Four-week individual caging of male ICR mice alters body composition without change in body mass

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Understanding the physiological implications of caging conditions for mice is crucial in improving the replicability and reliability of animal research. Individual caging of mice is known to alter mouse psychology, such as triggering depression-like symptoms in mice, suggesting that caging conditions could have negative effects on mice. Therefore, we hypothesized that individual caging could affect the physical composition of outbred mice. To investigate this, dual X-ray absorptiometry (DXA) was used to compare the mass, bone mineral content (BMC), bone mineral density (BMD), lean tissue percentage and fat tissue percentage between group and individual caged mice. We also conducted open field test to compare mouse activities in different caging conditions. Our results showed significantly reduced BMD and lean tissue percentage and significantly increased fat tissue percentage in individually-caged male mice. Furthermore, there were no differences in body mass and activity between the grouped and individual mice, suggesting that these physical alterations were not induced by group-related activity. In this study, we conclude that individual caging could alter the body composition of mice without affecting external morphology.

For drug discovery research, most drug candidates have been administered in animals using a mg/kg approach, where the dosage of the drug is determined by the animal’s body weight. The efficiency of drugs is dependent on four fundamental pathways characterized as the ADME process – absorption into the bloodstream, distribution to the tissues, metabolism of drug particles, and excretion from the body1. One of the main factors that contribute to ADME is body composition2,3; therefore, changes in the body composition can lead to unreliable results of animal testing.

Mice, being social animals, are thought to be negatively affected by isolation4. This has been supported by a previous study that has shown depression-like behavior in individually-caged mice5. While the psychological implications of solitude in mice have been relatively well established, the implications of social isolation on the body composition parameters of mice remain uncharacterized.

Therefore, to investigate the effect of group or individually caging on the physical composition of mice, we conducted a study comparing the body composition parameters of all mice. In this study, all eight-week-old ICR male mice (n = 22) were caged in groups (n = 12, 4 per cage) or individually (n = 10, 1 per cage) for four weeks. This study provides evidence of a significant differences in bodily content in group-caged and individually-caged mice, highlighting the influence of such external factors on mice physiology. We obtained the values of body mass, BMC, BMD, lean and fat tissue from InAlyzer using DXA analysis (Medikors Inc., Korea). Then we measured the locomotive activity of mice (n = 20) after four weeks of caging in group- (n = 16, 4 per cage) or individually-caged (n = 4, 1 per cage).

Results
BMD and lean percentage is decreased, fat percentage is increased in individually-caged mice. After four weeks of caging in groups or individually (Fig. 1a), the physical effects of caging were observed. To assess these effects, the measurements of body mass, BMD, BMC, lean tissue percentage, and fat...
In this study, we report a significant increase in fat tissue percentage and a decrease in BMD and lean tissue percentage in individually-caged mice compared to group-caged mice (Fig. 2). This suggests that using different cage conditions – group and individual – may influence the outcome of animal studies, and therefore caging conditions may be made under the same conditions – group or individual. In addition, we observed these physiological changes after a short span of four weeks. This suggests that, if animal studies were conducted over a longer period of time, the different caging conditions will show more noticeable differences in body compositions.

We also report that these alterations in body composition of individually caged mice are not caused by reduced activities (Fig. 4b). Recent studies show that bone degradation occurs in the absence of stimulus, suggesting that the lack of social interactions in individually-caged mice may also contribute to BMD reduction. Another study suggests that individual caging of mice may affect the circadian rhythm, including changes in hormone levels in response to stress. These alterations in the central nervous system and immune system may also cause changes in body composition of mice. Thus, further investigation is warranted to stimulus, stress, and other factors that may influence physiological changes in mice belonging to different caging conditions.

Consistent with our outbred ICR mice results, a significant reduction in BMD and lean tissue percentage were previously reported in individually caged young inbred C57BL/6J mice. This confirms that individual caging of mice can affect body composition despite having different mouse strains. However, our results do not show

**Figure 2.** All mice were caged individually or together for four weeks. Eight-week-old male ICR mice (n = 22) were caged in groups of four or individually (n = 12 and n = 10, respectively).
a change in body mass (Fig. 2a) nor a higher variance in fat tissue percentage and BMD for group-caged mice (Supplementary table S3). We also observed significantly increased fat tissue percentage in individually caged mice.

The ADME process, in particular the metabolism of drugs, is linked to tissue distribution; an increase in fat tissue percentage would interfere with the metabolism of drugs. The alteration of metabolism could hinder drug clearance and absorption, which may cause variation and affect the replicability of pharmacological studies. This suggests that if mice, with similar weights but different body compositions, were administered with equal dosages, the effects on each mouse would vary from one another.

The current study shows significant differences in body composition between group- and individual-caged mice. To maintain consistency in animal studies and results, all mice should be placed under identical caging conditions. From the basis of the current study, further investigation is warranted to determine the primary cause of physiological alterations and to observe the long-term effects of differing caging conditions.

**Materials and Methods**

**Materials.** 2,2,2-Tribromoethanol (avertin) and 2-Methyl-2-butanol were obtained from Sigma-Aldrich (Missouri, United States). Saline was purchased from JW Pharmaceutical (Seoul, Korea). The animal marker was purchased from Muromachi Kikai Co., Ltd (Tokyo, Japan).
Animals. For DXA analysis, eight-week-old male Imprinting Control Region (ICR) mice (n = 22) were purchased from Orient Bio Inc. (Seoul, Korea). Twelve mice were caged together in groups of four and the remaining was caged individually for this study. Three cages were prepared for group caging (n = 4 per cage) and ten cages were prepared for individual caging (n = 1 per cage). For open field test, eight-week-old male Imprinting Control Region (ICR) mice (n = 20) were purchased from Orient Bio Inc. (Seoul, Korea) and caged under the same conditions as before. Sixteen mice were caged together in groups of four and the remaining was caged individually. All mice were caged in the same size cages (260 (W) × 420 (D) × 180 (H) mm) for four weeks under regulated conditions with 12-hour light-dark cycles and food and water were provided ad libitum. The animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University and performed in accordance with the National Institutes of Health guide for the care and use of laboratory animals.

Body composition measurement and analysis. The body composition of the mice was measured after four weeks of caging. To prevent subjects from moving during measurement, mice were anesthetized with 4% avertin (v/v) prior to measurement. Body composition parameters of body mass, BMC, BMD, lean and fat tissue of mice were measured using the InAlyzer (Medikors Inc., Korea), which uses dual X-ray absorptiometry (DXA) to provide accurate measurements of bone and body composition for animals. After anesthetization, mice were...
immediately placed in the center of the InAlyzer scanning area in the prone position with arms and legs extended out to the side. Imaging scans were taken and measurements were obtained using the InAlyzer software.

**Open field test.** All mice \((n = 20)\) were caged in groups or individually for four weeks and transported to the testing room to acclimate for an hour. Brightness was maintained at approximately 20 lux inside the testing room and barriers were placed in between the individual and grouped cages to prevent interaction. All mice were allowed to roam for 15 hours, and their activities were monitored by an overhead tracking camera, and velocity and distance were tracked using EthoVision 3.1 (Noldus, Netherlands). An animal marker (FG2200B, blue) was used to stain the back of one randomly selected mouse in each group cage. (b) There are no differences in distance moved and velocity in both groups of mice. All the error bars represent the SEM.

**Statistical analysis.** Graphs were obtained with GraphPad Prism 6 and statistical analysis were performed with Student's *t*-tests \((^*P < 0.05, ^{**}P < 0.01, ^{***}P < 0.001\)) The error bars represent the SEM.

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Author Contributions
Y.K. designed and supervised all experiments and analyses. J.S., J.W., Y.C. and Y.H.C. performed animal experiments. J.S., Y.H.C., N.N.S. and Y.K. wrote the manuscript.

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