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Nutrition, metabolism, and targeting aging in nonhuman primates

Priya Balasubramanian[1], **Julie A. Mattison**[2], and **Rozalyn M. Anderson**[1,3]

[1]Department of Medicine, School of Medicine and Public Health, University of Wisconsin, Madison WI

[2]Translational Gerontology Branch, National Institute on Aging, Baltimore MD

[3]Geriatic Research, Education, and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison WI

Summary

This short review focuses on the importance of nonhuman primate nutrition and aging studies and makes the case that a targeted expansion of the use of this highly translatable model would be advantageous to the biology of aging field. First, we describe the high degree of similarity of the model in terms of aging phenotypes including incidence and prevalence of common human age-related diseases. Second, we discuss the importance of the nonhuman primate nutrition and aging studies and the extent to which the outcomes of two ongoing long-term studies of caloric restriction are congruent with short-term equivalent studies in humans. Third, we showcase a number of pharmacological agents previously employed in nonhuman primate studies that display some potential as caloric restriction mimetics. Finally, we present nonhuman primates as an important model for translation of mechanisms of delayed aging identified in studies of shorter-lived animals. Proof of efficacy and safety of candidate longevity agents in nonhuman primates would be a cost-effective means to bring these exciting new avenues a step closer to clinical application.

Keywords

Aging; nutrition; nonhuman primates; caloric restriction; mimetics; translational research

Rhesus monkeys as a model for human health and disease

Nonhuman primate species are an excellent model for human biology due to their genetic and physiological similarity to humans. Nonhuman primate studies bring the promise that the insights into aging biology gleaned will be highly translatable to human aging biology. The rhesus monkey (*Macaca mulatta*) genome shares ~93% sequence identity with the human genome[1]. Similarity between monkeys and humans at the genomic level extends to

numerous aspects of anatomy, physiology, neurology, endocrinology, immunology, and behavior[2]. Rhesus monkeys develop and age in similar ways to humans but on a compressed time-scale[3,4]. In captivity, the median lifespan for rhesus monkeys is ~26 years of age and the maximum lifespan of a captive rhesus monkey is ~40 years. A reasonable rule of thumb considers macaques aging at a rate of two and a half to three times that of humans[5,6], with the caveat that not all aging and developmental milestones are paralleled. For example, females are reproductively fit relatively early and maintain menarche relatively longer than humans. Nonhuman primate studies have considerable advantages over human studies in terms of experimental design; the environment, dietary intake, and medical oversight can be fully defined, thus limiting confounding issues arising due to lack of uniformity in these parameters. Unlike rodents, rhesus monkeys display patterns of eating and sleeping behavior that mirror those of humans. Yet unlike human subject studies, rhesus monkey studies can be designed to facilitate comprehensive monitoring of subjects and strict adherence to the study protocol. Given the high degree of translatability and the tractability in study design, nonhuman primates are a vital link between basic research and clinical application. The links between aging and adiposity in nonhuman primates has been reviewed recently[7], so here we will focus on caloric restriction (CR) and putative CR mimetics. We present that increased understanding of the biology of aging in rhesus monkeys will be extremely illuminating for human aging, and efforts to understand causative elements in rhesus monkey aging and age-related disease vulnerability are highly likely to reveal novel approaches for application in preventative human health care.

Delayed aging by caloric restriction in rhesus monkeys

Caloric restriction is the only environmental intervention that repeatedly and strongly increases maximum lifespan and delays biological aging in laboratory rodents[8]. Over the last 20 years, astonishing progress has been made in defining longevity pathways and identifying factors that contribute to age-related changes in short lived species[9]. In many of these studies CR is viewed as the gold-standard model of delayed aging and the reference to which other models of delayed aging are compared. The translatability of mechanistic insights from the study of CR in shorter-lived species hinges on the effects of CR being conserved in primates including humans and nonhuman primates. To address this, three independent rhesus monkey studies were initiated in the late 1980's. Two of these studies are ongoing: one at the National Institute on Aging (NIA)[10] and the other at the Wisconsin National Primate Research Center based at the University of Wisconsin (UW)-Madison[11]. The third study, performed at the University of Maryland reported favorable effects of CR, although the study was focused on obesity and glucoregulation with only a small cohort designated to CR[12]. At the UW, the CR intervention in a cohort of 76 adult monkeys was associated with significant improvements in morbidity and mortality[13]. These findings contrasted with the report from the parallel NIA study, where a difference in survival was not observed between groups within the cohort of 121 monkeys, although a trend towards lower morbidity was reported for CR monkeys compared to controls[14]. Two major differences in study design included the timing of onset of CR where CR was implemented in adults at UW and in juveniles and advanced-age animals at NIA, and in the implementation of the diet including feeding protocols and diet composition. Subsequent analysis indicated that a direct

comparison of longitudinal data from both studies is warranted[15]. A joint initiative from both UW and NIA research teams has been developed to directly compare the two studies with a view to uncovering the basis for differences in outcome and the publication of this work is highly anticipated.

Caloric restriction impacts health indices in rhesus monkeys

Similar to humans, rhesus monkeys undergo changes in body composition with age including increased adiposity and a redistribution of body fat[16]. Not surprisingly, animals on caloric restriction tend to be smaller than their control fed counterparts and this is disproportionately evident in the reduction in adiposity[17–19]. Along with these favorable changes in body composition, improved glucoregulatory function was one of the first identified benefits of CR in rhesus monkeys, including lower circulating glucose levels and improved insulin sensitivity[12,20,21]. Incidence of insulin resistance and diabetes are significantly lower in CR animals[14,22]. Similar to humans, obesity in rhesus monkeys is associated with a number of risk factors for disease including insulin resistance and elevated serum triglycerides and cholesterol[23–25]. CR lowers circulating levels of triglycerides and improves lipoprotein profiles where levels of HDLs are higher with CR and levels of VLDLs are lower[26–28]. These outcomes are consistent with improved metabolic homeostasis and reduced risk for diabetes and cardiovascular disease.

Further evidence for delayed aging in rhesus monkeys on CR comes from studies focused on specific tissues including skeletal muscle, brain, bone, and the immune system. Sarcopenia is the age-related loss in skeletal muscle mass and function and begins in middle age in rhesus monkeys[29,30]. The onset and progression of this age-related condition is delayed in CR monkeys[31], where cellular atrophy and muscle fibrosis are both attenuated[32,33]. Aging of skeletal muscle in rhesus monkeys is gradual, similar to humans, and the onset set of aging phenotypes is linked to changes in mitochondrial activity and redox metabolism[34]. The age-related decline in physical activity is also attenuated in rhesus monkeys on CR and the intervention is associated with improved metabolic cost of movement[35]. Measures of resting metabolic rate suggest that it is lower with CR[35,36], however, analysis of the data is complicated by overt difference in body composition, and it is unclear how meaningful the small differences reported might be. Brain aging is also delayed by CR. MRI based studies of brain volume reveal preservation of white matter and neuronal volume, and markers of inflammation are significantly lower[13,37,38]. The caudate nucleus and putamen regions are vulnerable to age-associated atrophy and are protected by CR[13,39]. The age related increase in iron deposition in the globus pallidus and the substantia nigra is also attenuated by CR[40]. Correlation analysis of brain volume against peripheral insulin sensitivity suggests a role for systemic homeostasis in protection against age-related atrophy[41]. CR has long been associated with lower bone mass and lower bone mineral density that, until quite recently, was viewed as a potentially negative outcome[42]. It has become clear that bone density is markedly influenced by body weight and in this light the lower bone density measured in CR animals could be viewed as an adaptive rather than a pathological outcome of the diet[43,44]. Although the starting point is different for control and CR monkeys, the rate of decline in bone mass and bone density is greater in the controls suggesting a protective effect of CR. The ability of CR to delay aging of the immune system has also been

investigated and reports suggest that CR delays senescence of T cells and preserves the naïve population and repertoire diversity[45,46]. Other reports indicate that certain types of age-related skin damage are lowered and wound healing capacity is somewhat augmented in monkeys on CR[47]. Finally, there is some indication that age-related changes in circadian rhythm are prevented by CR where a youthful 24 hour cortisol periodicity is preserved in old monkeys on CR although this outcome was observed in males only[48]. These disparate studies together indicate that the impact of CR on aging is pervasive, with evidence of multiple indicators of disease vulnerability being either attenuated or abrogated in monkeys fed a CR diet.

The translatability of the benefits of CR to humans is suggested in outcomes of a multicenter clinical study designed to evaluate CR in humans in the CALERIE (Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy) study. The study outcomes are reviewed elsewhere in this issue; however, in terms of the findings reported above a short-term 6 month trial indicated that CR induced favorable changes in body weight, body composition, glucoregulatory function and serum risk factors for cardiovascular disease[49–54], consistent with the findings in nonhuman primates, and the beneficial metabolic effects were sustained out to 2 years of CR[55–58]. These clinical studies demonstrate the general translatability of CR's effects with parallel outcomes in human and nonhuman primate studies, but issues related to compliance in humans studies suggest that in depth mechanistic studies might best be performed in the nonhuman primate model, where tightly controlled environmental conditions and adherence to a uniform study protocol are ensured. Studies using tissues and data from the longitudinal rhesus monkey studies will provide crucial understanding of the mechanisms behind the beneficial effects of CR and these insights gleaned will almost certainly prove to be highly translatable to human health and human aging. The ideal design would identify mechanisms of CR using data and specimens from nonhuman primate studies and then seek confirmation of their conserved impact in the outcomes of CR in humans.

Translational intervention studies in rhesus monkeys

In addition to investigation of the mechanisms of CR, a major line of inquiry in aging research is to identify CR mimetics[59]. These are pharmacological agents that are intended to mimic the delayed aging effects of CR without requiring a reduction in caloric intake. The Interventions Testing Program (ITP) is an NIH/NIA initiative launched in 2000 with the initial goal of uncovering pharmacological agents that extend lifespan in laboratory mice[60]. The intent was to engage the aging-focused research community to propose novel or repurposed existing agents for trials in mice to determine their ability to promote longevity. This program has been a resounding success and has identified several novel longevity agents, including rapamycin, an immunosuppressant; acarbose, an inhibitor of complex carbohydrate absorption in the gut; 17-alpha estradiol, a non-feminizing estrogen ortholog; and nordihydroguaiaretic acid, an antioxidant[61–63]. Another compound of considerable interest to the aging community is resveratrol, a polyphenol that has been shown to positively regulate longevity in non-mammalian species and positively influence health in mammalian species[64].

There have been several difficulties in translating rodent treatments directly to humans, including issues related to differences in genetics and physiology, dosing, and species specificity in the pharmacokinetics. This translational gap might be bridged by rhesus monkey studies that could confirm the ability of agents identified in shorter-lived species to regulate longevity in long-lived nonhuman primates. The absence of beneficial effects might not necessarily indicate that the intervention is not of value in a primate species, rather it may point to the need for primate specific adaptation or optimization in the approach. Here we select a few agents with proven efficacy in nonhuman primates that are strong candidates as CR mimetics due to their target regulatory pathways (Figure 1).

Resveratrol

Resveratrol is a polyphenolic compound known for its anti-aging and anti-tumorigenic properties[64]. The biological effects of resveratrol are attributed to activation of a variety of mediators including, but not limited to, SIRT1 (nicotinamide adenine dinucleotide (NAD)-dependent histone deacetylase) and AMPK (AMP-activated protein kinase), which in turn leads to activation of PGC1a and inhibition of NF-kB and mTOR signaling[65]. Resveratrol is thought to ameliorate dietary and age-related metabolic complications through modulation of these diverse signaling factors and transcriptional regulators that are nutritionally regulated and nutrient responsive. Studies in rhesus monkeys have demonstrated pleiotropic effects of resveratrol. Resveratrol supplementation improved insulin sensitivity, decreased adipocyte size and attenuated inflammation in the visceral adipose tissue of monkeys fed a high-fat, high-sugar diet fed monkeys[66]. In these monkeys, resveratrol treatment also prevented dedifferentiation of β-cells in islets[67]. In the blood vessels, resveratrol supplementation prevented high fat diet-induced macrophage and lipid accumulation, preserved endothelial cell integrity and decreased inflammation-driven stiffening of arterial wall[68]. Resveratrol treatment also counteracted high fat diet-induced fiber type switching from type I to II fibers in the soleus muscle[69]. In terms of the central nervous system, resveratrol conferred neuroprotection against diet-induced neuroinflammation and cerebral vascular dysfunction[70]. Together these studies are strongly supportive of a beneficial impact of resveratrol on health in over-nourished primates, boding well for its application in humans as preliminary studies suggest[71].

Rapamycin

Rapamycin, an inhibitor of the nutrient sensitive kinase mTOR, has been demonstrated to extend lifespan and delay the onset of age associated diseases in rodents[72] Rapamycin is FDA-approved as an immunosuppressant in organ transplant patients and some forms of cancer. Even so, extending the translational potential of rapamycin as an anti-aging intervention in humans is met with resistance due to adverse metabolic changes such as glucose intolerance, insulin resistance and hyperlipidemia that occurred with chronic treatment in mice[73–75]. Furthermore, recent studies suggest that the metabolic effects of rapamycin are quite variable depending on the duration of treatment (transient vs long term), dosing regimen (intermittent vs continuous), age and sex of the animals being treated[76–78]. Clarification of the long-term effects of rapamycin in nonhuman primates could be a valuable next step before attempting to broaden the clinical applications of rapamycin in

humans. A recent study in common marmosets, *Callithrix jacchus*, showed that long term (14 months) oral treatment of rapamycin did not affect body weight, blood lipids or glucose tolerance[79]. The authors did report a transient decrease in fat mass during the initial months of rapamycin treatment, but there was no difference in fat mass at the conclusion of the intervention period. The authors also reported evidence of induced autophagy in the muscle and adipose tissue, but not in the liver of rapamycin treated marmosets, suggesting rapamycin modulates proteostasis[80] It will be extremely informative to extend these studies that we might know whether prolonged rapamycin treatment can impact healthspan or other indices of longevity in nonhuman primates.

PPAR agonists

Peroxisome proliferator-activated receptors (PPARs) are known to regulate lipid and glucose homeostasis in a variety of metabolically active tissues like adipose tissue, liver, skeletal muscle, and heart[81]. Synthetic ligands for PPARs such as thiazolidinediones (PPARγ agonists, also known as insulin sensitizers), fibrates (PPARα agonists, lipid lowering drugs) and dual agonists (PPARα/γ) are currently used in type 2 diabetic patients to manage hyperglycemia and hyperlipidemia to reduce cardiovascular risk factors associated with diabetes. Nonhuman primates have served as an excellent preclinical model for testing and validating the safety and efficacy of many of these existing PPAR agonists. Rosiglitazone or pioglitazone (both PPARγ agonists) treatment in obese diabetic rhesus monkeys improved peripheral insulin sensitivity, increased insulin clearance, decreased circulating triglyceride and cholesterol levels, and increased HDL and adiponectin levels[82–86]. The insulin sensitizing action of thiazolidinediones in monkeys is attributed to increase in muscle AMPK activity and atypical protein kinase C (aPKC). PPARα agonists such as fenofibrate or K-111 also improve circulating lipid profiles and insulin sensitivity[25,87,88]. Dual peroxisome proliferator-activated receptor α/γ agonists may be even more efficient in reducing cardiovascular events in diabetic patients as they confer both the insulin sensitizing effects of PPARγ agonism and lipid lowering effects associated with PPARα agonism. In diabetic rhesus monkeys, dual PPAR α/γ agonists like Aleglitazar and TAK559 have been shown to correct hyperinsulinemia and result in a less atherogenic lipoprotein profile without adversely affecting liver function, body weight or fluid retention as observed with other selective PPAR agonists[89,90]. The beneficial effects of these classes of drugs on lipid profiles and glucoregulatory function in the context of metabolic dysfunction are reminiscent of the outcomes of CR[91,92]. Unfortunately, long-term treatments with these types of compounds have fallen into disfavor clinically due to the occurrence of unappealing side effects, including increased risk for bone fractures, congestive heart failure, edema, and inflammation of the liver[81]. It may be possible to design PPAR agonists that impinge on only a subset of downstream functions avoiding these confounding side effects. With a better understanding of the underlying biology, a PPAR-based strategy might prove to be the ultimate CR mimetic.

Fibroblast growth factor-21 analogs

Fibroblast growth factor-21 (FGF21) is a peptide hormone secreted by metabolic tissues like liver, adipose tissue, pancreas and skeletal muscle with both paracrine and endocrine actions.

Rodent studies have shown that FGF21 administration decreases body weight, corrects hyperglycemia, improves insulin sensitivity, and reduces circulating triglyceride and LDL-cholesterol levels[93]. FGF21 signaling occurs through AMPK and its mechanism of action involves the adipokines, adiponectin[94–96] Interestingly, overexpression of the FGF21 transgene confers longevity in mice[97]. Complementary to rodent data, studies in NHPs have also documented the beneficial metabolic effects of FGF21. In diabetic rhesus monkeys, administration of human recombinant FGF21 improved fasting glucose, insulin, glucagon and triglyceride levels[98]. LY2405319 (an engineered FGF21 variant) also showed a similar metabolic effect, in addition to decreasing leptin and increasing adiponectin levels, this compound had a lipid-lowering effects in late stage diabetic monkeys[99]. Elevated circulating levels of adiponectin are consistently observed in animals on CR[100–103] and may be mechanistically important due to its established ability to stimulate AMPK and PPARs[104–106] To circumvent the dosing difficulties associated with a shorter half-life of FGF21, several long-acting analogs have been developed and tested in nonhuman primates. Fc-FGF21 (RG), the first-long acting analog of FGF21 generated by fusion of Fc to human recombinant FGF21, showed greater efficacy than human recombinant FGF21 in improving the metabolic status of obese rhesus monkeys[107]. A monoclonal antibody mimAb1 that mimics FGF21 and activates βKlotho/FGFR1c (FGF receptor 1c) receptor signaling mimicked FGF21's metabolic actions and resulted in an improved glycemic and lipid profile in obese cynomolgus monkeys[108]. Obese cynomolgus monkeys treated with the bispecific Avimer polypeptides anti-FGFR1c/β-Klotho protein decreased body weight, lowered triglyceride levels and lowered fasting insulin levels[109]. A complicating issue with the use of FGF21 to treat metabolic disease is that circulating FGF21 levels are higher in the obese suggesting the possibility of FGF21 resistance[110]. This is unlikely to be a problem in healthy individuals and places renewed emphasis on the impact of FGF21 on aging in otherwise healthy humans and nonhuman primates.

The concept that aging itself might be a suitable drug target in a clinical context is very new but there is considerable interest in bringing this idea to fruition[111,112]. Until now, the emphasis for each of the interventions described above, apart from rapamycin, has been on correcting metabolic dysfunction. Mechanistically, each one is predicted to show at least some similarity to CR, making a strong case for their use as agents for anti-aging. With a clearer set of biomarkers derived from studies of aging and delayed aging by CR, it should be possible to test these promising agents in nonhuman primate studies to determine their ability to impinge on the aging process.

Final remarks

In conclusion, it is resoundingly clear that nonhuman primate studies fill a vital need in aging research. A high-resolution molecular understanding of aging and delayed aging in rhesus monkeys could be used as a framework against which other interventions might be tested. With this groundwork laid, the possibility exists to conduct effective translational studies, and in the short term, offset the usual high-cost impediment placed on nonhuman primate work. The advantages of conducting translatability studies of longevity agents in primates are manifold: first, acting as a filter to identify the "most-likely to work" candidates; second, to uncover potential requirements for optimization in the move from

rodents to primates; and third, to identify biomarkers of efficacy that will ensure progress and productivity in the implementation of human trials. With all this promise, it would seem remiss not to engage in some serious monkey business.

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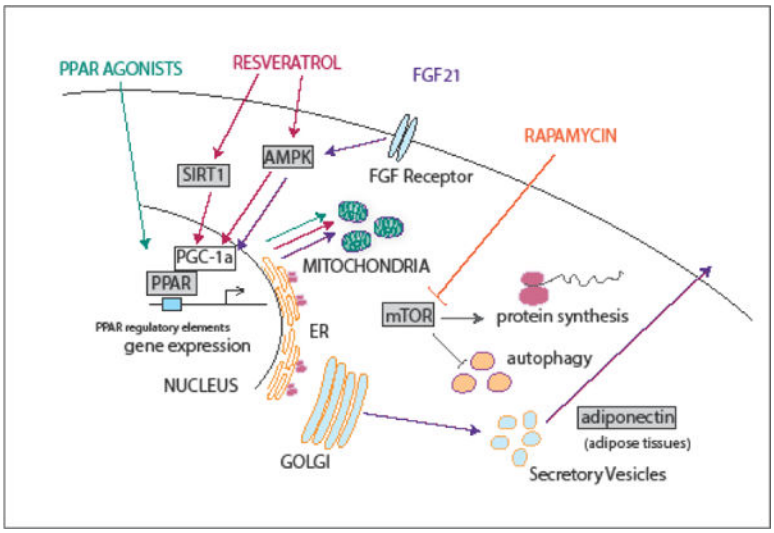

Figure 1.

Target pathways and processes for putative CR mimetic agents resveratrol, rapamycin, PPAR agonists, and FGF21. Apart from rapamycin, nuclear receptor activation and mitochondrial activation are shared outcomes of treatments. Key signaling molecules include AMPK, SIRT1, PGC-1a, and mTOR, all of which have been associated with regulation of longevity in the context of CR.