Ageing Research Reviews xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review

Calorie restriction in rodents: Caveats to consider

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ARTICLE INFO

Article history:
Received 7 February 2017
Received in revised form 30 May 2017
Accepted 31 May 2017
Available online xxx

Keywords:
Nutrition
Diet restriction
Protein restriction
Fasting
Low calorie diets
Longevity
Mortality
Lifespan
Healthspan
Chronic disease

ABSTRACT

The calorie restriction paradigm has provided one of the most widely used and most useful tools for investigating mechanisms of aging and longevity. By far, rodent models have been employed most often in these endeavors. Over decades of investigation, claims have been made that the paradigm produces the most robust demonstration that aging is malleable. In the current review of the rodent literature, we present arguments that question the robustness of the paradigm to increase lifespan and healthspan. Specifically, there are several questions to consider as follows: (1) At what age does CR no longer produce benefits? (2) Does CR attenuate cognitive decline? (3) Are there negative effects of CR, including effects on bone health, wound healing, and response to infection? (4) How important is schedule of feeding? (5) How long does CR need to be imposed to be effective? (6) How do genotype and gender influence CR? (7) What role does dietary composition play? Consideration of these questions produce many caveats that should guide future investigations to move the field forward.

Contents

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http://dx.doi.org/10.1016/j.arr.2017.05.008
1568-1637/

1. Introduction

Eight decades have passed since the publication of the paper by McCay et al. (McCay et al., 1935) describing the impressive pro-longevity effects of retarding the growth of rats by restricting food available to them. This paradigm of calorie restriction (CR), also known as diet restriction (DR), has emerged over that period to become one of the most widely used tools of biogerontologists for dissecting biological mechanisms of aging. The appeal of the paradigm is its robustness as evidenced by the wide number of invertebrate and vertebrate species exhibiting prolongevity effects in response to a wide variety of CR regimens. Moreover, its appeal is strengthened because the beneficial effects on lifespan typically also encompass positive effects on healthspan. The latter includes delay in onset and reduction in incidence of many chronic diseases as well attenuation of many age-related functional declines, including mobility and cognition.

In response to the familiar refrain describing the robustness of the CR paradigm that has been the focus of many past reviews, we will couch the current review within a context of denting and tarnishing its reputation by presenting several major caveats that now need to be considered in moving the field forward. We believe that such an approach is timely and certainly necessary. Consistent with our charge in this endeavor, the review will be limited to rodent studies of CR, but the points we raise certainly apply across the wide range of approaches and animal models that use this paradigm. Moreover, the points raised in the review are certainly relevant to considerations of how to apply the CR paradigm to human health.

To this end, we will attempt to summarize what we know and what we do not know regarding CR in rodents, and we will focus primarily on effects of CR on lifespan and healthspan. Thus, a deep dive into mechanisms of CR is not the main objective of this effort. The product will best be viewed within the context of other reviews provided in this Special Issue as well as recent reviews appearing elsewhere that offered critiques of the CR paradigm (Roth and Polotsky, 2012; Sohal and Forster, 2014a).

2. At what age does CR No longer produce benefits?

One of the first caveats to consider regarding the robustness of CR for retarding aging in rodents is the age at which it is imposed. This consideration raises important practical questions regarding the relevance of CR as an intervention in humans. Without going into details regarding this issue, there remains considerable controversy regarding the health benefits of dieting for elderly persons (Porter Starr et al., 2014; Waters et al., 2013). However, even within the context of CR research in rodents, the question remains: Is there an age at which CR loses its effectiveness in terms of significantly increasing lifespan and/or healthspan?

By far, the most applied paradigm in rodent research involves the initiation of CR shortly after weaning but typically post pubescent, ranging from 4 to 12 weeks of age. This paradigm remains the most robust regarding effects of lifespan and healthspan, although later sections of the review will raise mitigating issues such as genotype and type of diet and consider possible negative effects on health and healthspan. The question is at what age does CR begin to lose its anti-aging benefits?

Early in the development of the CR field using rodent models, a critical question was whether the prolongevity effects of CR required retarding development. McCay et al. (McCay et al., 1941) addressed this issue in an early study in which rats ranging in age from 200 to 450 days were subjected to their CR paradigm adjusted to maintain body weight. Because no statistical analysis was conducted in this early study, Ingram and Reynolds (1983) (Ingram and Reynolds, 1983) reanalyzed the data presented in the original article to confirm that increased lifespan in the CR group begun at mature ages. Other early studies corroborated these findings by showing that mature rodents responded nearly as well to CR as young post-weaning animals. For example, Yu et al. reported significant increases in lifespan in Fischer-344 (F344) rats placed on 40% CR at 6 mo of age (Yu et al., 1985). Similarly, Weindruch and Walford reported significant increases in lifespan in two long-lived mouse strains (C57BL/6 and B10C310) when put on a 40% CR regimen at 12 mo of age(Weindruch and Walford, 1982). Pugh et al. confirmed this longevity effect of CR in male C57BL/6 (B6) mice treated at 12 mo of age and also reported significant reductions in cancer incidence in the CR group compared to controls (Pugh et al., 1999b).

Many subsequent rodent studies have confirmed that CR begun up to 12 mo of age promotes significant prolongevity effects. However, even this conclusion must be tempered with considerations of genotype and feeding schedule. For example, Goodrick et al. employed every-other-day (EOD) feeding to impose CR in A/J, C57BL/6J, and B6AF1/J mice at different ages (Goodrick et al., 1990). When initiated at 1–2 months of age, the regimen of intermittent feeding (IF) produced significant increases in lifespan in all three strains. However, when initiated at 6 mo, IF had significant prolongevity effects only in B6 and the hybrid strain. Moreover, when started at 10 mo of age, IF significantly reduced lifespan in A/J mice, and no had significant effects on lifespan in the other two strains. Forster et al. noted similar age x genotype interactions with a regimen of 60% CR (Forster et al., 2003a). When initiated at 4 mo of age, CR increased lifespan in C57BL/6Nnia and B6D2F1/Nnia mice; however, there was no significant lifespan effect in DBA/2Nnia mice. When initiated at 24 mo, CR reduced lifespan in all three strains, with the greatest reduction in DBA mice. Similar negative effects on lifespan were reported by Ross in which CR was initiated in 300 day old Sprague-Dawley (SD) rats (Ross, 1977). Lipman et al. noted no significant lifespan effects when 33% CR was initiated in Long-Evans (LE) rats at 18 mo of age (Lipman et al., 1995). In a more extensive study, Lipman et al. (1998) reported no significant lifespan effects or reductions in tumor burden when 32% CR was introduced to 18- and 26-mo old male F344xBN F1 rats (Lipman et al., 1998).

Thus, at question is whether there are ages in rodents at which CR is no longer effective or even detrimental to lifespan and healthspan? Dhahbi et al. initiated CR (~40%) in 19-mo old male C3B6F1 mice and reported significant increases in lifespan accompanied by reduced tumor rates as well as a global gene transcriptional profile that resembled life-long CR(Dhahbi et al., 2004). Lee et al. also confirmed that CR (40%) initiated in 14-mo old male B6C3F1 mice produced a global gene expression profile that indicated slower aging(Lee et al., 2002). As one caveat, it is possible that mice are more responsive than rats to late-life CR. Additionally, even among mice it is now clear that genotypes respond differently to the same level of CR. For example, in an extensive survey of age-related lesions in mice on 40% CR since early life, Harbison (Harbison et al., 2016) observed that male and female C57BL/6Nnia mice experienced much greater disease reduction in response to CR compared to DBA/6Nnia mice. In p53-deficient mice that have greater susceptibility to spontaneous and inducible tumors, 40% CR as well as a 1-day fast per week significantly reduced tumor burden when initiated at 10 mo of age (Berrigan et al., 2002).

In addition to the positive effects of late-life CR on cancer risk, there are other studies suggesting beneficial effects on many other indices of aging at a molecular level. For example, Goto et al. (summarized in Goto et al.) have published several studies examining the effects of CR (30–40%) induced in late-life over short periods (2–3.5 mo) (Goto et al., 2007). A few illustrative findings are as follows: (1) Half-lives of numerous proteins were increased in mouse hepatocytes taken from 23-mo old animals subjected to 2 mo CR;

Please cite this article in press as: Ingram, D.K., de Cabo, R., Calorie restriction in rodents: Caveats to consider. Ageing Res. Rev. (2017), http://dx.doi.org/10.1016/j.arr.2017.05.008