

Effects of low level and severe dietary restriction on age-related sarcopenia

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Abstract

Aging is associated with a progressive loss of muscle mass and strength, a condition also known as sarcopenia of aging. Progressive muscle wasting has been observed in rodents, non human primates and humans. Caloric restriction is a very robust anti-aging intervention, whose effect may depend on level and duration. A severe caloric restriction was said to attenuate the loss of muscle mass and function with age. We have evaluated the effects of age and mild (one day of fasting every week) or severe caloric restriction (3 days fasting every week) on the weight of different muscles (extensor digitorum longus, soleus, tibialis anterior and diaphragm) of male rats. Our data show that sarcopenia of aging does not necessarily occur in all muscles (diaphragm appears to be immune) and that only a severe caloric restriction counteracts sarcopenia of aging. Functioning of diaphragm is related to energy consumption and may not decrease significantly per unit of weight with age. Thus our findings are in line with the hypothesis that both severe dietary anti-aging intervention and uninterrupted exercise in the physiological range, may counteract sarcopenia of aging.

Key Words: aging, sarcopenia, caloric restriction

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Aging is characterized by deteriorative changes with time during postmaturational life and by a progressive inability to withstand stresses which make the organism vulnerable to disease and increase the risk of death [21]. An age-related loss of muscle mass and function occurs in the skeletal muscle of a variety of species. This process is referred to as sarcopenia of aging and is reflected by a 25-35% decrease in the cross-sectional area of several limb muscles due to atrophy and loss of muscle fiber [20]. From a histological perspective, sarcopenia in humans is characterized by an early decrease in fiber size and number, with a preferential loss of type II (fast twitch, glycolytic) muscle fibers [17]. The general mechanisms of this age-associated hypoplasia and atrophy are not clearly defined, and multiple components such as neuronal and hormonal changes, inadequate nutrition, low-grade chronic inflammation, and physical inactivity are among the sources believed to contribute [8, 26, 31]. At the subcellular and the molecular level events responsible for sarcopenia are unknown, mitochondrial DNA mutation, alteration of cellular and systemic redox status and altered apoptotic signaling might be key factors driving the onset and progression of muscle loss [6, 25].

Caloric restriction (CR) is the most effective experimental intervention known to consistently slow the aging process and extending median and maximum life span with positive effects on health span in different organisms, from invertebrates to mammals [22]. Observational studies suggest that CR may have beneficial effects also on human longevity [11, 16]. Studies on non-human primates were started and although it will take several more years to obtain evidence of life span alterations, some changes in physiological profiles similar to those seen in rodents have been reported [15, 29]. These preliminary observations suggest that CR will have beneficial effects on morbidity and mortality [23]. There is evidence that severe CR has multiple beneficial effects on skeletal muscle: CR attenuates decline in function, slows the loss of muscle mass and prevents loss in fiber with age [9, 27, 28]. It was said that these protective effects probably stem from the ability of CR to reduce mitochondrial abnormalities, oxidative stress, and the age-related elevation of proapoptotic signaling in skeletal muscle [7, 9, 18, 28, 33]. Since duration and level of CR have an important influence on anti-aging effects, in the present study we have evaluated the effects of milder and more severe CR regimens on

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Table 1. Body weight and food consumption at various time intervals on study for male Sprague Dawley rats fed *ad libitum* (AL), mild caloric restriction (FW) and severe caloric restriction (EOD).

	AL	FW	EOD
Body weight (g)^{a,b,c}			
3 months	429 ± 17.0 ^d	429 ± 17.0 ^d	429 ± 17.0 ^d
6 months	529 ± 37.4 ^{ew}	514 ± 37.0 ^{ew}	451 ± 32.3 ^{dey}
12 months	627 ± 48.4 ^{fw}	599 ± 48.2 ^{fy}	497 ± 42.5 ^{fz}
24 months	633 ± 58.4 ^{fw}	621 ± 49.6 ^{fw}	508 ± 58.4 ^{fy}
29 months	581 ± 43.6 ^{efw}	547 ± 13.5 ^{efgwy}	480 ± 90.0 ^{fy}
36 months	-	-	336 ± 71.6 ^g
Food intake (g/day)^{a,b,c}			
3 months	21.8 ± 0.24 ^d	21.8 ± 0.24 ^d	21.8 ± 0.24 ^d
6 months	21.6 ± 0.73 ^{dw}	20.9 ± 1.28 ^{dw}	16.8 ± 0.30 ^{efy}
12 months	23.6 ± 0.54 ^{dw}	21.4 ± 0.84 ^{dy}	17.6 ± 0.48 ^{fyz}
24 months	22.3 ± 1.18 ^{dw}	19.4 ± 0.49 ^{dey}	16.5 ± 0.94 ^{efz}
29 months	22.4 ± 0.33 ^{dw}	18.5 ± 0.54 ^{ey}	15.9 ± 0.25 ^{efz}
36 months	-	-	15.3 ± 1.71 ^e

Values represent the mean ± SD; ^a significant age effect, $P < 0.01$; ^b significant diet effect, $P < 0.01$; ^c significant age by diet effect, $P < 0.01$. ^{defg} Means in the same column across age groups with different superscripts are significantly different ($P < 0.05$). ^{wyz} Means in the same row across diet groups with different superscripts are significantly different ($P < 0.05$).

different types of muscles (extensor digitorum longus, soleus, tibialis anterior and diaphragm) of male rats in order to evaluate the effectiveness of nutritional interventions designed to counteract the sarcopenia of aging.

Materials and Methods

Animals and diets

The original founder stocks of the Sprague Dawley rats were obtained from Harlan Italy (S. Pietro al Natisone, Udine, Italy) and the breeding colony established from these animals has been maintained for 7 years in a conventional environment at the Center for Gerontological Research of the University of Pisa. The average body weight of rats in this colony has remained constant. The male rats that were used in this study were maintained at 20-22°C, and conditioned to a 12h light/12h dark cycle with lights on from 6:00 to 18:00h daily. An *ad libitum* (AL) feeding regimen was used for all animals from the time they were weaned (at 3 weeks of age) until the time they entered the experimental protocol. The animals were housed in standard rat cages, and received Teklad diet (Harlan Italy) and water *ad libitum*. At 3 months of age, the test animals were separated into three groups: a control group that continued to receive food AL, and two CR groups which were fed either AL 6 days and fasted one day every week (FW) or fed AL every-other-day (EOD). 25 animals of each group (AL, FW, EOD) were included in the following survival analysis that lasted until the age of the tenth percentile survivors.

Other groups of animals remained in their respective nutritional groups until the age of sacrifice. On the day of *ad libitum* feeding, rats were offered more food than they could consume in 24 h. Weight of withdrawn food was recorded and food consumption was computed by the difference. Food was withdrawn 16 h before sacrifice. Rats on the EOD restricted diet were sacrificed on the day of fasting. Rats were anaesthetized by the intraperitoneal injection of pentobarbital (50 mg/kg body weight), the extensor digitorum longus (ELD), soleus, tibialis anterior and diaphragm muscles were removed and weighted. The Official Italian Regulation No. 116/92 for the care and use of laboratory animals was followed.

Statistical analysis

One-way or two-way ANOVA, and Tukey's test for post hoc analysis were used. P values of 0.01-0.05 were considered as significant.

The effects of CR regimens on survival were estimated with the pair-wise log-rank statistics to test the null hypothesis of equality in overall survival among groups.

Results

Effect of caloric restriction on life span

The differences between the survival curves for AL, FW and EOD rats were analyzed by the pair-wise comparisons performed using long-rank statistics. As shown in Fig. 1, survival was significantly increased by a severe dietary restriction only (EOD group). A

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beneficial effect of the milder diet (FW group) was visible until 24 months of age and disappeared thereafter.

Body weight and food consumption

Body weight data and food consumption at all age groups for AL, FW and EOD rats are reported in Table 1. There was a decrease in body weight and food intake at a given age group as the level of CR increased. In addition to a significant overall age and CR effect among all of the individual age and CR groups, there was also an age and CR interaction both in body weight and in food intake.

Effect of age and CR on different muscles

Effect of age and mild and severe caloric restriction on ELD, soleus, tibialis anterior and diaphragm are shown in Fig. 2. There was a significant decrease in muscle/body weight ratio on limb muscles with increasing age. This effect was counteracted by a severe caloric restriction, no significant difference was found between AL and FW rats, with the possible exemption of the soleus of AL and FW rats by age 29 months.

No effect of age and diets was observed on diaphragm.

Discussion

In humans, age-related muscle loss is thought to be a multi-factorial process composed of events such as physical activity, nutritional intake, oxidative stress, inflammatory insults and hormonal changes [1, 3, 30]. Mechanisms of rodent muscle loss are said to be similar to those in humans [5]. Use of animal studies eliminates secondary effects of disease caused by old age and gives strength to investigation on anti-aging intervention. In this perspective, the present study was designed to evaluate the effects of a severe and a milder (and more tolerable) CR (one day fasting every week) on the age-related muscle loss in a rodent model. Effects of a different very mild protocol (8% daily caloric restriction) were reported very recently [14]. As regards to survival, best results were obtained with severe CR as previously reported [10, 22]. The milder restriction (a day of fasting a week) showed only a tendency to extend median survival and had no effect on maximum lifespan. On the other hand, other types of mild restriction (a reduction of daily food consumption) were shown to delay the age of onset and the progression of most age-associated diseases [21, 22].

Our findings show that the severe every other day CR delays muscle mass loss with age in limb muscle whereas the milder CR has no significant effect (with the possible exception of the soleus). Several studies have consistently shown that a severe reduction of food intake significantly attenuates the rates of functional

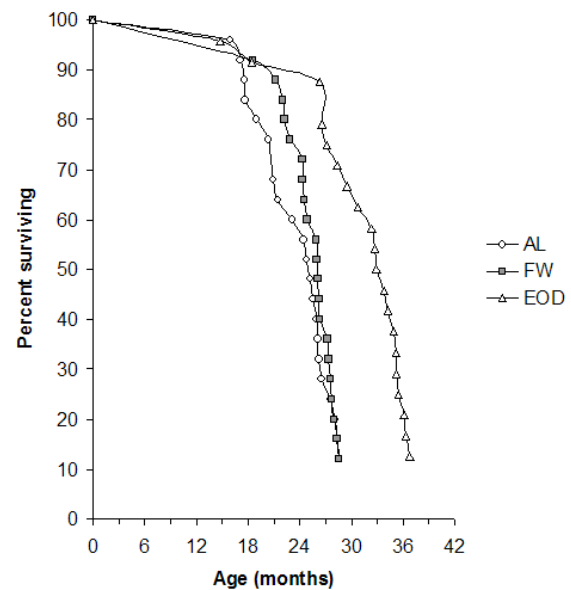


Fig. 1 Survival curves for male Sprague-Dawley rats fed ad libitum (AL), mild caloric restriction (FW) and severe caloric restriction (EOD). Curves are generated from 25 rats for each group. Results of pair-wise comparisons, testing homogeneity of the survival curves among different groups, performed using log-rank statistics: AL vs FW $P=0.56$; AL vs EOD $P=0.0001$; FW vs EOD $P=0.0001$.

decline and loss of muscle fiber number with age [7, 9, 27, 28]. Recently, Kim et al. have reported that lifelong 8% caloric restriction had no effect on the plantaris mass/body mass ratio if not combined with wheel running of old rats [5]. So far, little is known about the mechanisms involved in muscle mass loss with age and potential treatments for sarcopenia, but environmental influence and epigenetic modifications represent important modulators in this process. It is customary to state that the protective effects of CR stem from its ability to reduce oxidative stress without affecting the metabolic rate per unit of lean body mass [24], and to enhance cell repair mechanisms at the molecular [12, 19] and cellular levels [2, 4] and that the tight coupling between nutrition and cell repair may be the Achilles' heel of well fed laboratory rodents as well as humans living in affluent western societies, who are prone to accelerated ageing and age-associated diseases. However, in regard to muscle maintenance, it may be worthwhile to mention that rats on a caloric restricted diet continue voluntary wheel running until senescence [32] and that the function of diaphragm, that seems to abstain from aging-associated sarcopenia, may not change significantly activity throughout life.

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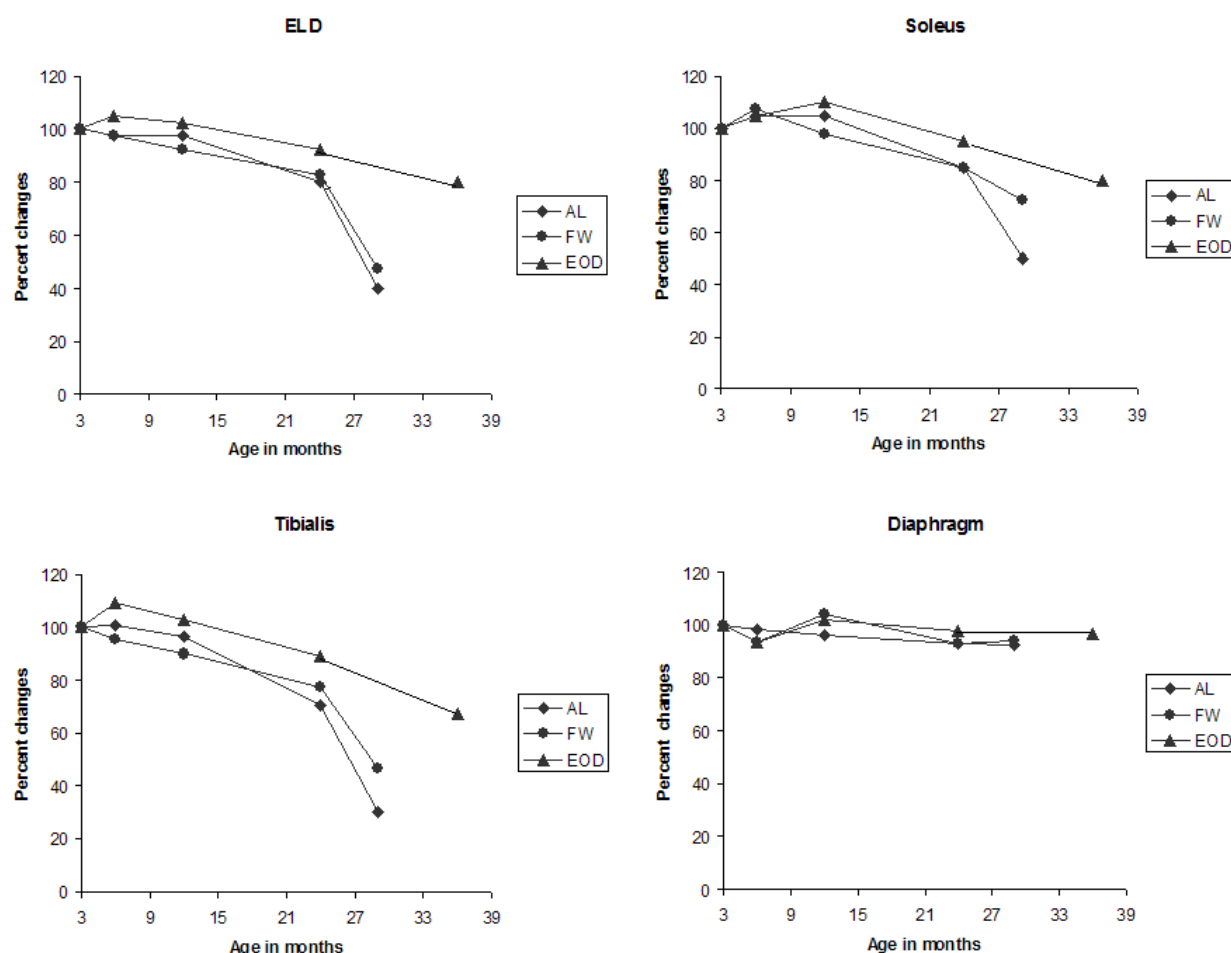


Fig. 2 Effect of aging and mild or severe caloric restriction on different muscles: extensor digitorum longus (ELD), soleus, tibialis anterior and diaphragm of male Sprague-Dawley rats. Data are given as percent changes of muscle/ body weight ratio. ELD, soleus and tibialis anterior: Results of two-way (age by dietary regimen) ANOVA: age main effect, $P < 0.01$; Tukey Test: 6 months vs. 24, 29 and 36 months; 12 months vs 24, 29 and 36 months; 24 months vs 29 and 36 months ($P_s < 0.05$). Dietary regimen main effect, $P < 0.01$; Tukey Test: EOD vs AL and FW ($P_s < 0.05$). Interaction age by dietary regimen was significant, $P < 0.01$. Diaphragm: Results of two-way (age by dietary regimen) ANOVA: age main effect, NS; dietary regimen main effect, NS; interaction age by dietary regimen, NS. AL: ad libitum. FW: mild caloric restriction. EOD: severe caloric restriction.

Perhaps, lifelong exercise might be the best intervention against age-related muscle loss until to very old age [13].

Abbreviations

CR, caloric restriction; AL, *ad libitum*; FW, fasted 1 day every week; EOD, every other day; ELD, extensor digitorum longus

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