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Calorie Restriction: Progress during mid-2005 - mid-2006

Rozalyn M. Anderson[1,2] and **Richard Weindruch**[1,2]

1*University of Wisconsin Madison, GRECC VA Hospital, 2500 Overlook Terrace, Madison, Wisconsin 53705, USA.*

2*Wisconsin National Primate Research Center, 1220 Capitol Court, Madison, Wisconsin 53706, USA.*

Overview

In keeping with the current trend, this year again has seen a substantial volume of literature on the topic of calorie restriction (CR). Work in widely diverse species has contributed to our understanding of CR and continues to provide important clues about the mechanism of lifespan extension by CR. Let us acknowledge at the outset that the scope of this review permits only a handful of these studies to be described, for this we apologize and list areas of progress that we view as most important.

Towards a mechanistic understanding

A number of pathways originally identified in longevity studies in invertebrates have come under further scrutiny, requiring some revision of previous models. The role of the Sirtuin family of NAD dependent deacetylases in longevity and in the mechanism of CR has drawn particular attention (Longo and Kennedy, 2006). It remains to be seen if the contribution of Sirtuins to the mechanism of CR is conserved in yeast, worms and flies. As a result, data from these organisms may not necessarily be predictive of a role for Sirtuins in the mechanism of lifespan extension by CR in mammals. The role of mitochondrial respiration in CR has also been brought into question by a study that demonstrates lifespan extension with CR in a respiratory deficient yeast strain (Kaeberlein et al., 2005a). The fact that alternate pathways promoting longevity are induced in strains lacking respiratory capacity does not negate a role for mitochondrial metabolism where the organelles are functional. The important finding from these studies on strain dependent differences is that CR may extend lifespan by impacting multiple pathways and that there may be a certain amount of mechanistic plasticity.

The TOR pathway has long been suspected of providing a link between nutrition, metabolism and longevity, and deficiency in TOR signaling extends lifespan in worms and flies. A large-scale analysis of over 500 single gene deletion strains in yeast has pointed to the involvement of the nutrient sensing TOR pathway in the mechanism of CR (Kaeberlein et al., 2005b). There is also evidence for down-regulation of the mTOR pathway in the long-lived Ames Dwarf mouse (Sharp and Bartke, 2005). The mammalian mTOR pathway has recently been shown to be involved in determining resting oxygen consumption and oxidative capacity in cultured cells (Schieke et al., 2006). mTOR affects mitochondrial function and the mitochondrial phosphoproteome independent of the regulation of ribosomal gene expression. The influence of TOR on mitochondrial function is of particular interest in the context of CR, because mammalian studies have demonstrated that mitochondrial metabolism and ROS generation are altered in tissues from restricted animals compared to controls (see below).

A significant challenge in the study of the mechanism of CR in invertebrates is presented by the lack of consensus in methodological approach. In worms, differences in the implementation of CR (bacterial dilution/anexic media/use of “genetic mimic”) cause complications in determining key factors involved. As a result, the extent of crosstalk between the insulin signaling pathway and CR is controversial, and the involvement of TOR and SIR-2.1 is still under investigation (Houthoofd et al., 2005). Nonetheless, the use of RNAi screens proves invaluable in the identification of factors that influence longevity and continues to provide new and exciting leads (Hansen et al., 2005). An important issue relating to methodology has been resolved in *Drosophila* studies where the standard CR regimens reduce calories by dilution of either the yeast or sugar component of the diet. By direct comparison of isocaloric diet in terms of lifespan extension and reversibility of effect, it appears that all calories are not created equal and the yeast component is the key determinant of lifespan in this organism (Mair et al., 2005).

The extent of overlap between established regimens that influence lifespan in the mouse model is also unclear. In rodents, CR extends the lifespan of the Ames dwarf but does not further extend the lifespan of the growth hormone receptor knockout (GHRKO) mouse (Bonkowski et al., 2006). The inference is not that the GHRKO mouse and CR extend lifespan by the same mechanism, rather that elements required in the mechanism of CR are absent in the GHRKO mouse. This is based on differences in gene expression profiles in tissues from GHRKO and CR animals that do not support an equivalent mechanism of lifespan extension.

A number of studies have demonstrated an anti-inflammatory effect of CR in rodents. In rats, CR and exercise have beneficial effects on circulating levels of C reactive protein, a marker of inflammatory tone (Kalani et al., 2006). Transcriptional profiling of mouse adipose tissue reveals a marked reduction in the expression of genes involved in inflammation with long term CR (Higami et al., 2006). While we do not yet know how CR brings about the anti-inflammatory effect, this phenotype may be an important contributor to the delay in onset of age-associated diseases.

Mitochondria

The role of mitochondria in the mechanism of CR continues to be an area of active research. Differences in experimental approach have yielded interesting and sometimes apparently contradicting data. In flies, CR does not appear to affect mitochondrial numbers in muscle but does appear to alter mitochondrial morphology and *in vitro* enzyme activities (Magwere et al., 2006). In mammalian cells grown in serum derived from CR rats, mitochondrial biogenesis is increased and bioenergetic efficiency is improved with concomitant reduction in reactive oxygen species (ROS) generation (Lopez-Lluch et al., 2006). A separate study reports increased mitochondrial biogenesis with CR based on increased transcription levels of nuclear encoded mitochondrial genes and increased mitochondrial DNA content in tissues from restricted mice compared to controls (Nisoli et al., 2005). A role for nitric oxide in CR is suggested by the finding that endothelial nitric oxide synthase (eNOS) is induced by CR, and the effect of CR on mitochondrial markers is reduced in eNOS null mutant mice. It will be interesting to see if reactive species in general play a signaling role in the mechanism of CR.

Studies in isolated mitochondria from rat skeletal muscle reveal a reduction in ROS production with CR that is not due to changes in proton leak (Bevilacqua et al., 2005). Separate studies demonstrate that CR opposes the decline in oxidative capacity of skeletal muscle mitochondria. This effect is independent of mitochondrial DNA integrity suggesting that there is a difference in mitochondrial function with CR that cannot be simply explained by differences in mitochondrial DNA damage (Baker et al., 2006). This same study demonstrated reduced activities of both citrate synthase and Complex IV of the electron transport system in extracts

from CR muscle, however; activities were maintained with age in CR tissues but declined below the CR levels in tissues from Control animals with age. Analysis of Complex IV activity in skeletal muscle *in situ* provides evidence that mitochondrial from CR animals have a higher affinity for oxygen (Hepple et al., 2005). This raises the possibility that data from isolated mitochondria may not reveal key differences in mitochondrial function with CR.

Studies in primates

A major goal of the field is to determine the potential of CR in humans. On route to this is to understand whether CR can slow the aging process in nonhuman primates that share close genetic makeup to humans. There are two studies (one at the USA's National Institute on Aging; the other at the University of Wisconsin) in rhesus monkeys that began in the late 1980s, which are examining the effects of CR on aging. In our study, we have previously reported that the monkeys on CR display signs of improved health (e.g. 70% less body fat, higher insulin sensitivity, favorable changes in circulating lipids). Further, CR imparts a complete protection from Type 2 diabetes and an emerging survival advantage compared to the age-matched controls (unpublished observations). As the rhesus monkeys at our Primate Center have an average lifespan of ~27 years and a maximum lifespan ~40 years, it may be another 25 years before we obtain full survival data from this population.

Significant progress has also been made on the effects of long-term CR in humans. Direct evidence comes from studies of cardiovascular aging in long-term practitioners of CR who were reported in 2004 to display markedly improved risk factor profiles for protection against developing cardiovascular disease, including core features of CR (e.g., reductions in circulating insulin and glucose levels). These individuals also display fewer signs of aging in heart (diastolic) function (Meyer et al., 2006). Additional progress in human CR is occurring as result of the USA's National Institute on Aging funding to conduct CR investigations in people. CR lowers body temperature and insulin levels (both of which happen in rodents on CR) in overweight subjects (Heilbronn et al., 2006). In addition, CR (as well as exercise) lowers adipocyte size and some negative outcomes such as lipid deposition in visceral and hepatic tissues and insulin resistance linked to large adipocytes (Larson-Meyer et al., 2006).

Final comment

Clearly, significant advances in understanding the biology of CR were gained over the period reviewed. A glimpse at the publications appearing subsequent to this period suggests that next year's review will convey even greater insights.

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