Abstract: Caloric Restriction has long been known to reduce disease and extend the life of mice and other animals. This paper reviews the major findings of caloric restriction experiments in several animal models such as rats, C. elegans, Drosophila, and rhesus monkeys. These models demonstrate that while caloric restriction may be feasible, we still have much to learn about the molecular mechanisms involved. As such, current experimental studies being done on humans may be premature.
A way of extending lifespan has long been unsuccessfully sought by humans. For example, Ponce de Leon is said to have discovered Florida while searching for a fountain of life.\(^1\) Nevertheless, modern scientific techniques are leading us closer to links between genetics and aging, as well as subsequent applications of this knowledge, however slowly. One area in which extensive research has been done surrounds caloric restriction and its implications for lifespan. While at first caloric restriction seemed to raise the possibility of extending lifespan simply by cutting calories, it now appears that the mechanisms involved are much more complicated and controversial. Should we be testing and implementing caloric restriction on humans despite these controversies and gaps in knowledge? By closely examining several key studies the answer becomes clearer.

As early as 1914, Francis Peyton Rous noted that decreasing food intake reduced the occurrence of tumors in rats and subsequently lengthened their life. Now this phenomenon is now one of the most well studied topics in the biogerontology field.\(^2\) Since Rous’s discovery in 1914, we have gained a significant amount of knowledge about caloric restriction and aging from animal models. For example, in the 1930’s, McCay et al. concluded that the longer length of life of rats on the restricted diets was due to the decreased rate of growth, although this was based little on the findings of their studies and more on their personal beliefs.\(^3\) However, McCay’s premonitions were confirmed by more airtight experiments done with rats in the 1960s and 1970s by Ross Morris. Morris found that from age 6 months to 24 months, the age-specific death rate was

\(^1\) http://sageke.sciencemag.org/cgi/content/full/2003/8/re2 (Masoro)
\(^2\) http://sageke.sciencemag.org/cgi/content/full/2003/8/re2 (Masoro)
\(^3\) http://sageke.sciencemag.org/cgi/content/full/2003/8/re2 (Masoro)
lower in food-restricted rats than in those fed freely.\textsuperscript{4} Since Morris’s work, the popularity of studying the effects of caloric restriction in animal models seemed to skyrocket.

Much of the work surrounding caloric restriction focuses on the GH/IGF-1 axis. GH, or growth hormone, declines with normal aging, and subsequently decreases IGF-1 levels.\textsuperscript{5} IGF-1, or insulin like growth factor 1, is a signaling system which stimulates growth in many different cell types, as well as blocking apoptosis. IGF-1 also acts as an intermediate for many growth hormone responses, and may stimulate the growth of some types of cancer.\textsuperscript{6} According to a 2009 paper by Berryman et al., “the natural declines in GH and IGF-1 that accompanies age-related degenerative processes implies that the GH/IGF-1 axis may be a causative determinant.”\textsuperscript{7} This axis has subsequently been explored in a variety of different studies and models.

What we now understand about the effects of caloric restriction has been significantly expanded by work done in other animal models. Animal models are useful for studying longevity because they can be relatively short lived and easy to manipulate.\textsuperscript{8} As previously discussed, the earliest caloric restriction studies were performed in rats. Currently, research has also been extensively performed in Caenorhabditis elegans, a small nematode animal. In Lakowski and Hekimi’s classic 1998 paper on caloric restriction in C. elegans, they mutated a number of genes to partially starve the organism. To briefly summarize their findings, mutations in many genes (\textit{eat genes}) resulted in partial starvation of the worm by disrupting function of its feeding organ.

\textsuperscript{4} http://sageke.sciencemag.org/cgi/content/full/2003/8/re2 (Masoro)
\textsuperscript{5} http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2631405/?tool=pubmed (Berryman)
\textsuperscript{6} http://www.biocarta.com/pathfiles/h_igf1pathway.asp (Croston)
\textsuperscript{7} http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2631405/?tool=pubmed (Berryman)
\textsuperscript{8} http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2631405/?tool=pubmed (Berryman)
Most of the mutations lengthened life span by up to 50%. C. elegans also have a distinct genetic mechanism which allows it to enter a dauer stage where it goes into stasis and does not develop. The study found that food restriction in C. elegans lengthens life span by a mechanism distinct from that of dauer-formation mutants. This study demonstrated that caloric restriction affected C. elegans in a manner similar to mammals like rats.

Additionally, more current research demonstrates that the daf-2 signaling pathway in C. elegans shares significant homology with metabolic pathways in flies and yeast that have also been reported to have profound effects on lifespan, namely the insulin like signaling pathway. More research shows that daf-16 is required for the daf-2 mutation to extend lifespan under normal food conditions, which suggests that caloric restriction in C. elegans is independent of the insulin like signaling, and is instead dependent on the daf-16 pathway. The image below also shows how it seems that under altered growth conditions such as caloric restriction, an insulin-like substrate binds to the daf-2-encoded receptor, initiating events like activation of the age-1, a homolog of phosphoinositide-3-OH kinase, or PI3K in mammals. PI3K activates the akt-encoded protein kinase B (PKB), which phosphorylates the daf-16 transcription factor, keeping it from moving into the nucleus. According to Carter’s 2002 article in Trends in Genetics, “disruption of the daf-2 pathway (including mutations in daf-2, age-1 or akt genes) prohibits the phosphorylation of the daf-16 transcription factor,

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9 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC23719/ (Lakowski)
10 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2340190/?tool=pubmed (Min)
permitting its translocation to the nucleus.” Therefore, *daf-16* transcription factor activity in the nucleus is believed to extend life in *C. elegans*.\(^\text{13}\)

![Diagram showing the insulin/IGF signaling pathway in C. elegans](image)

The main take away message about research done in *C. elegans* is that caloric restriction is possible, but not necessarily fully understood, and as such might not be by the same mechanisms as humans.

Another important animal in the study of caloric restriction is *Drosophila melanogaster*, a type of fly. A Swiss study in 2003 demonstrated that the forkhead transcription factor FOXO “is a crucial mediator of insulin signaling in *Drosophila*, mediating the reduction in cell number in insulin-signaling mutants.”\(^\text{14}\) However, a 2008 article in *Aging Cell* stated that “it is still thought that insulin/IGF and the

\(^{13}\) http://www.sciencedirect.com/science/article/pii/S0168952502026963 (Carter)
\(^{14}\) http://www.ncbi.nlm.nih.gov/pubmed/12908874 (Junger)
mechanisms of dietary restriction (DR) might as yet function through overlapping mechanisms.” The same study found that that over-expression of dFOXO in head fat body extends lifespan and “reduces steady-state mRNA abundance of insulin-like peptide-2 (ilp2) under conditions of high dietary yeast, but not when yeast is limiting.”

This suggests success in the caloric restriction of this animal through the FOXO transcription factor. In contrast to the ilp2 pathway, conditions of diet restriction that increase lifespan changed the mRNA abundance of only insulin-like peptide-5 (ilp5). To see whether reduction of ilp5 was required for DR to extend lifespan the researchers then blocked the diet-dependent changes with RNAi, and found that caloric restriction still lengthened longevity. They also tested capacity of CR to lengthen life without dFOXO, the insulin/IGF-responsive transcription factor. Restriction of the diet was equally effective among genotypes with and without dFOXO. From this study and many others, it is clear that in Drosophila insulin/IGF plays an integral role in controlling growth and metabolic responses to nutrition.  

Some research has also been done on caloric restriction in rhesus monkeys, a significantly more long lived species than either C. elegans or Drosophila. The National Institute on Aging (NIA) initiated an extensive study in 1987 to investigate the effects of a 30% caloric restriction in male and female rhesus macaques across age ranges. They found that similarly to rodents, lifespan was extended for the monkeys undergoing caloric restriction. More specifically, results from the NIA study have shown that CR decreases body weight and fat mass, improves glucoregulatory function, decreases blood pressure and blood lipids, and decreases body temperature. The younger males

15 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2340190/?tool=pubmed (Min)
exhibited delayed skeletal and sexual maturation, while adult bone mass was not affected by CR in females, nor were several reproductive hormones or menstrual cycling. They also found that “CR attenuated the age-associated decline in both dehydroepiandrosterone (DHEA) and melatonin in males.” Below is a graph demonstrating how male and female monkeys experienced changes in body weight as well as longevity based on caloric restriction17

The success of caloric restriction in lengthening life in a longer lived mammalian species provides insight into the possibility of caloric restriction in humans.

The image below compares what research suggests about the GH/IGF-1/insulin signaling pathway in worms, flies, and mammals.18 As you can see, the pathways have some similarities and some differences. In any case, we can see that the pathway is highly complex and

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18 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2631405/?tool=pubmed (Berryman)
too lengthy for the scope of the paper. Let’s examine a few of these complexities and how they factor into the controversial nature of caloric restriction in humans.

Major concerns and lack of knowledge surrounds SIRT1, an enzyme that deacetylates proteins that contribute to cellular regulation, as well as the phenol resveratrol. According to a 2011 paper published in Free Radical Biology and Medicine, “accumulated evidence suggests that SIRT1 may be actively involved in CR-induced signaling pathways.” Additionally, resveratrol, known for the “French paradox,” seems to have similar effects as caloric restriction. According to the aforementioned paper, “while the deacetylase activity of SIRT1 is important for the beneficial effects of resveratrol, resveratrol-induced SIRT1 activation has recently been challenged by the observations that resveratrol could not induce SIRT1-mediated deacetylation of native substrates in vitro.” Resveratrol seems to extend the life span of mice fed a high-calorie diet in a similar manner as CR, and as such, both CR and resveratrol could theoretically induce SIRT1 expression and presumably make the deacetylase activity of SIRT1 even stronger. As you can see in the picture below, AMPK, or AMP-activated protein kinase, is suggested as a pathway for resveratrol to activate SIRT1, based on in vivo studies done in the mice. Also in the image below, the red arrows represent pathways that need to be studied. 

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19 [http://www.uniprot.org/uniprot/Q96EB6](http://www.uniprot.org/uniprot/Q96EB6)
The SIRT1 enzyme and resveratrol are one example of how we have much to learn about the molecular mechanisms which promote longevity. In general, research demonstrates that the molecular determinants of lifespan are very complex and based on multiple factors. Additionally, as stated by Marquez et al., “advances in the genetics and molecular biology of longevity will require interdisciplinary approaches if...an extension of both lifespan and health span is to be achieved.” An article written in 2011 goes as far as to say “the underlying mechanism [of caloric restriction] remains poorly understood.” These statements demonstrate that we still have much to learn about how genetics and molecular processes determine lifespan. You may be shocked to know that despite all we have left to understand, caloric restriction experimentation is currently being performed on humans.

Controversial human experimentation is currently being performed on human volunteers through the National Institutes of Health. The study is known as Calerie, which stands for Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy, and the subjects were recruited by researchers from Washington University, Tufts, and the Pennington Biomedical Research Center. These participants enroll in the

study for 2 years during which they reduce their caloric intake by 25 percent. According to a New York Times article on the study, “Essentially, the study asks whether calorie restriction allows people to grow older in better health — with less disease, fewer drugs and shorter hospital stays — through a method that neither medicine nor scientific technology have yet come close to approximating.”24 This statement presents a major problem with the study, and caloric restriction testing on humans in general. Medicine and technology have so much to learn about the mechanisms involved in caloric restriction, as well as how to study this, making such a large, expensive study premature. Additionally, Susan Roberts, a professor of nutrition and psychiatry who is in charge of the Calorie team at Tufts, told the New York Times Journalist Glenn Croston that they are also seeking to find out “are there unacceptable side effects that you wouldn’t pick up in animals that you would pick up in humans?”25 This statement presents a dangerous reality of doing human experimentation like this. There is potential for finding undesirable or dangerous side effects to caloric restriction, especially because of our gaps in understanding about the molecular mechanisms.

Several hundred members of a “Calorie Restriction Society” have been self-inducing caloric restriction for many years now. They have gained considerable fame, and have even published a book called “The C.R. Way.”26 A question that few seem to be asking in the midst of this research and dietary medication is should we be trying to lengthen human lifespan in the first place? People in this century are already living much longer than ever before, and as a result, many financial and healthcare resources

24 (Croston)  
25 (Croston)  
26 (Croston)
are stretched to their limits. We should answer this question before we continue seeking to extend human lifespan.

This paper has overviewed some of the major discoveries about how caloric restriction influences lifespan in several animal models. While these discoveries have remarkable potential, I would argue that based on the consensus that we do not fully understand the mechanisms by which caloric restriction can increase lifespan, it is premature to be experimenting with humans. Seeking to increase human longevity also raises a multitude of ethical issues. Our earth is already overpopulated and starved of resources. It may be more prudent to decide as a society whether or not we should really be trying to extend lifespan before we aim for that goal.
Reference List: (Coded to footnotes)


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