estrogens exert a variety of neuroprotective effects, reduce risk of cardiovascular complications, and influence the risk of autoimmune disease, as well as neoplasia in the reproductive system and mammary glands, while androgens can suppress immune function, promote progression of some cancers, and increase the risk of atherosclerosis. It is therefore conceivable that major differences between circulating estradiol 17beta and testosterone levels in reproductive age females, as compared to males, could modify responses to treatments designed to slow the aging rate, reduce inflammation including brain gliosis, and protect from age-related disease. Searching for mechanisms of the male-specific effect of 17aE2 on longevity, Garratt et al [69-71] compared the effects of this compound in intact and castrated males. Most of the beneficial (presumably anti-aging) effects of 17aE2 on glucose homeostasis, hepatic mTOR complex 2 (mTORC-2) signaling, and circulating metabolites, as well muscle composition and function, were either absent or drastically diminished in castrated animals. These findings imply that the extension of longevity in 17aE2-treated males depends on the presence of the testes and most likely on testosterone or other secretory products of the male gonad. Intriguingly, the same investigators have shown that some of the beneficial effects of 17aE2 seen in intact males could also be detected in ovariectomized females [71]. Apparently, the impact of the ovaries (most likely mediated by estradiol 17beta) on the response of aging-related traits to 17aE2 is opposite to the effects of the testes. In a more recent study, Debarba et al [72] have shown that the protective effects of 17aE2 on hypothalamic neuroinflammation, which were seen in males but not in females, are reduced by castration before the 17aE2 exposure. This adds to the evidence that the

anti-aging effects of this intervention are at least partially dependent on testicular function.

Sex differences in the pattern of GH release from the anterior pituitary produce major differences in the profile of hepatic gene expression leading to sexual dimorphism in the levels of enzymes that metabolize drugs and endogenously produced toxicants and in the regulation of fat and carbohydrate metabolism [73,74]. These sex differences in hepatic function could be expected to influence responses to drugs, diet, and interventions designed to produce beneficial metabolic changes.

Numerous other mechanisms are also likely to be involved. Recent search for genetic regulators of longevity in UM-HET3 mice identified different candidate loci in females and males [75]. As was mentioned earlier in this article, reduction of IGF-1 signaling by genetic manipulations that suppress the levels of circulating IGF-1 [40] or the number of IGF-1 receptors [41] extends longevity in female but not in male mice. Effects of genetic deletion of IRS1 on longevity are apparently greater in females [42,76]. In contrast, suppression of blood glucose levels with acarbose has a much greater effect on male longevity [64]. Collectively, these findings suggest that in spite of signaling via the same or overlapping cascades (including IRS1, IRS2, Akt, and PI3K), IGF-1 and insulin may have different effects on aging, with IGF-1 [40] signaling apparently playing a greater role in females and insulin signaling/glucose homeostasis being more important in regulation of male aging, as previously suggested by Huffman (personal communication). Interestingly, CR and suppression of GH signaling reduces both IGF-1 and insulin levels, along with increasing insulin sensitivity, and extends longevity in both sexes.

Several studies were specifically directed at identifying mechanisms responsible for sex differences in the responses of UM-HET3 mice to 17 α E2, acarbose, and rapamycin. Treatment with 17 α E2 extends longevity only in males [64], acarbose extends longevity in both sexes, but the effect is much greater in males [64], while the beneficial effects of rapamycin on longevity are greater in females [54]. Interestingly, some of the beneficial metabolic effects of these drugs exhibit sexual dimorphism similar to their effects on longevity, while others are seen in both sexes. For example, treatment with 17 α E2 reduced phosphorylation of a key component of cap-dependent translation, eIF4E, by opposing the age-

related increase in the MEK1-ERK1/2-MNK1/2 signaling in males only [77]. This contrasted with the effects of acarbose and rapamycin on the same signaling cascade which were seen in both sexes [77]. Interestingly, the same study has shown that the age-related increases in MEK3-p38MAPK-MK2 pathway were suppressed by rapamycin, acarbose, and 17 α E2 in both females and males [77]. A study conducted in a different strain of genetically heterogeneous mice detected sex-specific stimulatory effect of 17 α E2 on hepatic and circulating IGF-1 levels along with an indication of uncoupling IGF-1 production from insulin sensitivity [78].

Recent demonstration that another anti-diabetes drug, a sodium-glucose transporter 2 inhibitor, canagliflozin, extends longevity in male, but not female, UM-HET3 mice [79] was followed by a study of the neuroprotective effects of this compound in males and females [80]. While improvement in insulin sensitivity and reduction of hypothalamic gliosis was seen in both sexes after canagliflozin treatment, hippocampal microgliosis and astrogliosis were reduced only in males. Moreover, exploratory and locomotor activity at old age (30 mo) were improved by canagliflozin only in males [80].

Sex differences related to presumed mechanisms (including hallmarks or pillars) of aging [81] invite more speculations on the etiology of sex differences in the effects of anti-aging interventions. In addition to hormonal differences mentioned earlier in this article, this includes differences in accumulation of somatic mutations [82], epigenomic instability, likely related to X-chromosomal inactivation [83,84], various aspects of inflammation and immune function [85,86], life course changes in adiposity and fat distribution [87], as well as paracrine interactions, including impact of adipose tissue on skeletal muscle [87]. Interventions directed at any of these characteristics could be expected to have sexually dimorphic impact on aging and longevity.

CONCLUSIONS

Numerous nutritional, genetic, and pharmacological interventions can significantly extend longevity of experimental animals. Importantly, this includes several species of mammals, raising hope that these or similar interventions may slow or postpone human aging. Somewhat unexpectedly, effects of many anti-aging interventions on longevity of laboratory mice are

strongly dependent on sex. This includes complete sexual dimorphism, that is presence of responses in only one sex. Ongoing research begins to identify molecular, cellular, and homeostatic mechanisms that could explain differences between sexes in responses to these interventions.

Although longevity is considered as only one of surrogate measures of aging, data concerning the impact of various anti-aging interventions on longevity suggest that the fundamental mechanisms of aging (or the relative importance of different aging mechanism) are not the same in females and males. This emphasizes the importance of using both sexes in studies of the biology of aging and in search for novel anti-aging interventions. It appears likely the results of ongoing and future studies may lead to different recommendations for preservation of health during aging in women and men, and perhaps also to development of sex-specific interventions in human aging. Further studies, including approaches that can uncouple the impact of gonadal sex from chromosomal sex [88,89], will undoubtedly lend to much progress in identification of mechanisms responsible for sex differences in response to anti-aging interventions.

Conflict of Interest

The authors have nothing to disclose.

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Author Contribution

Conceptualization: AB. Funding acquisition: AB, EH, KH. Writing – original draft: AB. Writing – review & editing: AB, EH, KH.

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