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Rapid communication

Long-term moderate caloric restriction and social isolation synergize to induce anorexia-like behavior in rats

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ABSTRACT

Moderate caloric restriction (CR) is an effective strategy to delay the onset of chronic disease states. Conversely, social isolation (SI) carries an increased risk of morbidity and mortality from several causes. The present studies were designed to investigate the long-term effect of the two combined exposures. Two-month-old male rats of the Fischer 344 strain were fed either ad libitum or under a regimen of CR, and each of the two animal sets were housed either in group or isolation. Food consumption and animal growth curves were as expected during the first 6 wk of observation. However, starting at 2 mo and continuing until the fifth month of follow up, rats exposed to both CR and SI showed signs of altered feeding behavior and were unable to complete their (already restricted) meal. Furthermore, altered behavior was accompanied by a corresponding decrease in growth rate until no further increase in body weight was observed. Restoration of group-housing conditions led to a reversal of this phenotype. We conclude that chronic moderate CR and SI synergize to induce anorexia-like behavior, representing a simple and reproducible model to study such an eating disorder.

Introduction

Dietary habits represent major determinants of human health and disease. Within this context, experimental observations spanning several decades have indicated that caloric restriction ([CR]; i.e., reduction of caloric intake without causing malnutrition) is an effective means to extend lifespan and delay the onset of phenotypic alterations that are typical of old age, leading to a decreased incidence of age-related morbidities [1–3]. For example, recent work from our research group has highlighted the protective role of CR on the emergence of the neoplastic-prone tissue landscape associated with aging (i.e., long-term CR is able to delay the occurrence of peculiar changes in the aged tissue microenvironment favoring the selective growth of [pre]-neoplastic cells) [4].

On the other hand, a lifestyle characterized by social isolation (SI) and/or a lack of social interaction has been linked to accelerated aging, and carries an increased risk of morbidity and mortality from several causes, including cardiovascular diseases [5]. This higher risk was reported to be comparable, in magnitude, with that associated with smoking or hypertension (i.e., major risk factors for human diseases) [6]. Furthermore, the prevalence of SI has attained epidemic levels in Western countries and become a very serious public health concern to the point that the first Minister for Loneliness was appointed in 2018 in the United Kingdom [7].

As part of our research efforts aimed at characterizing the links between aging and cancer, we planned a series of long-term studies to investigate possible interactions between CR and SI, with the basic rationale that CR could counter, at least in part, the detrimental effects of SI on morbidity and mortality. During these studies, CR was noted to synergize with SI and cause a significant reduction in food intake and growth rate in exposed rats.

Methods

Animals (male rats of the Fischer 344 strain) were maintained on an alternating 12 h light/dark cycle in a temperature- and humidity-controlled environment, with water available ad libitum (AL). The rates were fed a standard rodent laboratory chow diet (Mucedola, Settimo Milanese, Italy, #4 RF21). The studies were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Cagliari, Italy. Eight-week-old male rats were randomly divided into 4 groups of 8 animals each. Two sets were fed AL and housed either in pairs (AL-P) or in isolation (AL-I), while two other groups were fed a CR diet (70% of the amount consumed by their respective AL controls) and again housed in pairs (CR-P) or in isolation (CR-I). Body weight was monitored weekly, and food consumption per cage was measured daily. No differences in growth curves between the pairs were evident throughout the experiment. All animals were euthanized at the

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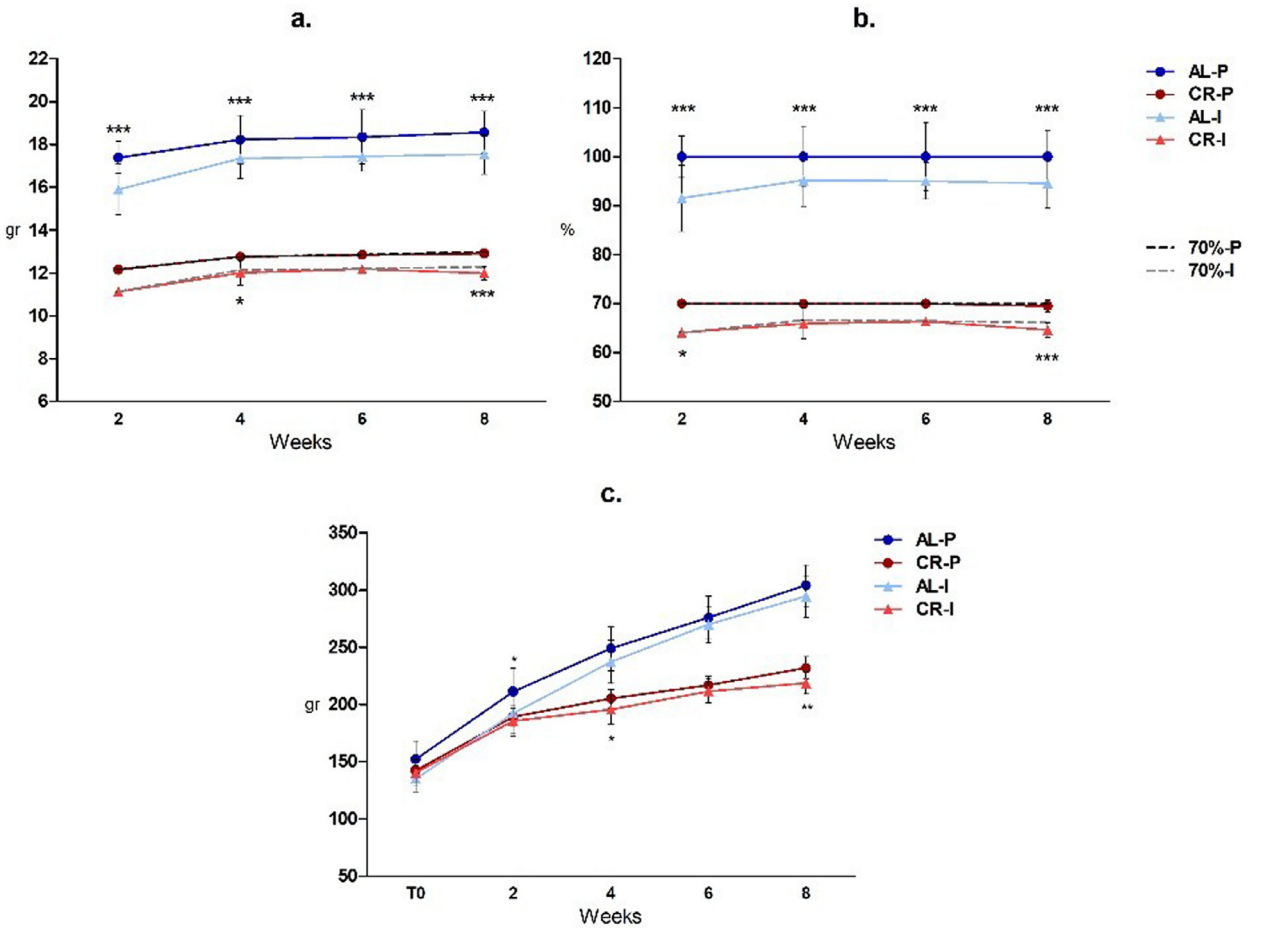

Fig. 1. Food intake and growth curves during the first 2 mo of follow up, with (A) absolute amounts of food intake (g), and (B) relative levels (%) compared with rats fed ad libitum (AL) and housed in pairs. Dotted lines represent the daily food ration delivered to rats fed a caloric-restriction (CR) diet and housed in pairs (black) and those fed a CR diet in isolation (gray). (C) Growth curves. Significantly different from their respective control (AL in isolation vs AL in pairs, and CR in isolation vs CR in pairs). $^{*}P < .05$, $^{\dagger}P < .01$, $^{\S}P < .001$. Other experimental details are given in the text.

end of 6 mo. The statistical analysis was performed using the 2-tailed Student *t* test or 1-way analysis of variance, with Dunnet's multiple comparison posttest (Graph Pad, La Jolla, CA).

Results

During the first 2 mo of follow up, food consumption and growth rate in the different experimental groups were largely as expected (Fig. 1). AL-I rats ate approximately 5% to 7% less compared with their AL-P controls, which is in agreement with previous findings [8]. On the other hand, both the CR-P and CR-I groups finished their respective food ration (70% of their respective AL controls) within 24 h (Fig. 1A, B), and generally completed their meal by 8 h after delivery, as commonly observed in rats with a CR regimen [9].

Growth curves mirrored patterns of food intake. A slight delay in body weight gain was evident in isolated groups compared with their respective pair-housed controls. Starting from the 3rd mo and even more during the fourth and fifth months of follow up, an important change in eating behavior was observed in rats from the CR-I group. Although given only 70% of the food ration consumed by their AL-I counterparts, the rats were unable to completely eat their meal within 24 h after delivery (Fig. 2A, B). Residual food was in the range of 10% to 20% and reached levels as high as one-third of the daily delivered amount.

Growth curves in these animals were similarly affected, with a widening gap in body weight gain compared with CR-P group (Fig. 2C). During the fifth month, there was almost no gain in body weight and a tendency to lose weight was apparent in the last 2 wk (Fig. 2C). In contrast, animals in other experimental groups, including those under the AL-I regimen, maintained their regular eating habits and growth curves throughout the study. Furthermore, the ratio between body weight and food intake was not significantly different between the CR-P and CR-I groups or between the AL-I and CR-I groups (data not reported).

In light of the latter findings, we tested whether the chronic, anorexic-like phenotype developed by animals exposed to the combined CR-I regimen could be reversed. To this end, the CR-I group was divided into 2 subgroups. One group continued on the same treatment, and the other was returned to group-housing conditions. The results obtained 4 wk later are presented in Figure 3. Rats maintained on the CR-I regimen continued to eat only part of their assigned meal, but those shifted to the CR-P protocol immediately returned to a normal feeding behavior (i.e., consumed their entire daily food ration). Moreover, the rats completed their meal within 8 h after delivery.

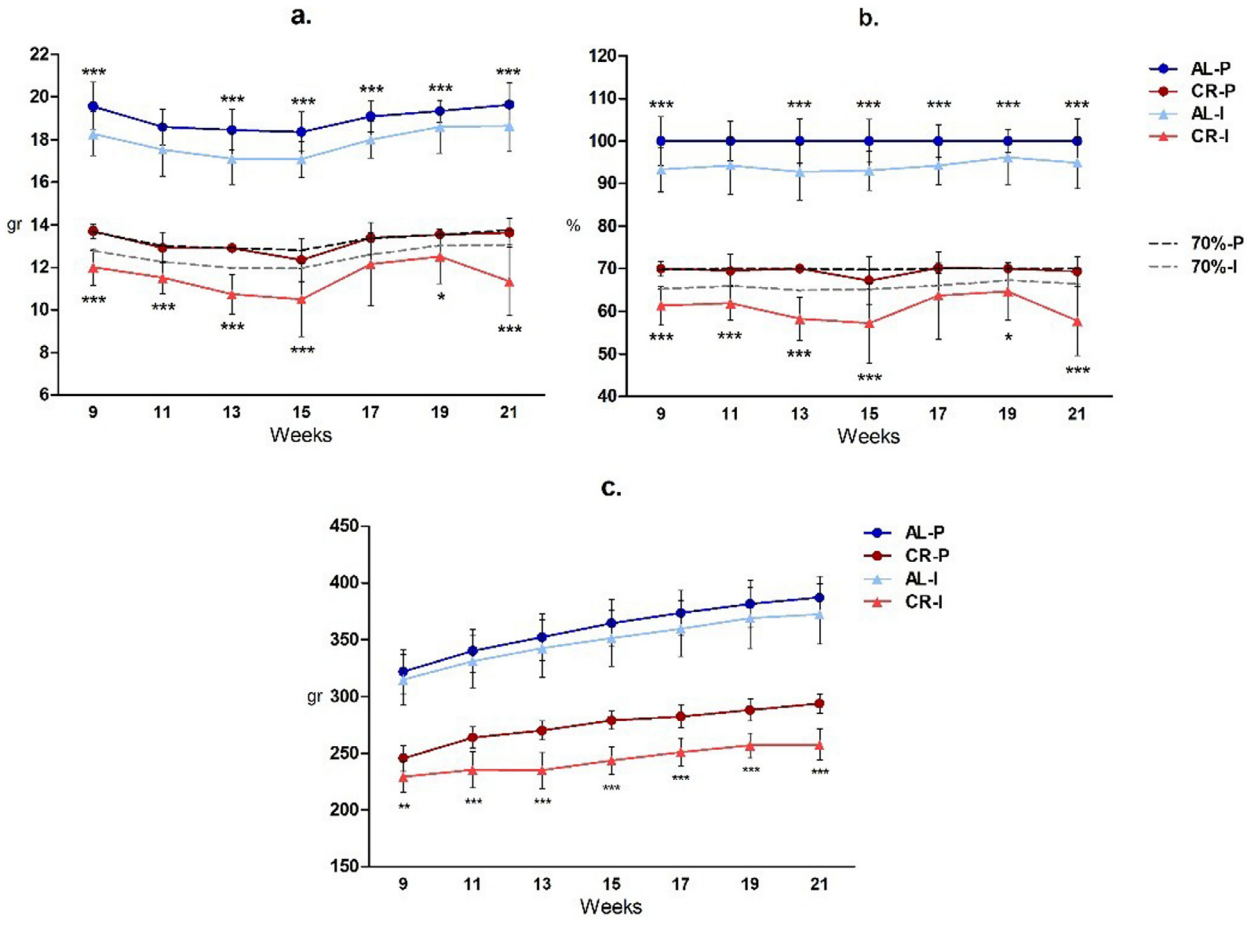

Fig. 2. Food intake and growth curves during months 3 to 5 of follow up, with (A) absolute amounts of food intake reported (g), and (B) relative levels (%) compared with rats fed ad libitum (AL) and housed in pairs. Dotted lines represent the daily food ration delivered to rats fed a caloric-restriction (CR) diet and housed in pairs (black) and those fed a CR diet in isolation (grey). (C) Growth curves. Significantly different from their respective control (AL in isolation vs AL in pairs, and CR in isolation vs CR in pairs). $^{*}P <$ .05, $^{\dagger}P <$ .01, $^{\S}P <$ .001. Other experimental details are given in the text.

Discussion

These results provide a clear indication that long-term exposure to a dietary regimen of reduced caloric intake coupled with SI can alter eating behavior in rats toward the acquisition of an anorexic phenotype. The emergence is not abrupt, suggesting that this phenotype results from the chronic, cumulative effect(s) of the combined treatments. In fact, during the first 2 to 3 mo of follow

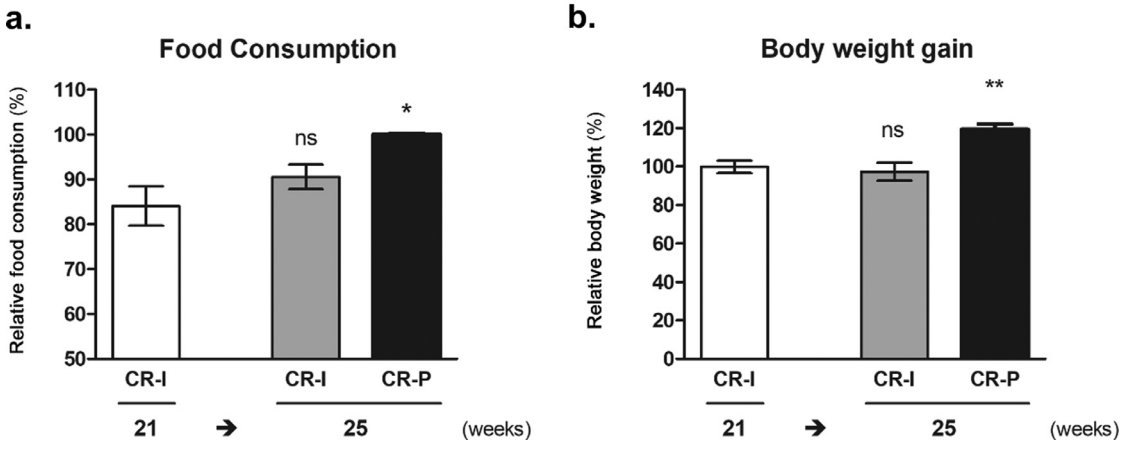

Fig. 3. Relative increase in (A) food intake and (B) body weight in rats exposed for 1 mo to the caloric-restriction (CR) regimen and housed in pairs after 5 mo of CR diet in isolation. A group maintained on the CR diet in isolation is shown as a control. A 1-way analysis of variance with Dunnet's multiple comparison posttest was applied. Significantly different from control. $^{*}P <$0.05, $^{\dagger}P <$ 0.01. Other experimental details are given in the text. No error bar is discernible in the column for rats fed the CR diet and housed in pairs (A) because individual values were very close (range, 98%–100%).

up, food consumption was largely as expected in all experimental groups. Rats housed in isolation and allowed free access to food throughout the day (AL-I) ate slightly less than their corresponding controls housed in group (AL-P). This result is in line with those from previous investigations [8], although some studies have reported opposite findings and an age-dependent effect [10]. On the other hand, both groups on the CR protocol (CR-P and CR-I) consumed their respective food ration within 8 h after delivery, as usually observed under such dietary regimen [9,11]. The residual food that was occasionally present in the cages of CR-I animals had been completely eaten by 24 h. Growth curves were consistent with this trend. Marked differences were present between the AL and CR groups, as expected. In addition, a slight delay in growth rate was seen in the rats housed in isolation compared with the respective group-housed controls, mirroring the minor differences in food intake previously mentioned.

Starting from the 3rd mo and, more prominently, during the 4th and 5th mo of follow up, an important alteration in the eating behavior of the CR-I group was noted. The rats were no longer able to consume their daily food ration within 8 h, as observed in the first 2 mo. Moreover, a sizeable amount of residual food (ranging from 10% to 20%) was still present at the end of 24 h when the next ration was delivered. Growth curves reflected such a decrease in food intake, as a significant gap in body weights (~15%) was observed between the CR-I and CR-P groups. This pattern persisted, with minor fluctuations, until the end of the 5th mo, prompting a discontinuation of the planned long-term study.

Conclusions

These results describe an experimental model that consistently induces a long-term, altered eating behavior mimicking an anorexic phenotype. Importantly, this is observed in animals with a normal genetic background. Numerous reports have suggested possible links between CR and/or SI on one side and eating disorders, including anorexia, on the other [12,13]. For example, dieting in adolescents increases the risk of obesity, binge eating, and extreme weight-control behavior years later [14]. Furthermore, experimental studies have documented the synergism of an acute exposure (10 d) to SI and CR in inducing aphagia in mice. However, this effect was marginal in wild-type animals, but became prominent in the presence of a specific brain-derived growth factor gene polymorphism, the BDNF-Val66 Met variant [13]. The latter is also implicated in the promoting effect of severe CR on binge eating behavior in adolescent girls [15]. In another study, mice exposed to SI and time-restricted feeding (with food availability limited to 2 h/d) for 10 wk were reported to develop symptoms observed in anorexia nervosa, despite the fact that food intake in these animals was close to that of AL-fed controls [16]. In addition, the short-term (2 wk) feeding of a CR diet coupled with SI was associated with aggressive behavior in mice, although the extent of CR was not specified [17]. Interestingly, both CR and SI represent 2 independent common changes that are often introduced in husbandry for rodents, particularly in neuroscience, with the first to motivate animals in reward-association tasks and the latter to accommodate individual feeding schedules or the need to reduce interactions because of implants. Thus, the multifaceted effects of these exposures, alone or in combination, should be carefully evaluated. For example, the duration of olfactory memory in male Long–Evans rats was increased by CR, but decreased after SI [18]. However, the effect of both treatments combined was not investigated.

SI has been linked to the development of depression [19], which in turn is a risk factor for the onset of eating disorders, including anorexia [20]. However, SI per se did not translate into an anorexic-like phenotype under our experimental conditions, which suggests that additional triggers elicited by CR are at play. Additional studies are warranted to address this important issue.

The apparent reversibility of the anorexic phenotype upon reversal of the housing conditions is another interesting finding in our studies, with 2 important implications: Emphasis on the role of SI in eliciting and sustaining this phenotype, and adding to the potential of this model for the analysis of biochemical and molecular pathways involved in the anorexic behavior.

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