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# NIA's intervention testing program at 10 years of age

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**Abstract** The previous 20 years of basic research on aging has identified a large number of genes and gene products whose expression can be manipulated in a variety of ways to increase the healthy life span of animal models such as yeast, nematodes, fruit flies, and mice. In an overt attempt to capitalize on this information, the National Institute on Aging (NIA) began a program in 2003 to identify nutritional and pharmaceutical interventions that could be safely employed to extend the healthy life span of mice. This program is called the Intervention Testing Program (ITP), and this article briefly describes the development of this initiative and some of the early success achieved during its first 10 years (2004–2014) of operation.

**Keywords** Aging · Gene expression · Animal models · Life span extension

## Introduction

The National Institute on Aging (NIA) was founded in 1974, and in 1984, I joined the NIA as a program administrator in the Molecular and Cell Biology Branch (MCBB) that included four grant programs in Cell Biology, Genetics, Molecular Biology, and Animal Models, with specific responsibility for the Molecular Biology Program. At that time in its early history, the research funded by MCBB was still fairly descriptive in nature, mostly involving research on phenomena such as age-related changes in protein and DNA structure, and age-related changes in levels of specific enzyme activities in various model organisms such as nematodes, fruit flies, and rodents. One notable exception to this general pattern was Tom Johnson's NIA-funded research on a long-lived nematode mutant designated *age-1* (Friedman and Johnson 1988). Although it was not clear at that time what to make of this long-lived mutant, the interpretation was confounded by evidence that there seemed to be at least one other mutation located near the *age-1* mutation in this mutant strain.

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## Can genetic changes positively influence longevity?

When I attended a meeting sponsored by the Jackson Laboratory in Bar Harbor, Maine, in 1988, Michael Rose described the results of his attempts to increase the average life span of a fruit fly population by repeatedly selecting the progeny of the older members of the population, and then re-breeding these, and again selecting the progeny of the oldest members of this population. After repeating this protocol five times, he was able to demonstrate that he did indeed have a population with a considerably longer average life span than the original parents, suggesting that longevity can be influenced positively by selectable genetic factors.

Because of these results, the director of our Genetics Program, Anna McCormick, set out to develop a research initiative to search for and identify such putative genes that might exist in a variety of animal systems. This initiative to search for what we referred to as Longevity Assurance Genes (LAGs) was launched in 1989 with a series of workshops attended by investigators whom we deemed likely to ultimately respond to a Request for Applications (RFA) in this area of research. The first RFA was issued during 1992, with funding beginning in 1993.

Although the first 5 years of funding did not immediately result in real breakthroughs, it did provide enough promising results to renew the initiative for five more years. This second RFA that was issued in 1998 not only led to the identification of several genetic changes that appeared to increase longevity but also attracted some new investigators who were not involved in the first 5 years of funding. Ultimately, a third RFA was issued in 2003. Overall, this 15 years of funding produced a list of about 50 genes whose expression could be manipulated to produce long-lived mutants of yeast, nematodes, fruit flies, and mice compared to their wild-type parents (Warner 2003). These included genes involved in insulin signaling, pituitary function, damage prevention and/or repair, and food consumption.

## Developing an Intervention Testing Program (ITP)

This success encouraged myself, and Donald Ingram of the NIA intramural research program, to consider whether the time had now come to develop a program that would rigorously test in a mammalian model longevity-increasing interventions that had been demonstrated in as few as a single non-mammalian species. However, these reports often lacked sufficient accompanying pathology data and/or survival data to support the conclusion that the intervention used does extend either the mean or maximum life span, or both, and what the mechanism might be if it does.

The procedure for starting such a new program at NIH is usually begun by convening a workshop of scientific experts to discuss whether such a concept has merit, and whether the field is ready for development, and such a workshop was convened in Texas in 1999 (Warner et al. 2000). If a concept does seem to have merit, and the field appears to be ready, a presentation is made to that effect at an annual retreat convened each year by the Director of the Institute, in this case Richard Hodes. At first, Dr. Ingram and I could convince few, if any, of our NIA colleagues that this was a worthwhile enterprise. Nevertheless, we tried again the following year, once more with only limited success, but on our third try, Dr. Hodes gave us the green light and some funds with which to proceed. The National Advisory Council on Aging concurred with issuing a RFA to fund three testing sites, and these were finally funded in 2004. The sites ultimately chosen from among nine applications included the University of Michigan, the Jackson Laboratory, and the University of Texas Health Science Center in San Antonio (UTHSCSA), and the three principal investigators (PI) were Richard Miller, David Harrison, and Randy Strong, respectively.

NIA then developed a two-level approval process for selecting interventions to test (Miller et al. 2007). The process begins each year when the NIA publishes a solicitation requesting suggestions from extramural scientists for interventions to be tested. Nominations must be accompanied by the rationale for the nomination, the recommended dose and method of intervention, if known, as well as any pertinent information about protocols for measuring circulating levels in the bloodstream of the compound being tested. These suggestions, usually 10–15 of which are received per year, are initially evaluated by the members of an Access Committee. The Access Committee is composed entirely of non-NIA extramural scientists with expertise in one or more of the following areas: gerontology, nutrition, pathology, aging biomarkers, pharmacology, or physiology.

These evaluations are then forwarded to a Steering Committee composed of both NIA and non-NIA scientists, including the three PIs, who make the final decisions about which interventions will actually be tested each year, and with what priority. Given the annual budget available to the ITP, usually only three to five interventions can be started per year, including controls, depending on the complexity of the intervention.

## Gender-specific interventions

All three sites carry out identical experiments to document reproducibility, using four-way cross genetically heterogeneous mice bred at each testing site. Survival curves are determined for a sufficient number of mice, usually 36 females and 44 males, to detect a 10 % increase or decrease in mean life span with 80 % power. These survival curves are accompanied by a limited number of assays for age-sensitive traits, as well as

pathological assessment; more details are provided in Miller et al. (2007).

The first published report described two interventions that showed limited promise, i.e., aspirin and nordihydroguarietic acid (NDGA). However, these results were preliminary as the experiments reported were not continued to determine maximum life span, and the benefits were male-specific. A later report from Strong et al. (2008) confirmed the male-specificity benefits of these interventions, and that they applied only to median life span and not maximum life span. The effect was slightly greater with NDGA than with aspirin. Much later, Harrison et al. (2014) confirmed these results with NDGA and obtained comparable results with acarbose, a caloric restriction mimetic. They also showed that neither 17-alpha estradiol nor methylene blue had significant effects on maximum life span in either male or female mice.

## Rapamycin

The partial success with NDGA reported in 2007 and 2008 was followed by a report in 2009 showing a more robust effect on mouse life span in response to including rapamycin in the diet. Harrison et al. (2009) showed that when rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR) pathway, is included in the diet of genetically heterogeneous mice, both median and maximum life spans are increased for both male and female mice. Of particular interest, this intervention can be delayed until the mice are at least 600 days old. This life-extending effect of rapamycin did not appear to be due to caloric restriction because the body weights of the mice exposed to rapamycin diets are similar to those of the untreated control mice (Harrison et al. 2009).

The rapamycin intervention was originally proposed by David Sharp, a professor at UTHSCSA. Rapamycin was already approved for clinical use in humans where it has immunosuppressive activity in tissue transplant patients. Several possible mechanisms have been suggested by Sharp and Strong (2010), including increasing autophagy, activation of nutrient-responsive gene expression, inhibition of cell senescence, and prevention, or at least a delay of onset, of a variety of age-associated diseases. This suggestion may have been prompted by the earlier report of Powers et al. (2006) showing that rapamycin increases the chronological life span of stationary phase cultures of yeast and also increases their resistance to heat and oxidative stress.

Harrison et al. (2009) reported that there are no obvious differences in the causes of death between control and rapamycin-treated mice. Wilkinson et al. (2012) then extended these findings by showing that many age-related changes occur more slowly in rapamycin-treated mice. These include age-dependent decline in spontaneous activity, as well as liver degeneration, tendon elasticity, and endometrial and a wide variety of other tumors. However, the news is not altogether positive, as rapamycin can also increase cataract severity and testicular degeneration.

## Clinical trials in other mammals, including humans

This success showing that rapamycin can delay aging in mice raises the question of its applicability in larger mammals, including humans (Sierra 2010), and Mannick et al. (2014) have reported that inhibition of mTOR improves immune function in the elderly. Hayden (2014) has described discussions between gerontologists Matt Kaeberlein and Daniel Promislow at the University of Washington who have been considering the potential value of a study in dogs living freely in the same environmental conditions as humans. They propose a small trial using 60 pet dogs that typically have life spans of 8–10 years, 30 of which would receive rapamycin and 30 would not. The trial would be started at 6 years of age, and the dogs would be monitored for cardiac function and other aging phenotypes such as development of type 2 diabetes and cancer until death. Such a study might reveal whether rapamycin retards aging per se, or acts principally by reducing late-life disease, but a larger study will probably be necessary to establish this unequivocally.

Kaeberlein and Kennedy (2009) have also considered human studies, but have pointed out that such studies may not be without risk due to rapamycin's immunosuppressive effects and its apparent effects on cataract formation and testicular function. Nevertheless, at least one very small clinical trial involving five men in their 80s and 90s has already been initiated by Dean Kellog, a clinician at UTHSCSA, and some limited success has been achieved so far (Leslie 2013). One of the participants has halved the time required to walk 12 m, from 18 to 8 s. Whatever the ultimate results are from such a trial, the results suggest that mTOR, a protein that helps coordinate nutrition, growth, and aging, may be a possible target for pharmaceutical intervention in humans.

**Table 1** Summary of results of interventions tested

| Intervention | Results | References |
|---|---|---|
| Rapamycin | Median and maximum life span extension in both males and females | Harrison et al. (2009), Miller et al. (2014) |
| NDGA, acarbose, 17-alpha estradiol | Median life span extended for males or females, or both | Strong et al. (2008), Harrison et al. (2014) |
| Aspirin, curcumin, green tea extract, NFP, 4-OH-PBN, oxaloacetic acid, resveratrol, simvastatin, median-chain TG, methylene blue | No or insignificant life span extension | Strong et al. (2008, 2013), Miller et al. (2007, 2011) |

*NDGA* nordihydroguarietic acid, *NFP* nitrofluorbiprofen, *4-OH-PBN* 4-hydroxy-phenylbutyl nitrone

### Treatment of age-related diseases in familial adenomatous polyposis (FAP) mice

Hasty and his colleagues at UTHSCSA (Hasty et al. 2013) have tested whether rapamycin might also extend the life span of mice heterozygous for the mutated adenomatous polyposis coli (APC) gene. Whereas these mice typically only live to about 170 days, rapamycin not only reduces the number of intestinal neoplasms in these mice but also extends their life spans to about 940 days, or about the same as rapamycin-treated normal mice. However, it is not clear whether the life-extending effect in these mice is due only to the reduction of tumors or also due to causes not related to tumor formation.

### Interventions that were not effective in mice

One of the goals of the ITP was also to identify interventions that are not effective in extending the life span of mice. Thus, the three testing sites are also committed to reporting negative results as well as positive results. Interventions so far shown not to be effective, only minimally so or sex-dependent, are thus summarized in Table 1, and these include aspirin, curcumin, green tea extract, nitrofluorbiprofen, 4-hydroxy-phenylbutyl nitrone, median-chain triglyceride oil, oxaloacetic acid, resveratrol, methylene blue, and simvastatin. However, usually only one dose was tested, and the outcome could be different if the dose chosen is not optimal for that intervention.

## Discussion and summary

The NIA's Intervention Testing Program was initiated in 2004 and has so far published test results on 14 non-genetic interventions for their ability to extend the maximum life span of genetically heterogeneous male and female mice. Of these 14 interventions tested, only rapamycin has consistently increased both median and maximum life spans in both genders of mice. Among individual experiments, the increase in median life span ranged from 14 to 26 % in females and 9 to 23 % in males. The relevance of these results obtained in mice to humans remains to be determined. The finding that four of the five treatments showing benefits were valid for only one sex was unanticipated and demonstrates the importance of testing both sexes independently.

However, the conclusions drawn from these studies must be considered to be preliminary because of the need to establish more solid information about optimal dose levels to use.

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