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Modulation of frailty syndrome by diet: A review of evidence from mouse studies

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ABSTRACT

Frailty is a progressive, aging-related syndrome of unknown etiology characterized by increased susceptibility and decreased resilience to external stressors. Understanding underlying mechanisms and identifying interventions to improve resilience are major challenges in biogerontology. Adequate nutrition is central to organismal health and wellbeing and can be modulated to improve longevity. While there are clear associations between poor nutrition (excess or deficient calorie intake) and frailty in humans, only recently has the link between frailty and nutrition been addressed in experimental model systems in which causality and molecular mechanisms can be explored. Here we review the evidence linking nutrition to the frailty syndrome, including individual aspects such as muscle function, body composition and chronic disease, with a specific focus on mouse models and experimental nutritional interventions including dietary restriction.

1. Introduction

One of the largest shifts in population demographics in the past century is the increase in the number of elderly individuals. By 2060, it is projected that the number of Americans aged 65 years and up will double from 46 million today to over 98 million, rising as a percentage of the total population from 15% to 24% (https://www.prb.org/aging-unitedstates-fact-sheet/). Aging is the number one risk factor for chronic diseases including the frailty syndrome, with an incidence of up to 50% in the 85 + age group (Buckinx et al., 2015). This increase in the elderly population poses a unique set of challenges. One is the increased cost of caring for frail individuals, which is estimated at up to 4 time the cost of non-frail individuals (Bock et al., 2016). Another is the required expansion of services and infrastructure to cope with the predicted increased demand.

Frailty has emerged as an important concept in clinical and experimental gerontology over the last 20 years, yet a consensus 'gold standard' definition remains elusive (Cesari et al., 2017). Generally, frailty is recognized as a state of progressive, multisystem physiological decline leading increased susceptibility and decreased resilience to adverse events, such as falls, resulting in disability. While distinct from any single aging-related disease (e.g. Alzheimer's disease, cancer), frailty is consistent with the definition of an aging-related medical syndrome: a constellation of symptoms and signs that when present in a critical mass predict characteristic outcomes (e.g. short (3 yr.) and intermediate (7 yr.) term mortality (Fried, 2016) and pathophysiology distinct from normal aging (Fried, 2016). Importantly more research is required to determine the relationship between disease process and frailty.

The development of specific tools to assess frailty in populations, such as the deficit accumulation scale from Rockwood and colleagues (Yannakoulia et al., 2017) and the Fried frailty phenotype criteria (Bonnefoy et al., 2015), have greatly advanced our understanding of how different components of ageing and health contribute to frailty status. A number of recent reviews (McArdle et al., 2019; Rockwood and Howlett, 2018; Clegg and Hassan-Smith, 2018; Ferrucci and Fabbri, 2018; Wilson et al., 2017) have also described in detail the associations between deregulation and/or dysfunction of physiological systems contributing to or associated with frailty. These include mitochondrial dysfunction, increased inflammation, altered energy metabolism, and

Abbreviations: CR, calorie restriction; MetR, methionine restriction; EOD, every-other-day feeding/fasting; IF, intermittent fasting; HFD, high fat diet; HFHSD, high fat high sucrose diet; WT, wildtype; KO, knockout; FI, frailty index; IL10$^{-/-}$, interleukin-10 knockout; Sod1KO, superoxide dismutase 1 knockout; AL, *ad libitum*; PFI, physiological frailty index; DR, dietary restriction; EAAs, essential amino acids; PA, physical activity; ADF, alternate day feeding/fasting; FMD, fasting mimicking diet; TRF, time restricted feeding; KD, ketogenic diet; PR, protein restriction; CON, control diet; INT, intermittent; MF, moderate fat; IGF-1, insulin like growth factor 1; FGF-21, fibroblast growth factor 21; CON, control; AL, ad libitum

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declines in muscle function and strength to name a few (Fried, 2016; McArdle et al., 2019; Rockwood and Howlett, 2018; Clegg and Hassan-Smith, 2018; Ferrucci and Fabbri, 2018; Wilson et al., 2017; Lorenzo-López et al., 2017).

Development of therapeutic strategies to address the frailty syndrome will have vast benefits on both individual and socioeconomic levels. Nutrition is a cornerstone of public health, but also a targetable intervention with the potential to modulate frailty risk. For example, protein malnutrition and deficiency of micronutrients including vitamin D, C and omega-3 fatty acids are associated with frailty, whereas healthy dietary patterns such as the Mediterranean diet might be able to prevent or delay frailty onset (Lorenzo-López et al., 2017; Yannakoulia et al., 2017; Bonnefoy et al., 2015; Bach-Faig et al., 2011). Optimal protein intake in ageing is a complicated issue. It is well established that sufficient protein intake is required to prevent sarcopenia (Nowson and O'Connell, 2015), however determining the 'optimal level' requires further investigations, particularly for those older frail adults with renal impairment. In this population, increased dietary protein in older adults with renal dysfunction is detrimental (Martin et al., 2005).

Conversely, overnutrition in the form of a high-fat or westernized diet is associated with increased risk of chronic diseases including frailty (Lorenzo-López et al., 2017; Michelon et al., 2006; Chang, 2017; Bollwein et al., 2013). Importantly, a number of these health outcomes show sexual dimorphism, suggesting that there is no 'one size fits all' solution and demanding further investigation into the complex association between gender, frailty and over/under-nutrition towards the eventual goal of evidence-based personalized recommendations (Regitz-Zagrosek, 2012). Furthermore, dietary approaches in older adults face other hurdles such as decreased taste with age, increased difficulty with eating, as well as social factors such as loneliness, and depression leading to unwillingness to eat (Seshadri et al., 2018). We refer the reader to a number of comprehensive reviews investigating the multifactorial role of nutrition and frailty in humans (Lorenzo-López et al., 2017; Landi et al., 2016; Donini et al., 2003).

Given the complex multifactorial interaction between nutrition and frailty, experimentally tractable rodent models of frailty or individual components of the frailty syndrome such as muscle function, physical performance, age-related hearing loss, and development of cataracts, have been developed. These model systems have been exploited both to reverse translate findings from human association studies, as well as to study proposed interventions with established anti-aging effects such as calorie restriction. In addition to testing causal relationships, such models are also ideally suited to identifying underlying molecular mechanisms in a depth that cannot be done with humans for practical or ethical reasons.

In this review, we focus specifically on those studies that have utilized a dietary intervention to modulate frailty in a mouse model. Inclusion and exclusion criteria for studies included in this review are shown in Table 1. Because this is still a young area of research with limited number of citations, we expanded the criteria to include dietary modulation of individual components of the frailty syndrome which are related to the musculoskeletal and nasal/ocular system as defined in the mouse clinical frailty index (Whitehead et al., 2014) in aged mice. While we acknowledge this is certainly not a comprehensive inclusive list of all categories of the frailty index, we believe this provides a sufficient basis for reviewing the available literature and provides a jumping off point for future research to expand upon the important field of evaluating how nutrition impacts frailty utilising frailty tools in preclinical models.

1.1. Use of mice as models of mammalian aging and the frailty syndrome

Rodents have been used as mammalian models in experimental medicine and biology for over a century. For example, one of the seminal discoveries in aging research linking reduced energy intake to extended longevity was made in rats, and remains the most reproducible way to extend lifespan and delay the onset of ageing/age related diseases in most species tested (Weindruch et al., 1986; McCay et al., 1989). Genetic tractability and smaller size/reduced cost, together with the ability to control environmental factors such as diet, has favoured the use of mice over the past decades. More recently, there has been a push to develop mouse models of the frailty syndrome in order to better understand underlying mechanisms and to test interventional approaches to ameliorate, reverse or even potentially prevent the development of frailty altogether. The development of reliable assays to measure a wide variety of functional endpoints on the organismal level (e.g. food intake, energy metabolism, motor behaviour) and in blood, urine, or tissue on the molecular level have made mouse models invaluable tools in the study of aging and aging-related syndromes such as frailty.

1.1.1. Genetic models frailty

The first mouse model proposed to mimic the human frailty syndrome was the interleukin-10 knockout (IL-$10^{-/-}$) mouse (Walston et al., 2008). Originally developed as a model of colitis, this mouse was soon identified as developing a frail phenotype including increased inflammation, decline in muscle strength, reduced fat mass, altered energy metabolism and elevated interleukin-6 (IL-6) (Walston et al., 2008; Westbrook et al., 2017; Ko et al., 2012). Importantly, as these phenotypes are present in both frail humans as well as in the frail $IL10^{-/-}$ mouse model, this has led to the IL-$10^{-/-}$ mouse being considered the gold standard for genetic models of frailty. However, it is important to note that there are some limitations to the use of this model including: (1) they do not show all of the features of the human frailty syndrome (i.e. no difference in bodyweight between wildtype and knockout mice (Walston et al., 2008)), (2) specific pathogen free housing is required to prevent the development of colitis and (3) some aspects of the human frailty syndrome have not been investigated in this model (i.e. endurance and walking speed) (Kane et al., 2016a). Nevertheless, the $IL10^{-/-}$ KO mouse represents an important model for understanding the frailty syndrome, with the caveat that it is unclear whether the mechanisms of frailty development would be the same as those seen in natural aging.

More recently, a mouse model lacking the antioxidant enzyme Cu/

Table 1
Inclusion and exclusion criteria for studies included in this review.

Inclusion criteria	Exclusion criteria
Use of a frailty tool/criterion in mice	Not specifying a frailty tool or set of criteria
If a validated frailty tool is not used, then inclusion of one of the following criteria used in the mouse clinical frailty index:	Rats, humans or other studies are excluded
(1) musculoskeletal: gripstrength, muscle function, exercise endurance	
(2) ocular/nasal: cataracts	
(3) ocular/nasal: hearing loss	
Must evaluate frailty in aged animals	Assessment of frailty in young animals (aged < 12mo)
Nutritional interventions including calorie restriction, methionine restriction, intermittent fasting or every-other-day feeding, altered micro/macronutrient composition, and high fat diet	Not specifying details of onset/length of the nutritional intervention

Table 2
Summary of studies included in the review stratified by nutritional intervention.

Study population	Intervention/diet	Frailty metric used	Study outcome	Reference
C57BL/6 J and DBA/2 J male and female mice; onset at 4mo age	AL or 40% CR; lifelong intervention	Mouse clinical FI	CR reduced FI in males but not females	(Kane et al., 2016b)
Male and female Ames dwarf mice and wildtypes; onset at 8 weeks of age	AL or 30% CR; lifelong intervention	None; examined features of the frailty syndrome including muscle function and fatigue	CR (and Ames Dwarf genotype) maximized grip strength and reduced fatigability in middle aged and old male mice, but there was no decline with age in females at these measured timepoints	(Arum et al., 2013)
Male and female NIH Swiss mice; onset at 52 weeks of age	AL HFD or rapamycin diet	None; used the PFI composed of 29 variables reflective of physical fitness (body weight and grip strength), cardiovascular system (systolic, diastolic and mean blood pressure, heart rate, tail blood flow and tail blood volume), total blood cell composition (white and red blood cell counts and differentials), plasma concentration of CXCL1/KC, triglycerides and glucose.	HFD significantly increased PFI in male but not in female mice	(Antoch et al., 2017)
Male C57BL/6 mice; onset at 16mo age	Four days of a diet that mimics fasting (FMD) two times per month	None; examined muscle function	Improved Rotarod performance and learning ability in mice fed the FMD when tested at 23mo age	(Brandhorst et al., 2015)
7-week old male CD-1 wild type mice; onset at 7wks age	IF or HFD; duration of 11mo	None; examined muscle function and cognition	Mice on IF had better exercise tolerance IF improved learning and memory assessed	(Li et al., 2013)
Male C57BL/6 J; onset at 4mo age	AL or ADF groups	None; examined muscle function	ADF mice had improved endurance capacity	(Marosi et al., 2018)
Male C57BL/6 J mice	Intermittent (INT) diet, alternating weekly between CR of a control diet and AL moderate-fat (MF) feeding until 24 months of age	None; activity measurements as surrogates	INT diet increased physical activity and improved survival relative to control mice	(Rusli et al., 2017)
Male C57BL/6 mice; onset at 21mo of age	AIN-93-M (control) or a diet low in micronutrients with antioxidant properties (50% of mouse recommended daily intake of vitamins A, E, B6, and B12, folate, selenium, and zinc); duration of 4mo intervention	None; evaluated muscle mass, grip strength, and physical activity as surrogates	Reduced dietary intake of vitamins A, E, B6, and B12, folate, selenium, and zinc resulted in a lower oxidative capacity and has major impact on muscle health as shown by decreased force production and PA, without effects on muscle mass.	(van Dijk et al., 2018)
Male C57/BL6 J mice; onset at 21mo age	AIN-93-M control diet or a diet reduced in vitamin A (retinol), vitamin E (α-tocopherol), selenium and zinc; 4mo intervention	None; activity, muscle strength as surrogates	Deficient diet mice had more muscle fatigue, decreased grip strength and impaired mitochondrial dynamics	(van Dijk et al., 2016)
Male C57BL/6 mice; onset at 6mo age	AL, DR or DR group that was supplemented with EAAs, without methionine; 18mo duration	None; muscle strength as surrogate	DR (and DR + EAAs) improved bodyweight-adjusted grip power and ameliorated age-dependent histological and functional alterations in slow-twitch muscle fibres	(Yoshida et al., 2018)
Male C57BL/6 J mice; onset at 3mo age	Lifelong EOD feeding	None; examined modified SHIRPA, hearing loss and motor function using rotarod and gripstrength	Increased distance and average speed in open field test in EOD mice No prevention in age-related changes in SHIRPA with EOD No effect of EOD on preventing age-related hearing loss Trend towards improvement in rotarod with EOD Small non-significant increase in gripstrength with EOD feeding in old age	(Xie et al., 2017)
64 non-consanguineous swiss-OF1 male mice; onset at 6 weeks age	EOD Feeding; 18 weeks duration	None; examined muscle function	No difference in bodyweight or locomotion between AL and EOD Increased time to exhaustion and running distance on treadmill	(Rodriguez-Bies et al., 2010)
Emory morse mice (does not specify sex); onset at 8-11 weeks of age	40% CR; lifelong	None; examined cataracts	Decreased cataract formation in restricted mice Extended lifespan in restricted mice	(Taylor et al., 1995)
Male C57BL/6 mice; onset at 20 weeks of age	10%, 20%, 30% and 40% CR; duration was 3mo	None; used PA (and core body temperature)	Higher PA in 40% CR at the end of the study, not significant though	(Mitchell et al., 2016b)
Male C57BL/6 mice; onset at 20 weeks of age	20%, 30% and 40% protein restriction (made up by increased carbohydrate); duration was 3mo	None; measured PA (and core body temperature)	Reduced physical activity in 40% PR at the end of the study, not significant	(Mitchell et al., 2016b)
Female CB6F1 mice; onset at 6 weeks of age	CON (0.45% methionine) vs MetR (0.1%*)	None; evaluated cataract formation	Significant reduction in cataract score at 18mo of age with MetR MetR delayed the age of onset of pathologies	(Miller et al., 2005)

(*continued on next page*)

Table 2 (*continued*)

Study population	Intervention/diet	Frailty metric used	Study outcome	Reference
Male C57BL/6 mice; onset at 11 months of age	CON (65% kcal from carb) or KD (89% kcal from fat); duration was lifelong	None; assessed locomotor speed and neuromuscular function	KD increased forelimb gripstrength and resistance to fall from a wire KD mice were also faster in the Locotronic speed test and had increased activity KD increased lifespan	(Roberts et al., 2017b)
C57BL/6 male mice; onset at 11mo of age	CON (10% protein, 13% fat, and 77% carbohydrates); KD (10% protein and 90% fat)	Mouse clinical frailty index and a 35-item composite healthspan score	Trend towards a reduction in FI with KD Significantly improved composite healthspan score and increased total movement in KD mice	(Newman et al., 2017)
Male C57BL/6 mice; onset at 7 weeks of age	CON (13% calories from fat, 65% carbohydrate, 22% Protein) or HFHSD (47% fat (7:1 lard-to-safflower oil ratio), 32% carbohydrate, 21% protein); duration was up to 60 weeks	None; measured bone health, activity, muscle function	HFHSD lowers bone density and strength HFHSD reduces physical activity and quadriceps weight	(Burchfield et al., 2018)
Male C57BL/6 mice; onset at 2mo age	26% CR; duration of 13mo	None; evaluated age-related hearing loss	CR prevented age-related hearing loss	(Someya et al., 2007)
Male C57BL/6 and CBA/N-slc mice; onset at 8 weeks of age	HFD; duration of up to 12mo	None; evaluated age-related hearing loss	12mo of HFD prevented age-related hearing loss in C57BL/6 mice but not in CBA/N-slc mice	(Fujita et al., 2015)

*initially 0.1%, but then increased at 4 months of age to 0.12% and again at 6 months of age to 0.15% to diminish the incidence of rectal prolapse and early death.

Zn superoxide dismutase (Sod1KO) has been proposed as a new genetic model of the frailty syndrome (Deepa et al., 2017). These mice show four of the criteria used to define human frailty, including weight loss, weakness, low physical activity and exhaustion. Moreover, they have increased inflammation and sarcopenia, as well as alterations in a number of pathways proposed in the aetiology of frailty in humans (oxidative stress, mitochondrial dysfunction, and cellular senescence) (Deepa et al., 2017). The Cu/Zn superoxide dismutase system has also been implicated in the aetiology of sarcopenia in motor neurons (Deepa et al., 2019). Deepa et al., (Deepa et al., 2019) show that sarcopenia begins in the motor neurons, where disrupted neuromuscular junctions lead to increased generation of ROS which has a positive feedback on the MNJs leading to more ROS and damage. This perpetuates a viscous cycle that eventually leads to loss of muscle fibres (Deepa et al., 2019) and sarcopenia. Certainly, this provides an important step forward in understanding the pathophysiology of sarcopenia in the context of frailty. Interestingly, despite some phenotypic overlap between these models and the frailty syndrome, there are differences which argue for careful consideration of the most appropriate genetic model of the frailty syndrome. Furthermore, there is no research to date assessing the potential impact of dietary interventions on phenotypes in genetics models of frailty.

1.1.2. Assessment of frailty in naturally aging mice

In addition to genetic models displaying symptoms of the frailty syndrome with high penetrance (Walston et al., 2008; Deepa et al., 2017), there is significant value in assessing frailty in naturally ageing mice. In recent years, a number of tools have emerged which have been proposed to be mouse equivalents to the human frailty tools. The two main tools are the mouse clinical frailty index (FI) (Whitehead et al., 2014) which is based upon the deficit accumulation tools used in humans (Mitnitski et al., 2001; Rockwood et al., 2005), and the mouse frailty phenotype assessment (Liu et al., 2014) based upon the Fried Criteria (Fried et al., 2001). In addition, a number of studies have used the SHIRPA scale, a semi-quantitative three-stage protocol involving an observational assessment, physical assessment as well as assessment of specific biological systems (Brandhorst et al., 2015). Although traditionally this assessment tool has been preferentially used in phenotyping transgenic mice (Brandhorst et al., 2015), it does share some similarities with the mouse clinical FI and phenotype assessment, suggesting that each tool can reliably measure frailty in the mouse. Both the mouse FI and phenotype assessment tools have been validated in several subsequent studies and shown to be highly related to the human scales (Rockwood et al., 2017). Several iterations of these two mouse indices have been developed since the initial description (Antoch et al., 2017; Graber et al., 2013; Kane et al., 2018; Gomez-Cabrera et al., 2017) including the adaptation of these indices for rats (Miller et al., 2017; Yorke et al., 2017). Further information on the development, implementation and application of these preclinical tools are reviewed in depth in this issue and thus beyond the scope of this review. However, we emphasize the value of implementing these tools in ageing studies in order to further our understanding of the aetiology and pathophysiology of the frailty syndrome.

1.2. Calorie restriction (CR)

While in humans there is a large body of research supporting the association between adequate nutrition and the prevention of frailty (Otsuka et al., 2019; Park et al., 2018; Parsons et al., 2019; Veronese et al., 2018), testing the causal relationship between diet and frailty in preclinical models has only been possible recently since the emergence of validated tools. The first study to demonstrate a causal relationship between diet and frailty using an established frailty tool in rodents was published in 2016. Kane and colleagues (Kane et al., 2016b) examined how lifelong reduced calorie intake impacted the mouse clinical frailty index (FI). They found that mice of different strains at the same

chronological age differed in their FI, and that short-lived mice (DBA2/J) of the same chronological age but presumably different biological ages were frailer compared to the standard C57BL/6 mouse. Moreover, CR reduced FI in a sex dependent manner with males having a significant reduction in FI when measured at 18mo of age (14mo on CR), while significance was not reached in females (Kane et al., 2016b). Such sexual dimorphism is common but poorly understood, including the morbidity-mortality paradox in humans in which females have better survival despite worse health than males (Kulminski et al., 2008), or an apparently opposite phenomenon in B6 females on 40% CR showing improved healthspan without lifespan extension (Mitchell et al., 2016a). While this ground-breaking study showed the relative ease with which non-invasive frailty measurements could be implemented into ongoing mouse studies, it also sets the stage for much needed mechanistic and interventional studies, including testing the interesting possibility of FI as a measure of biological rather than chronological age.

Although Kane and colleagues were the first to employ the FI in the assessment of CR on frailty, they were not the first to study the effects of CR on measures of healthspan included in the mouse frailty tools (Table 2). For example, it is well established that CR can prevent or delay the onset of presbycusis, or age-related hearing loss, when the intervention is started at a young age (Willott et al., 1995; Someya et al., 2007; Sweet et al., 1988). However much like Kane et al (Kane et al., 2016b) found, the effect of CR on presbycusis is strain specific, which implies that the mechanism of prevention (or lack thereof) is strain specific (Willott et al., 1995; Someya et al., 2010). Moreover, Arum et al., examined neuromuscular frailty phenotypes in genetically long-lived hypopituitary Ames dwarf and wildtype mice subjected to lifelong ad libitum (AL) or 30% CR at middle age (82 ± 12 weeks old) or old age (128 ± 14 weeks old) (Arum et al., 2013). Although the authors did not explicitly examine frailty *per se*, they reported on features of the frailty syndrome including muscle function and fatigue. Consistent with Kane et al., they found that CR (and the Ames dwarf genotype) maximized grip strength and reduced fatigability in middle-aged and old male mice, but that there was no decline with age in females at these timepoints (Arum et al., 2013). This suggests that the timepoint for decline in female mice either occurs at a different point (i.e. later in life), or that these particular tests are not sensitive enough to detect a decline in function in female mice. It also highlights the need for more in-depth testing of male and female wildtype mice to determine a reference range, as well as how anti-aging interventions affect frailty. While this was recently done in a cross-sectional manner (Fischer et al. (2016)), longitudinal measures using the FI could provide additional insight into the trajectories of individual animals over time.

1.2.1. Alternative calorie restriction regimens

A major limitation to translating CR to humans is that reducing daily food intake for extended time periods is an insurmountable hurdle for most people, making long-term applications clinically irrelevant. Nonetheless, there are a number of different feeding paradigms in rodents that increase lifespan and/or healthspan without enforced daily food restriction. Such regimens include intermittent feeding/fasting cycles (IF), such as every-other-day fasting/feeding (EOD), or the use of intermittent cycles of a fasting mimicking diet (FMD) interspersed with ad libitum food intake. While no study has explicitly examined the effects of such intermittent regimens on frailty using the newly established mouse frailty tools, it is encouraging to see that these regimens can improve aspects of the frailty syndrome and other chronic diseases which contribute to increased risk of frailty (Brandhorst et al., 2015; Wei et al., 2018; Li et al., 2013; Anson et al., 2003; Marosi et al., 2018; Rusli et al., 2017). However, there is not a universal improvement in the frailty categories that contribute to the overall score, as it has been demonstrated that EOD feeding does not slow age-related hearing loss in C57BL/6 J male mice when started at 2mo of age (Xie et al., 2017), while CR does (Willott et al., 1995; Someya et al., 2007; Sweet et al., 1988). This points to important differences in the mechanisms by which different nutritional interventions impact frailty and argues for increased studies into these areas. Table 2 describes how alternative restriction paradigms impact frailty outcomes in mice using established criteria, or surrogates such as activity, muscle function/strength, hearing loss and cataract formation.

1.2.2. Altered protein/amino acid composition

In addition to daily or intermittent calorie restriction regimens, there are experimental dietary interventions that improve lifespan and/or healthspan in rodent models by lowering protein to carbohydrate ratios or restricting particular essential amino acids (Miller et al., 2005; Solon-Biet et al., 2014). Although poorly characterized in humans, such diets hold promise because they can be eaten on an ad libitum basis without any enforcement of total calorie restriction.

More than 20 years ago, Orentreich and colleagues reported that a reduction in the concentration of a single dietary essential amino acid, methionine (from 0.86% to 0.17% w/w), resulted in a 30% longer lifespan of male Fisher-344 rats (Orentreich et al., 1993). Methionine restriction (MetR) has many overlapping benefits with CR including reductions in bodyweight, fat mass and oxidative stress coupled with improvements in insulin sensitivity as well as changes in many circulating hormones such as leptin, adiponectin, insulin like growth factor 1 (IGF-1) and fibroblast growth factor 21 (FGF-21) (Dong et al., 2018; Ables and Johnson, 2017). Although not specifically examined in the context of a validated frailty tool, studies have shown that MetR results in improvements in the domains that are important in frailty, such cataract formation and lens aging (Miller et al., 2005), muscle function (Yoshida et al., 2018) and chronic diseases (Liu et al., 2017; Sinha et al., 2014) which lead to increased survival (Miller et al., 2005). Unfortunately, it remains unknown if lifelong MetR reduces frailty to the same extent as 14mo of CR in male mice (Kane et al., 2016b), or if either approach can improve FI within shorter time periods, or potentially even reverse frailty with late-life application.

Although the *ad libitum* nature of methionine restriction regimens is a potential improvement over daily CR, the synthetic nature of current experimental MetR diets is still a formidable roadblock to translation to humans. With this in mind, a recent series of studies found that particular dietary compositions consisting of low protein and high carbohydrates within the overall geometric framework of potential protein:carbohydrate:fat ratios significantly improved insulin sensitivity, cardio-metabolic health, memory and cognition, obesity and immune function, ecology of the gut microbiota and longevity (Solon-Biet et al., 2014, 2016; Solon-Biet et al., 2015a, b; Wahl et al., 2018). While the authors unfortunately did not measure frailty directly, their data certainly point to improvements in multiple domains expected to result in less frailty and improved health outcomes. Future studies should investigate this further using a validated frailty tool.

1.3. Micronutrient deficiency/deficiencies

Inadequate intake of micronutrients has been associated with a higher risk of frailty in older adults. While much research has focused on vitamin D deficiency, other micronutrients have also been postulated to play a role in the aetiology of reduced physical activity and impaired muscle strength with advanced age (Polly and Tan, 2014).

To investigate how deficiency in micronutrients with antioxidant properties might impair muscle strength with age, 21-month male mice were fed with either AIN93 G or a diet with a 50% reduction in the mouse daily recommended intake of vitamins A, E, B6, and B12, folate, selenium, and zinc. After 4 months, mice on the micronutrient deficient diet showed a reduction in bodyweight without a change in food intake. Muscle mass was also not changed but plantaris cross sectional fibre area was reduced and the force production and fatigue resistance were significantly reduced in the extensor digitorum longus (EDL) muscle (van Dijk et al., 2018). In a different study, re-supplementation after 4

months of a diet low in vitamins A/E, selenium and zinc with the missing micronutrients reversed muscle-related phenotypes, as did supplementation of leucine-enriched whey protein (van Dijk et al., 2016). However, caution is urged when directly translating this to humans due to the inherent risks of a high protein diet in an older population with higher incidence of renal failure (Martin et al., 2005) than what is seen in mice.

Taken together, limited data on age- and frailty-associated traits in mouse models suggest that micronutrient deficiencies can exacerbate frailty, and that these can be potentially reversed by dietary supplementation.

1.4. High fat diet (HFD)

Classically, the consumption of a high fat diet (HFD) or western diet leads to obesity and metabolic syndrome (Schafer et al., 2016). In mice, a HFD increases inflammation and reduces lifespan (Mitchell et al., 2014), yet interestingly can protect against age-related hearing loss in male C57BL/6 J mice (Fujita et al., 2015). Restricting feeding of an HFD to a proportion of the day (time restricted feeding, TRF), or in reduced amounts (i.e. HFD in a CR regimen or on-off periods of HFD feeding), can overcome these detrimental metabolic consequences (Smith et al., 2018; Chaix et al., 2018; Hatori et al., 2012). Moreover, TRF can ameliorate the HFD-induced decline in motor function and muscle strength in male mice (Chaix et al., 2018), however these outcomes remain to be determined in female mice. Similar to HFD, ketogenic diets (KD) which are primarily fat with a small percentage of protein (no carbohydrates) show improved longevity and healthspan parameters (muscle function, memory) in male mice (Newman et al., 2017; Roberts et al., 2017a). In the one study which compared KD against control diet in aged male mice, they noted a trend ($p = 0.07$) towards a reduction in FI with the KD (Newman et al., 2017). Certainly, these results are very promising, however further testing in female mice and different strains is required using validated frailty tools in order to fully understand how these diets can modulate frailty and components of the frailty syndrome.

1.5. Future directions

Nutrient/energy intake plays a profound role in health and is a potentially modifiable risk factors for many chronic diseases in both mice and humans. Understanding how nutrient/energy intake can modulate the frailty syndrome in preclinical models represents an important but understudied area of research. In recent years the development of validated frailty tools for use in preclinical models have allowed us to advance further our understanding of the underlying mechanisms of frailty with the hope of developing interventions to halt or reverse the frailty processes. Calorie restriction without malnutrition remains the best-established intervention to improve health and lifespan, and has now been demonstrated in both in mouse models (Kane et al., 2016b) and non-human primates (Yamada et al., 2018) to improve frailty. While this represents an important step forward in understanding how nutrition can increase healthspan and decrease frailty, whether all such pro-longevity diets also reduce frailty, how calories and/or nutrients regulate frailty-related processes on a molecular level, whether such interventions work late in life to prevent or reverse frailty and whether frailty can be used as a marker of biological age, all remain to be rigorously established in preclinical models. We further emphasize the importance of including both males and females within studies, as well as looking at diverse genetic backgrounds as biological outcomes differ by both of these variables.

2. Conclusions

Nutrient/energy intake plays a crucial role in maintaining organismal homeostasis, with both over- and under-nutrition being detrimental to the health and wellbeing. These statements are supported by a large body of human nutrition studies. Frailty is a well-established syndrome of multifunction decline, which can be modulated by nutrition. However, there is limited research in preclinical models investigating how nutrient/energy intake can be used to modulate the frailty syndrome. The development of preclinical tools to assess frailty in mice, rats, and now non-human primates provides exciting opportunities to harness the power of preclinical models to better understand the interaction between nutrient/energy intake and frailty and elucidate underlying molecular mechanisms.

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