Accepted Manuscript

Title: Effects of a Post-Caloric Restriction Weight Maintenance Period on Skeletal Muscle and Adipose Tissue Mass in Male Mice

Type: Short Communications

Tomohiro Iwata[1,2], Takanaga Shirai*[3,4], Kazuki Uemichi[2,4], Riku Tanimura[4,5], Katsuyuki Tokinoya[6], Tohru Takemasa*[7]

1; Faculty of Health and Medical Sciences Department of Health and Sports Sciences, Kyoto University of Advanced Science, 1-1 Nanjyo Otani, Sogabe-cho, Kameoka-shi, Kyoto 621-8555, Japan

2; Faculty of Sport and Health Science, Ritsumeikan University, 1-1-1 Nojihigashi, Kusatsu, Shiga 525-8577, Japan

3; Department of Human Sciences, Kanagawa University, 3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama-shi, Kanagawa, 221-8686 Japan

4; Research Fellow of Japan Society for Promotion Science

5; Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, 305-8574, Ibaraki, Japan

6; College of Education, Yokohama National University. 79-2 Tokiwadai, Hodogaya-ku, Yokohama, Kanagawa, 240-8501 Japan

7; Institute of Health and Sport Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, 305-8577, Ibaraki, Japan

Number of Figures: 2

Number of Tables: 0

Running Title: Post-CR Weight Maintenance and Muscle–Fat Mass

Corresponding Author:

Takanaga Shirai, Ph.D.

Department of Human Sciences, Kanagawa University

3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama-shi, Kanagawa, 221-8686, Japan

Email: wtk-0598di@kanagawa-u.ac.jp

Tohru Takemasa, Ph.D. and Dr. Med. Sci.

Institute of Health and Sport Sciences, University of Tsukuba

1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8574, Japan

Email: takemasa.tohru.gm@u.tsukuba.ac.jp

論文題目：カロリー制限後の体重維持期間が雄マウスの骨格筋および脂肪量に与える影響

著者：岩田　知大[1,2]、白井　隆長[3,4]、上道　和毅[2,4]、谷村　陸[4,5]、時野谷　勝幸[6]、武政　徹[7]

所属：

1; 京都先端科学大学健康医療学部　621-8555 京都府亀岡市曽我部町南条大谷 1-1

2; 立命館大学スポーツ健康科学部　525-8577 滋賀県草津市野路東 1 丁目 1-1

3; 神奈川大学人間科学部　221-8686 神奈川県横浜市神奈川区六角橋 3-27-1

4; 日本学術振興会

5; 筑波大学人間総合科学学術院　305-8574 茨城県つくば市天王台 1-1-1

6; 横浜国立大学教育学部　240-8501 神奈川県横浜市保土ヶ谷区常盤台 79-2

7; 筑波大学体育専門学群　305-8574 茨城県つくば市天王台 1-1-1

概要：アスリートは体重管理の手段としてカロリー制限（caloric restriction: CR）を用いることが多い。しかし、CR 後の体重維持期が骨格筋量および脂肪量に及

ぼす影響は十分に明らかにされていない。本研究では、マウスを用いて、60%のCR を 2 週間（2CR 群）または 8 週間（8CR 群）実施し、骨格筋および脂肪組織への影響を検討した。その結果、体重および骨格筋湿重量は 2CR 群で有意に高く、鼠径白色脂肪組織（iWAT）の重量は 8CR 群で有意に高かった。これらの結果から、初期の体重減少後に体重維持期を含む 8 週間の CR は、2 週間の CR と比較して、大きな体重減少を示すものの、骨格筋量の減少および脂肪量の増加を引き起こす可能性が示唆された。なお、本研究は 8 週齢の雄マウスを対象としており、2CR 群と 8CR 群の差は、CR 期間の長短そのものではなく、異なる成長段階でカロリー制限を実施したことに起因する可能性がある。

Abstract

Athletes often use caloric restriction (CR) to manage body weight. However, the effects of post-CR weight maintenance on skeletal muscle and fat mass remain unclear. This study investigated the effects of 60% CR for either 8 (8CR) or 2 (2CR) weeks on skeletal muscle and adipose tissue in mice. In the 8CR group, weight loss slowed after 2 weeks. However, body weight and wet skeletal muscle weight were significantly higher and inguinal white adipose tissue weight was significantly lower in the 2CR group compared with the 8CR group. These findings suggest that 8 weeks of CR, including a weight maintenance phase after initial weight loss, may result in a greater loss of skeletal muscle mass and an increase in fat mass compared to 2 weeks of CR. As this study was performed in 8-week-old male mice, the differences between the 2CR and 8CR groups are likely to reflect not simply the length of CR but rather the influence of conducting CR at different stages of growth.

Key words: Calorie restriction, weight reduction, skeletal muscle, adipose tissue

1. Introduction

Athletes must undergo weight reduction to meet the weigh-in requirements for competitions with weight classes and to maintain their desired physique in aesthetic events [1]. In this context, the primary objective is to decrease adipose tissue, which serves as an energy reservoir, while simultaneously safeguarding the quantity of skeletal muscle, an essential component for exercise-related contractions.

Athletes frequently employ caloric restriction (CR) as a means of weight management. CR is a dietary strategy that entails reducing calorie intake to approximately 60–80% of the typical daily amount; a CR of up to 40% has been shown to extend lifespan in organisms ranging from yeast to mammals [2,3]. Studies have indicated that CR effectively reduces both body weight and fat mass when compared to an ad libitum (AL) diet. However, it is worth noting that CR also leads to a reduction in skeletal muscle mass [4].

In sports, athletes autonomously establish their weight loss pace and period, aligning them with their sport-specific attributes and training regimens. A study on the rate of weight loss revealed that an expedited reduction in weight via a 3-day fasting regimen led to a more pronounced loss of skeletal muscle mass when compared to a slow weight loss over 14 days, achieved through a 70% CR [5]. Therefore, a 14-day CR is preferable for athletes than rapid weight loss through fasting. However, alterations in body composition following a period of weight maintenance after weight loss have not yet been studied. Athletes may employ a conditioning strategy that involves losing weight and then maintaining it in preparation for a competition [1]. Therefore, this study aimed to compare the effects of long-term and short-term CR on skeletal muscle and adipose tissue, particularly focusing on the influence of a weight maintenance period following early weight loss. Although this study was conceptually motivated by weight reduction practices in athletes, direct experimental studies in athletes are not feasible; therefore, it was conducted as a basic experiment using untrained mice as a simplified model. A weight maintenance period following CR may function as a less intensive strategy, analogous to the difference observed between a 14-day CR and a 3-day fasting [5]. We therefore hypothesized that incorporating a maintenance period would contribute to the preservation of skeletal muscle while still facilitating the reduction of adipose tissue.

2. Materials and Methods

2.1. Animals

All experimental procedures performed in this study were approved by the Institutional Animal Experiment Committee of the University of Tsukuba (Approval no.: 20–407). Male C57BL/6j mice aged 7 weeks (Tokyo Laboratory Animals Science Co., Tokyo, Japan) were kept in temperature (22 ± 2◦C)- and humidity (55 ± 5%)-controlled facilities under a 12/12 h light/dark cycle. The mice were randomly assigned to groups after 1 week of acclimatization: an age-matched AL group, which was allowed ad libitum access to food (protein:fat:carbohydrate = 23.1%:5.1%:71.8%; Oriental, Japan) for 8 weeks; a 2CR group, which was fed ad libitum for 6 weeks, followed by a 60% CR for the subsequent 2 weeks; and an 8CR group, which was subjected to 60% CR for the entire 8-week period. In the CR groups, 60% of the amount calculated from the age-matched AL group was provided to the animals at 19:00 every day, and the food intake of all groups was measured every 24 h from the start of the experiment [6]. The mice were anesthetized at the end of the intervention after the experiments. The soleus, plantaris, and gastrocnemius muscles, along with the epididymal white adipose tissue (eWAT) and inguinal WAT (iWAT), were excised and weighed. We first conducted an experiment with AL and 8CR groups, as reported previously [7], in which analyses were restricted to skeletal muscle and serum. We subsequently conducted an additional 2CR cohort. The present study integrates these datasets and extends the analysis to adipose tissue during the post-CR weight-maintenance period.

2.2. Statistical analysis.

Data are expressed as the means ± standard deviation (SD). A two-way analysis of variance (Time × Diet) was performed on food intake and body weight, followed by Bonferroni's multiple-comparison tests when significant interactions were observed. For comparisons between groups, two-tailed unpaired t-tests were performed. Statistical significance was defined as $p < 0.05$. All statistical analyses were conducted using GraphPad Prism 8 (GraphPad, Inc.). This study was conducted without blinding.

3. Results

3.1. Food Intake and Body Weight

Food intake and body weight were assessed weekly to determine the occurrence of a weight maintenance phase during the prolonged CR (Fig. 1). In the 8CR group, the body weight had plateaued by the second week of the CR period (Fig. 1A). At the end of the experimental period, body weight was significantly higher in the 2CR group than in the 8CR group (Fig. 1B).

3.2. Tissue Weight

The wet weights of the soleus, plantaris, and gastrocnemius muscles, both absolute and body weight–normalized, were significantly higher in the 2CR group than in the 8CR group (Fig. 2). In contrast, eWAT showed no significant difference between the two groups, whereas iWAT was significantly lower in the 2CR group, both in absolute and normalized weights.

4. Discussion

This study is the first to examine the effect of a post-CR weight maintenance period on body composition. An analysis of body weight changes revealed that, in the 8CR group, weight reduction reached a plateau after the initial 2 weeks. However, at the end of the experimental period, body weight was significantly higher in the 2CR group than in the 8CR group. These results indicate that, although the 8CR group underwent a six-week weight maintenance phase following the initial weight loss, the final body weight differed between the 8CR and 2CR groups; therefore, the aim of this study was only partially achieved. However, we analyzed tissue weights normalized to body weight and showed that they underwent similar changes as absolute weights. The results suggest that the addition of a weight maintenance period after CR can lead to an increase in fat mass and a decrease in skeletal muscle mass.

In this study, consistent changes were observed across all skeletal muscles analyzed, whereas distinct effects were detected between eWAT and iWAT. Previous research has shown that iWAT exhibits higher sympathetic innervation and greater responsiveness to interventions, including browning, compared with eWAT [8)]. Accordingly, the differences in CR duration identified in this study were evident only in iWAT.

It is also important to note several limitations of the present study. First, this study did not examine hormonal or signaling changes. Previous studies have shown that hormones associated with the regulation of skeletal muscle mass are altered during caloric restriction [9-11]. Moreover, several studies have reported that skeletal muscle mass is altered during caloric restriction through changes in anabolic and catabolic signaling pathways [5,6]. Future research should include such analyses to clarify the mechanisms that contributed to the observed phenotypic changes.

Second, there are limitations in the feeding protocol and sampling strategy. In this study, CR was implemented by providing food once daily, which is consistent with the methodology used previously [12]. However, tissue sampling was conducted at a single time point; therefore, transient changes in molecular signaling associated with feeding and fasting cycles during the day may not have been captured. Moreover, the skeletal muscles analyzed in this study—the soleus, plantaris, and gastrocnemius—are antigravity muscles. The observed decrease in wet weight in the 8CR group alone may have resulted from a delayed effect of reduced mechanical loading due to body weight loss.

Finally, the choice of experimental animals represents another limitation. In this study, 8-week-old male mice were used as experimental animals. While this age is widely adopted in experimental research, some developmental processes are still reported to be ongoing at this stage [13]. Therefore, the phenotypic differences observed between the 2CR and 8CR groups may reflect not only the duration of the interventions but also the timing of growth. Furthermore, the difference in body weight at the final week between the groups may have also influenced the phenotypic differences observed in this study. This study was limited to male mice; however, as substantial sex differences have been reported in the effects of conventional CR, further experiments in female mice are warranted to evaluate the influence of the maintenance period after CR [14].

In summary, the findings of the present study suggest that the inclusion of a weight maintenance period in CR protocols may differentially influence body composition. Further studies are needed to elucidate the underlying mechanisms.

Acknowledgments

We are grateful to Dr. Yuki Aoki (University of Tsukuba) for constructive feedback and general comments that supported the development of this research. This work was supported by JSPS KAKENHI grants 20J15222.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could appear to have influenced the work reported in this article.

Author Contributions

TI and TS conceptualized the study design and protocol. TI, TS, KU, RT, and KT conducted the investigation and collected the data. TI and TS curated the data. TI carried out the data analysis and drafted the manuscript. TT supervised the study and reviewed and edited the manuscript. All authors critically reviewed, revised, and approved the final version of the manuscript.

Reference

1) Manore, M. M. 2015. Weight Management for Athletes and Active Individuals: A Brief Review. *Sports Med*. 45 Suppl 1.Suppl 1. S83-92. doi;10.1007/s40279-015-0401-0
2) Colman, R. J., Beasley, T. M., Kemnitz, J. W., Johnson, S. C., Weindruch, R. and Anderson, R. M. 2014. Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. *Nat Commun*. 5.3557. doi;10.1038/ncomms4557
3) Wei, M., Fabrizio, P., Hu, J., Ge, H., Cheng, C., Li, L. and Longo, V. D. 2008. Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. *PLoS Genet*. 4.1. e13. doi;10.1371/journal.pgen.0040013
4) Heymsfield, S. B., Gonzalez, M. C., Shen, W., Redman, L. and Thomas, D. 2014. Weight loss composition is one-fourth fat-free mass: a critical review and critique of this widely cited rule. *Obes Rev*. 15.4. 310-321. doi;10.1111/obr.12143
5) Nonaka, Y., Urashima, S., Inai, M., Nishimura, S., Higashida, K. and Terada, S. 2017. Effects of rapid or slow body weight reduction on intramuscular protein degradation pathways during equivalent weight loss on rats. *Physiol Res*. 66.5. 823-831. doi;10.33549/physiolres.933502
6) Shirai, T., Uemichi, K., Hidaka, Y., Kitaoka, Y. and Takemasa, T. 2021. Effect of lactate administration on mouse skeletal muscle under calorie restriction. *Curr Res Physiol*. 4.202-208. doi;10.1016/j.crphys.2021.09.001
7) Shirai, T., Iwata, T., Uemichi, K., Tanimura, R., Iwai, R. and Takemasa, T. 2023. The effect of serum from calorie-restricted mouse on mTOR signaling in C2C12 myotubes. *J Endocrinol*. 259.2. doi;10.1530/JOE-23-0111
8) Seale, P., Conroe, H. M., Estall, J., Kajimura, S., Frontini, A., Ishibashi, J., Cohen, P., Cinti, S. and Spiegelman, B. M. 2011. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J Clin Invest*. 121.1. 96-105. doi;10.1172/JCI44271
9) Levay, E. A., Tammer, A. H., Penman, J., Kent, S. and Paolini, A. G. 2010. Calorie restriction at increasing levels leads to augmented concentrations of corticosterone and decreasing concentrations of testosterone in rats. *Nutr Res*. 30.5. 366-373. doi;10.1016/j.nutres.2010.05.001
10) Smith, S. J., Teo, S. Y. M., Lopresti, A. L., Heritage, B. and Fairchild, T. J. 2022. Examining the effects of calorie restriction on testosterone concentrations in men: a systematic review and meta-analysis. *Nutr Rev*. 80.5. 1222-1236. doi;10.1093/nutrit/nuab072

11) Kraemer, W. J., Ratamess, N. A., Hymer, W. C., Nindl, B. C. and Fragala, M. S. 2020. Growth Hormone(s), Testosterone, Insulin-Like Growth Factors, and Cortisol: Roles and Integration for Cellular Development and Growth With Exercise. *Front Endocrinol (Lausanne)*. 11.33. doi;10.3389/fendo.2020.00033

12) Acosta-Rodriguez, V. A., de Groot, M. H. M., Rijo-Ferreira, F., Green, C. B. and Takahashi, J. S. 2017. Mice under Caloric Restriction Self-Impose a Temporal Restriction of Food Intake as Revealed by an Automated Feeder System. *Cell Metab*. 26.1. 267-277 e262. doi;10.1016/j.cmet.2017.06.007

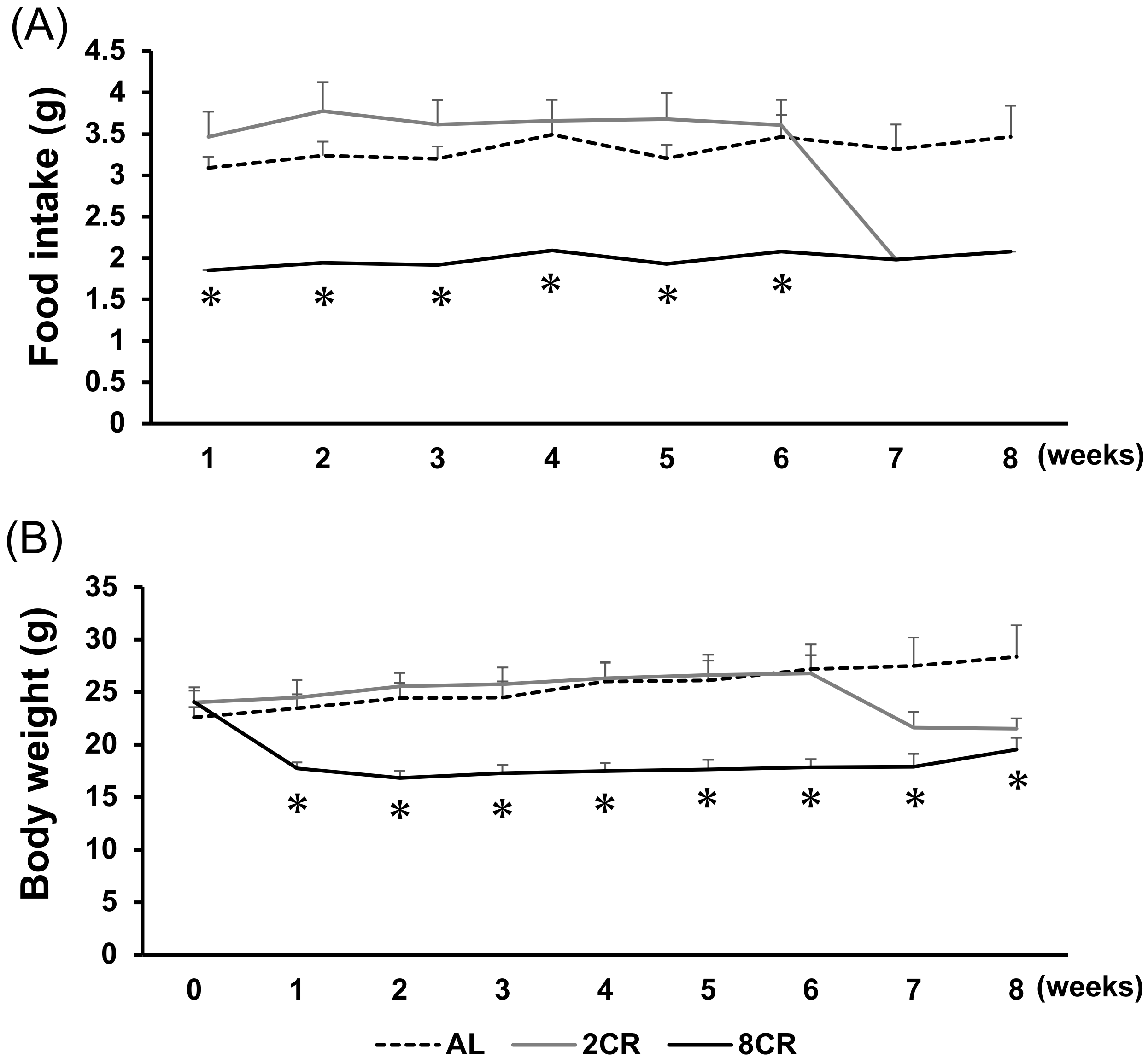
13) Jackson, S. J., Andrews, N., Ball, D., Bellantuono, I., Gray, J., Hachoumi, L., Holmes, A., Latcham, J., Petrie, A., Potter, P., Rice, A., Ritchie, A., Stewart, M., Strepka, C., Yeoman, M. and Chapman, K. 2017. Does age matter? The impact of rodent age on study outcomes. *Lab Anim*. 51.2. 160-169. doi;10.1177/0023677216653984

14) Kane, A. E., Sinclair, D. A., Mitchell, J. R. and Mitchell, S. J. 2018. Sex differences in the response to dietary restriction in rodents. *Curr Opin Physiol*. 6.28-34. doi;10.1016/j.cophys.2018.03.008

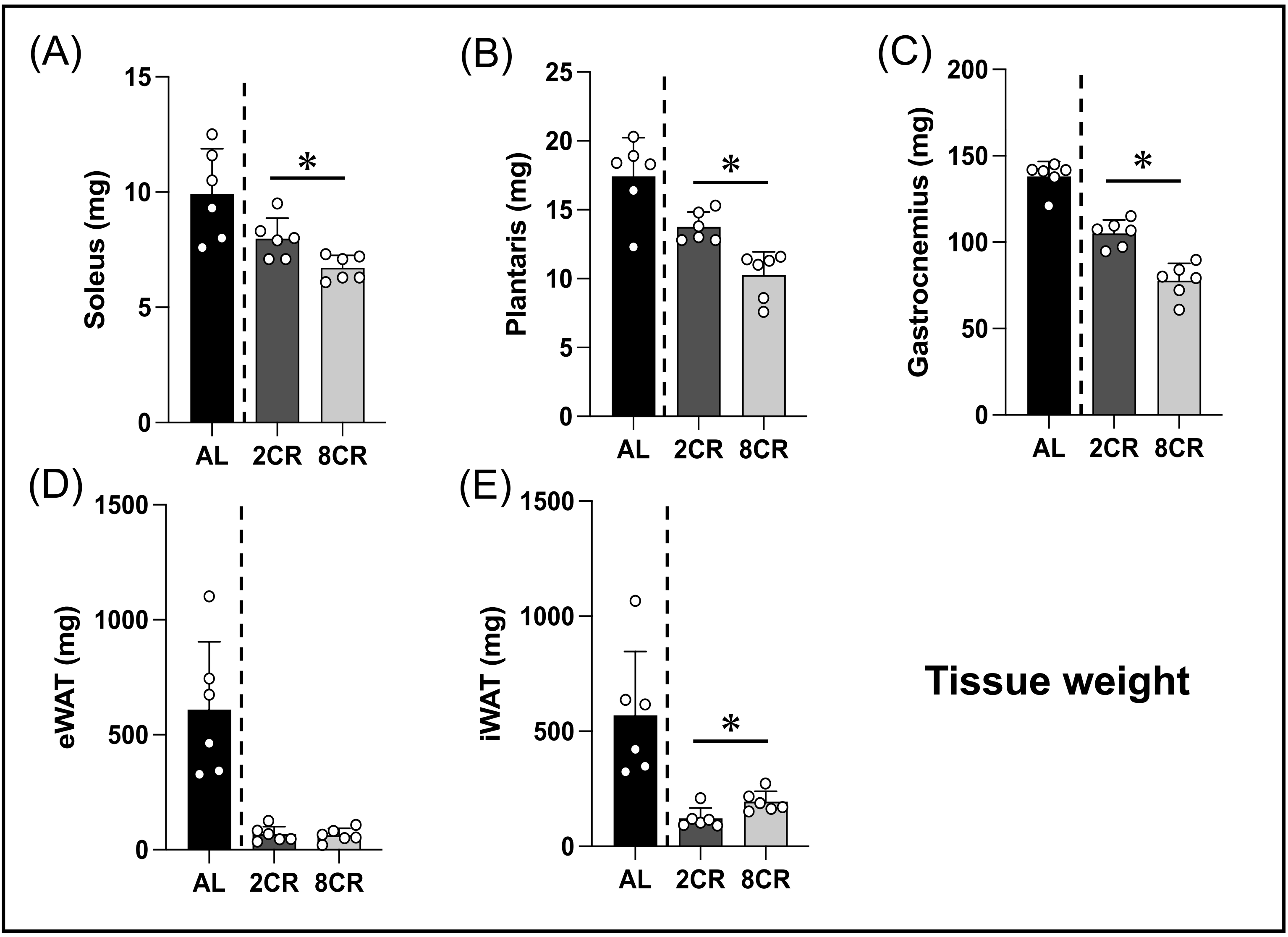
Figures and legends

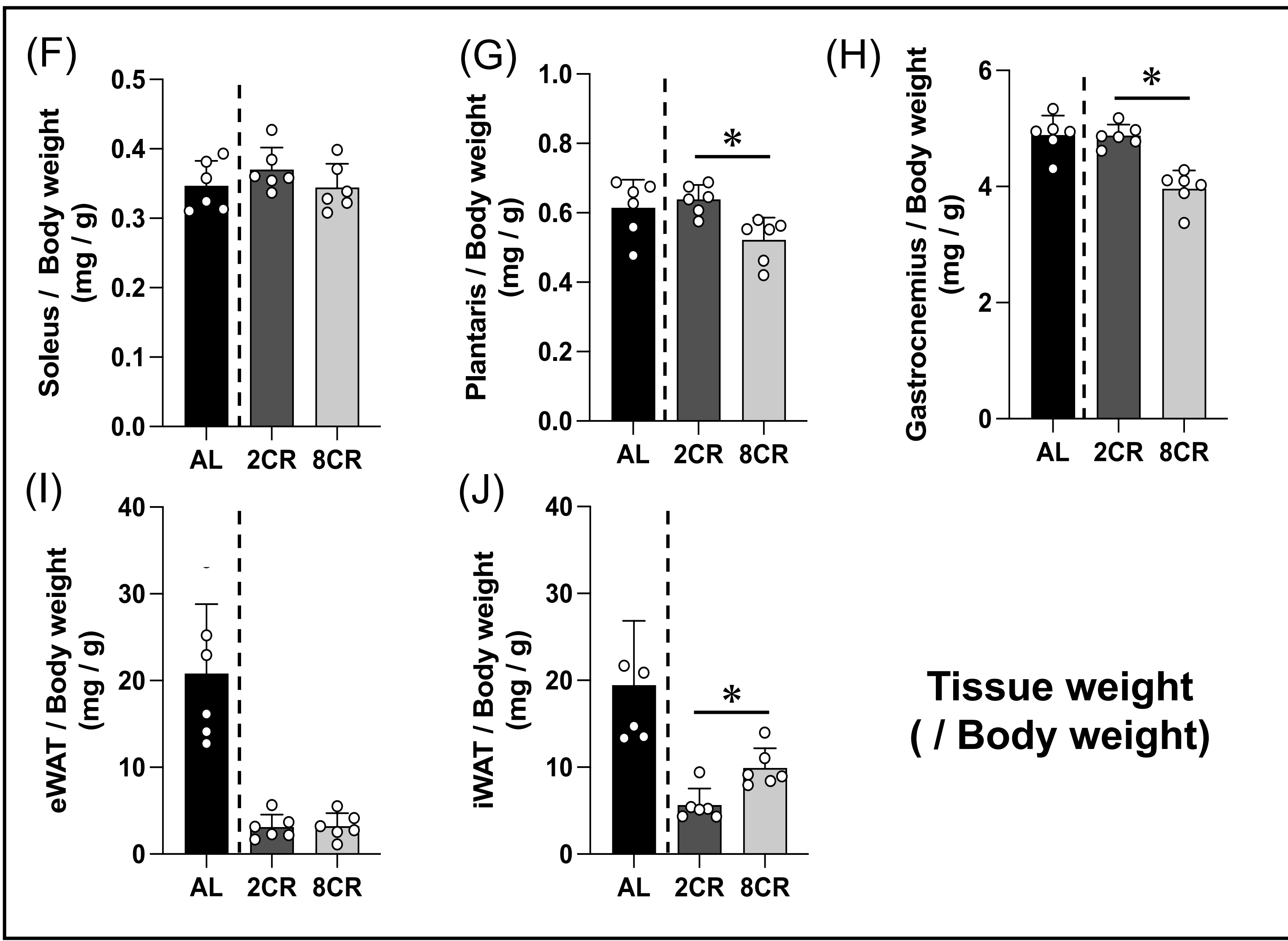
Fig 1. Food intake and body weight. (A) Changes in food intake and (B)body weight. Data are presented as mean + SD (n = 6 per group). *p<0.05 vs AL group, #p<0.05 vs 2CR group.

Fig 2. Skeletal muscle wet weight and adipose tissue weight. (A) Soleus wet weight, (B) plantaris wet weight, (C) gastrocnemius wet weight, (D) epididymal white adipose tissue weight and (E) inguinal white adipose tissue weight, (F) Soleus wet weight /Body weight, (G) plantaris wet weight/Body weight, (H) gastrocnemius wet weight/Body weight, (I) epididymal white adipose tissue weight/Body weight and (J) inguinal white adipose tissue weight/Body weight. Data are present as each plot and + SD (n = 6 per group). *p<0.05 between groups.



(A)
Food intake (g)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
*
*
*
1
2
3
4
5
6
7
8
(weeks)
(B)
Body weight (g)
35
30
25
20
15
10
5
0
*
*
*
*
*
*
*
*
0
1
2
3
4
5
6
7
8
(weeks)
AL
2CR
8CR


(A)
Soleus (mg)
(B)
Plantaris (mg)
(C)
Gastrocnemius (mg)
(D)
eWAT (mg)
(E)
iWAT (mg)
AL
2CR
8CR
*
Tissue weight


(F)
Soleus / Body weight (mg / g)
(G)
Plantaris / Body weight (mg / g)
(H)
Gastrocnemius / Body weight (mg / g)
(I)
eWAT / Body weight (mg / g)
(J)
iWAT / Body weight (mg / g)
AL
2CR
8CR
*
Tissue weight
(/ Body weight)