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Title: Female rats require a greater energy deficit for body weight reduction than males

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Running head: Sex differences in body weight reduction

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Abstract

Whether decreases in adipose and lean tissue during body weight (BW) reduction differ between males and females is unclear. To investigate sex differences in changes in body composition and energy deficits during BW reduction, sexually mature 15-week-old rats were divided into a group euthanized before BW reduction (n = 6 males, n = 7 females) and a group euthanized after three days of fasting (n = 6 males, n = 6 females). Energy expenditure and BW were measured during the study period. Losses of lean tissue (ΔLT) and adipose tissue (ΔAT) were calculated using simultaneous equations based on ΔBW, which is the sum of ΔLT and ΔAT, and the energy lost from the body is the sum of the energy lost from ΔLT and ΔAT. BW reduction was significantly greater in males than in females, total energy expenditure was significantly greater in males than in females, and the energy required to reduce BW by 1 kg was significantly less in males (3304.8 kcal/kg [SD 327.5]) than in females (3893.0 kcal/kg [SD 356.5]). Both ΔLT and ΔAT were significantly greater in males than in females. The ΔLT:ΔAT ratio was significantly different between males (67:33) and females (57:43). Sex differences exist in the metabolic responses to BW reduction, and females lose more energy-dense adipose tissue during BW reduction than males, demonstrating that a greater energy deficit is required to reduce BW in female rats than in male rats.

ラットにおける体重減少時の体組成の変化およびエネルギー負債の性差

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減量中の脂肪組織と除脂肪組織の減少が性差で異なるかは不明である。体重減少時の体組成の変化およびエネルギー負債の性差を検討した。性成熟した 15 週齢のラットを、減量前（雄：6 匹、雌： 7 匹）と、3 日間の絶食後（雄：6 匹、雌：6 匹）に安楽死させた。実験期間中は、エネルギー消費量と体重を測定した。除脂肪組織（ΔLT）と脂肪組織（ΔAT）の減少量は、ΔLT と ΔAT の合計が体重減少量であることと、LT と AT から失われたエネルギーの合計が体から失われたエネルギーであることに基づく連立方程式を用いて算出した。体重減少量は、雄が雌より有意に大きく、3 日間のエネルギー消費量も雄が雌より有意に大きかった。体重減少 1k g あたりのエネルギー負債は、雄（-3304.8 kcal/kg, SD 327.50）が雌（-3893.0 kcal/kg, SD 356.46）より有意に少なかった。重量の減少は LT も AT も雄が雌より有意に大きかった。ΔLT:ΔAT 比は、雄 (67:33) と雌 (57:43) で異なった。体重減少に対する代謝反応には性差が存在し、雌は雄よりも減量中により多くの、エネルギー密度の高い脂肪組織を失うことから、雌が雄より体重を減少させるためにより多くのエネルギーが消費される必要のあることが示された。

Introduction

The body is divided into fat-free mass (FFM) and fat mass (FM), and the energy densities of FFM and FM are 1,020 kcal/kg and 9,500 kcal/kg, respectively [*1,2*]. The body is divided into lean tissue (LT) and adipose tissue (AT). We previously analyzed the whole-body protein, total lipid, and glycogen content of several organs and tissues of rats and calculated their energy densities. The energy densities were found to be 1.4 kcal/g for skeletal muscle, 1.7 kcal/g for liver, 1.4 kcal/g for small intestine and stomach, and 7.9 kcal/g for AT [*3*].

Energy restriction reportedly decreases not only AT but also LT [*4,5,6,7,8*]. Tai et al. [*3*] reported that both 3-day fasting and 16-day energy restriction reduced the weight of adipose tissue and internal organs, such as the liver and small intestine. However, because the energy densities of AT and LT are different, the energy lost from the body during body weight (BW) reduction differs depending on the ratio of the decrease in LT and AT.

Forbes [*9,10*] reported that the greater the amount of body fat prior to BW reduction, the greater the loss in FM and the smaller the loss in FFM during BW reduction. Experiments in humans [*11,12,13*] and rats [*14,15*] have also reported that the greater the amount of body fat prior to BW reduction, the greater the FM loss owing to energy restriction. Therefore, it can be inferred that the ratio of AT and LT loss during BW reduction differs depending on the amount of body fat present prior to BW reduction and that the energy deficit required for BW reduction also differs. In general, the body fat percentage is higher in females than in males, suggesting that females lose more FM than males during BW reduction because of energy restriction.

Females reportedly have less BW reduction than males due to energy restriction in humans [*16,17*]. However, previous studies on the subject did not examine the mechanism by which females experience less BW reduction than do males. Therefore, the underlying mechanism remains unclear. Less BW reduction in females than in males suggests that a greater amount of energy-dense AT may be lost in females than in males. Since losing energy-dense AT requires a greater amount of energy deficit, females may need to achieve a greater energy deficit than males in order to reduce their BW.

Therefore, we examined the sex differences in body composition changes and energy deficits during BW reduction in rats. The hypothesis of this study was that the energy deficit to reduce BW and body fat loss would be greater in females than in males.

Methods

Animals and outline of the experiment

To examine sex differences, 12 sexually mature (15-week-old) male and 13 female Sprague-Dawley rats were used (CLEA Japan, Osaka, Japan). The temperature of the animal room was set at 23 ± 1 °C, with a dark period from 08:00 to 20:00 and a light period from 20:00 to 8:00. The rats were divided into a Pre-group (six males and seven females), which were euthanized before BW reduction, and a Post-group (six males and six females), which were euthanized after three days of fasting. The Post group fasted from 09:30 on the first day of fasting and was allowed to drink water *ad libitum*. The BW, water consumption, and oxygen expenditure were measured daily during the BW reduction period.

This study was approved by the Experimental Animal Committee of the Research Integrity Committee of Osaka University of Health and Sports Science (approval No. 02-1).

Measuring energy expenditure

Energy stored in the body expanded as the rats fasted throughout the study. Therefore, the total energy expenditure during the study period was the energy loss of the body. The rats in the Post group were placed in a chamber (22 cm × 34 cm × 14 cm) to measure energy expenditure. To acclimatize the rats to the measurement environment, they were allowed to feed *ad libitum* for two days and then fasted. Oxygen expenditure was measured using an open-circuit system [18]. The chamber was ventilated at 4,000 mL/min for male rats and 3,000 mL/min for female rats. A portion of the ventilated air (150 ml/min) was collected in a 250 L Douglas bag (Yagami, Osaka, Japan) every day for 23 h 45 min. The oxygen concentration was measured using a portable gas monitor (VO2000; S&ME, Tokyo, Japan). Oxygen consumption was calculated by multiplying the difference in oxygen concentration between room air and sampled air by the ventilation rate of the chamber, and the energy expenditure was calculated as 4.8 kcal/L oxygen. The energy expenditure was converted per 24 h.

Calculation of changes in LT and AT

Changes in the LT and AT were calculated using the following simultaneous equations:

$$\begin{cases} \Delta AT\ (g) + \Delta LT\ (g) = \Delta BW\ (g) & (1) \\ \Delta\ AT\ (g) \times 7.4\ (kcal/g) + \Delta LT\ (g) \times 1.25\ (kcal/g) = \text{Energy loss (kcal)} & (2) \end{cases}$$

Equation 1 indicates that BW reduction is the sum of the reductions in AT and LT. ΔBW was calculated using BW without gastrointestinal content in equation 1 because the gastrointestinal contents were measured as a portion of BW, but the gastrointestinal contents were not body tissues, such as LT or AT. Therefore, it was necessary to use a weight that did not include gastrointestinal content in this calculation. The gastrointestinal content weight used for this calculation was obtained from the tissue collection, as described below. The BW without the gastrointestinal contents of the rats in the Post group at the start of the study was 95.6% in males and 94.0% in females of their BW because gastrointestinal content accounted for 4.4% of the BW in males and 6.0% of that in females in the Pre group.

The values of 7.4 kcal/g, and 1.25 kcal/g in equation 2 are the energy densities of AT and LT, respectively, indicating that the sum of the energy reduced from AT and the energy reduced from LT is the energy lost from the body. The energy densities of AT and LT were obtained as follows. Six 7-week-old male SD rats (CLEA Japan) were sacrificed, and the liver, stomach, small intestine, kidneys, gastrocnemius muscle, soleus muscle, perirenal adipose tissue, and retroperitoneal adipose tissue were collected. After freeze-drying, the tissue was pulverized into powder and homogenized. The tissue energy densities of the liver, stomach, small intestine, and kidneys were defined as visceral organs, those of the gastrocnemius and soleus muscles were defined as skeletal muscle, and those of the perirenal and retroperitoneal adipose tissues were defined as AT. The samples used for the analysis were prepared by pooling

0.5 g of visceral organs, skeletal muscle, and AT from each rat. The energy densities measured by bomb calorimetry (Japan Food Research Laboratories, Osaka) were 1.38 kcal/g for visceral organs and 1.23 kcal/g for skeletal muscles. In our previous study, skeletal muscles accounted for approximately 90% of LT and visceral organs accounted for approximately 10% of LT [19]. Therefore, the energy density of LT was set to 1.25 kcal/g, and that of AT was 7.4 kcal/g.

Tissue collection

The rats were euthanized by drawing blood from the abdominal aorta under isoflurane anesthesia. Internal organs (heart, liver, kidneys, adrenal glands, and intestines), skeletal muscles (flexor hallucis longus, soleus, gastrocnemius, and plantaris), and ATs (perirenal, genital, posterior abdominal wall, and mesentery) were collected and weighed. After removing the intestinal contents, the intestines were weighed. The collected blood, internal organs, skeletal muscles, and ATs were returned to the abdominal cavity of the carcasses and frozen for biochemical analysis.

Biochemical analyses

The water content of the collected samples was calculated from the weight difference after drying in a high-temperature oven (HTO-450S; As One, Osaka, Japan) at 60 °C [20] to avoid the loss of short-chain fatty acids. The dried sample was pulverized into a powder using a mill (Vita-Max Absolute Blender; Osaka Chemical, Osaka, Japan) and homogenized before analysis. The whole-body protein content was calculated by multiplying the nitrogen content measured using the Kjeldahl method by 6.25 [21], the total lipid content was determined using the Folch method [22], and the glycogen content was determined using the method of Lo et al. [23].

The energy content of the whole body was calculated from the whole-body protein, total lipid, and glycogen content in the whole body multiplied by the physical combustion energy (9.5 kcal/g for fat, 5.7 kcal/g for protein, and 4.1 kcal/g for glycogen) [24].

Statistical analyses

The effects of sex and BW reduction were tested using two-way analysis of variance (ANOVA), with sex and BW reduction as two factors. Values before and after BW reduction were assessed using an unpaired *t*-test. Statistical significance was set at $P < 0.05$. IBM SPSS Statistics software program, Version 27 (IBM Japan, Tokyo, Japan), was used for statistical analyses.

Results

The pre-BW was significantly higher in males (513.7 g [standard deviation {SD} 14.5]) than in females (302.7 g [SD 21.6]), as was the post-BW (456.1 g [SD 19.7] vs. 261.7 g [SD 19.5]). BW reduction was significantly greater in males (-57.6 g [SD 2.7]) than in females (-41.0 g [SD 3.1]) (*Figure 1*). *Figure 1*

Energy expenditure decreased with each passing day, with total energy expenditure over the three-day period significantly higher in males (-153.0 kcal [SD 6.9]) than in females (-121.0 kcal [SD 5.0]) (*Figure 2*). *Figure 2*

To calculate the energy required to reduce BW by 1 kg, the BW reduction obtained using the BW without gastrointestinal contents as described in the methods section was used, and the BW reduction was -46.5 g (SD 3.3) for males and -31.3 g (SD 3.5) for females. The energy required to reduce BW by 1 kg was significantly lower in males (-3304.8 kcal/kg, [SD 327.5]) than in females (-3893.0 kcal/kg, [SD 356.5]).

The loss of LT and AT weights was significantly greater in males than in females (*Figure 3a*), while the proportion of AT loss among total BW reduction was significantly greater in females (43%) than in males (33%), and the proportion of LT loss among total BW reduction was significantly greater in males (67%) than in females (57%). *Figure 3a*

The LT and AT energy losses were also significantly greater in males than in females (*Figure 3b*), whereas the proportion of AT energy among total energy loss was significantly greater in females (81%) than in males (75%), and the proportion of LT energy among total energy loss was significantly greater in males (25%) than in females (19%). *Figure 3b*

Tables 1-1 and *1-2* show tissue weights before and after BW reduction, respectively. *Tables 1-1* and *1-2* Two-way ANOVA showed that males weighed significantly more than females, except that the adrenal glands were significantly heavier in females than males, and no significant differences were found in epididymal/parametrial AT weight. The carcass weight was also significantly higher in males than in females. Fasting significantly reduced the weight of the liver, kidneys, pancreas, spleen, total internal organs, gastrocnemius, plantaris, flexor digitorum longus, total skeletal muscles, mesenteric and total ATs, and carcasses of both sexes. According to the two-way ANOVA, fasting significantly reduced the weight of the liver, kidneys, pancreas, spleen, total internal organs, gastrocnemius, plantaris, flexor digitorum longus, total skeletal muscles, mesenteric and total ATs, and carcass in both sexes. A *t*-test showed that the weights of the pancreas, spleen, gastrocnemius, plantaris, perirenal AT, parametrial AT, and total LT were significantly reduced after BW reduction in females, but not in males. The weight of the mesenteric AT decreased significantly after BW reduction in males but not in females.

Tables 2-1 and *2-2* show tissue weights per 100 g BW. A two-way ANOVA revealed that the weights of the heart, adrenal gland, pancreas, gastrointestinal tract, total internal organs, gastrocnemius, soleus, flexor digitorum longus, and epididymal/parametrial AT were significantly higher in females than in males, whereas the weight of the skin was significantly lower in females than in males. Fasting significantly decreased the weights of the liver, total internal organs, and mesenteric AT but significantly increased the weights of the gastrointestinal tract, gastrocnemius, skin, and carcass. The perirenal AT weight decreased significantly after BW reduction in females but remained unchanged in males, whereas the adrenal gland and gastrointestinal tract weights increased significantly after BW reduction in females, but not in males. The heart, gastrocnemius, and skin weights increased significantly in males, but not in females.

Tables 2-1 and *2-2*

Table 3 shows whole-body protein, total lipid, and glycogen contents in the whole body. A two-way ANOVA showed that the weight of whole-body protein, total lipid, and glycogen were significantly higher in males than in females and decreased after BW reduction. After BW reduction, the weight of whole-body protein and glycogen decreased significantly in males but not in females, whereas the weight of total lipid decreased significantly in females but not in

Tables 3

males. The percentages of whole-body protein and glycogen in BW were higher in females than in males, but the percentage of total lipids did not differ markedly between the sexes.

Discussion

In this study, males had a greater BW reduction after three days of fasting than females, while the energy required to reduce BW was lower in males than in females. This was because males lost a greater amount of low-energy dense LT than high-energy dense AT, whereas females lost a greater amount of high-energy dense AT than low-energy dense LT. It is possible that females reduce BW less than males.

Forbes [*9,10*] reported that a greater amount of body fat prior to BW reduction is associated with a greater loss in FM and a smaller loss in FFM during BW reduction. In the present study, the weight of collected AT and the amount of body fat obtained by a whole-body analysis were both greater in males than in females. However, the body fat percentage calculated by the total lipid content of the body obtained by a biochemical analysis divided by BW and the weight of collected AT per BW were comparable between males and females. Because the amount of body fat was greater in males than in females in the present study, a smaller loss of LT and greater loss of AT were expected in males than in females. As expected, the loss of AT was greater in males than in females, but contrary to expectations, the loss of LT was greater in males than in females. Regarding the changes in the proportion of LT and AT, the proportion of AT loss was greater in females than in males. In addition, whole-body total lipid levels significantly decreased during the three-day fast in females, whereas no marked change was noted in males. Furthermore, the energy required for BW reduction was greater in females than in males. These results indicate that a greater proportion of the energy loss during BW reduction was from AT than from LT in females, suggesting that females expend more fat for BW reduction than do males. Therefore, it is inferred that factors other than body fat amount prior to BW reduction are responsible for the sex differences in body composition changes during BW reduction.

One possible factor associated with sex differences is the difference in muscle fiber composition between males and females. Matoba et al. [25] reported that the ratio of slow-twitch oxidative (SO) fibers in the skeletal muscle was higher in female mice than in male mice. SO fibers oxidize more fat than other muscle fibers do. This suggests that the higher ratio of SO fiber in females may be associated with the fact that females use more fat as energy during exercise than males [26,27,28,29]. The higher ratio of SO fibers in females may also be associated with the larger loss of AT in females than in males observed in the present study.

FFM is the weight that consists of water, protein, minerals, etc. in the body other than fat, whereas the LT used in this study is tissue consisting of approximately 20% protein and most of the rest is water. FM is the weight of fat in the body, whereas AT is tissue consisting of approximately 80% fat while also containing protein and water. In the present study, we examined the changes in the LT, such as skeletal muscles and internal organs, and AT during BW reduction. The energy density of FFM is lower than that of LT, and the energy density of FM is higher than that of AT. Therefore, the changes in the weight or energy are less when the energy density of FFM is used than when the energy density of LT is used. Conversely, the changes in the weight or energy are greater when the energy density of FM is used compared to when the energy density of AT is used. We calculated changes in FFM and FM with the simultaneous equations used in the present study using the energy densities of FFM (1,020 kcal/kg) and FM (9,500 kcal/kg). In females, the loss of FFM (-16.9 g) was less than the loss of LT (-18.0 g), whereas the loss of FM (-14.5 g) was greater than the loss of AT (-13.3 g). In males, the loss of FFM (-29.4 g) was less than the loss of LT (-31.1 g), while the loss of FM (-17.2 g) was greater than the loss of AT (-15.4 g). However, the changes were comparable to those calculated using the energy densities of LT and AT, as shown in *Figure 3*.

A limitation of this study is that the energy density of LT and AT increased owing to the decrease in body water after BW reduction, which may have caused errors in the calculation results. In humans, fluid intake is often restricted during BW reduction. Tai et al. [3] observed no marked changes in the water content of LT and AT when rats were fasted for three days. Unlike BW reduction in humans, Tai et al. allowed the rats to drink water *ad libitum* during a three-day fast, so the water content in LT and AT of the rats might not have changed. In the present study as well, the rats were allowed to drink water *ad libitum* during a three-day fast. Therefore, we assumed that the water content in LT and FT would not change, so the energy density would also not change in the present study.

In conclusion, sex differences were observed in the changes in body composition and energy deficits during BW reduction in rats. Females lose more energy-dense AT during BW reduction than males, suggesting that females require a greater energy deficit for BW reduction than males.

Conflict of Interest Self-Reporting

None to be declared.

AUTHOR CONTRIBUTIONS

KO and MK designed the research, KO and MK conducted the research, MK analyzed the data, and KO and MK wrote the paper. The MK was primarily responsible for the final content. All authors have approved the final version of the manuscript for publication.

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Figure legends

Figure 1. BW changes during the study.

BW was significantly higher in male rats than in females, and the BW reduction was significantly greater in male rats than in female rats. *$p<0.05$ vs. Male

Figure 2. Energy expenditure during the study.

Energy expenditure decreased during the three-day fasting period, and the energy expended during the three-day fasting period was significantly higher in male rats than in female rats. *$p<0.05$ vs. Male

Figure 3. Changes in lean and adipose tissue.

Reductions in LT and AT weights and energy were significantly greater in male rats than in females, while the proportion of AT loss among total BW reduction and total energy loss was significantly greater in female rats than in males, and the proportion of LT loss among total BW reduction and total energy loss was significantly greater in male than in female rats. *$p<0.05$ vs. Male

Table 1-1. Tissue weight before and after BW reduction (g)

	Male				Female				Two-way ANOVA		
	Pre		Post		Pre		Post		Sex	BW reduction	Sex × BW reduction
	mean	SD	mean	SD	mean	SD	mean	SD			
Organ											
Heart	1.15	0.06	1.12	0.12	0.79	0.15	0.70	0.08	*		
Liver	16.65	1.85	11.03	1.13†	9.55	1.20	6.31	0.48†	*	*	*
Kidneys	3.51	0.26	3.10	0.30†	2.03	0.22	1.69	0.20†	*	*	
Adrenal	0.04	0.01	0.03	0.00	0.05	0.01	0.05	0.01	*		
Pancreas	1.71	0.27	1.64	0.29	1.50	0.22	1.09	0.17†	*	*	
Spleen	0.89	0.08	0.75	0.13	0.57	0.07	0.46	0.03†	*	*	
Intestines	7.13	0.45	6.77	0.65	5.27	0.46	5.48	0.53	*		
Total	31.10	1.89	24.45	1.74†	19.76	1.83	15.76	1.10†	*	*	

*$p < 0.05$, Two-way ANOVA. †$p < 0.05$, vs. Pre (t-test).

SD, standard deviation; ANOVA, analysis of variance

Table 1-2. Tissue weight before and after BW reduction (g)

	Male				Female				Two-way ANOVA		
	Pre		Post		Pre		Post		Sex	BW reduction	Sex × BW reduction
	mean	SD	mean	SD	mean	SD	mean	SD			
Skeletal muscle											
Gastrocnemius	5.41	0.21	5.09	0.24	3.36	0.26	2.98	0.18†	*	*	
Soleus	0.31	0.03	0.30	0.02	0.21	0.02	0.19	0.03	*		
Plantaris	0.95	0.08	0.88	0.07	0.62	0.04	0.52	0.06†	*	*	
Flexor hallucis longus	1.32	0.04	1.24	0.10	0.83	0.07	0.75	0.05	*	*	
Total	7.99	0.30	7.51	0.32†	5.04	0.36	4.44	0.29†	*	*	
Adipose tissue											
Retroperitoneal	5.18	2.21	4.40	1.03	2.91	1.20	1.87	0.53	*		
Perirenal	1.44	0.46	1.35	0.36	1.28	0.57	0.64	0.33†	*		
Epididymal / parametrial	6.25	1.15	5.78	1.05	5.68	1.86	4.13	1.03†			
Mesenteric	5.40	1.66	3.23	1.35†	3.49	1.78	1.93	0.90	*	*	
Total	18.26	5.25	14.77	3.52	13.36	4.96	8.56	2.59†	*	*	
Skin	92.5	2.93	87.3	6.02	47.3	6.85	44.7	3.54	*		
Carcass	308.4	9.18	288.4	10.51†	177.7	12.54	165.4	11.79	*	*	

*$p < 0.05$, Two-way ANOVA. †$p < 0.05$, vs. Pre (t-test). SD, standard deviation; ANOVA, analysis of variance

Table 2-1. Tissue weight before and after BW reduction (g/100 g)

	Male				Female				Two-way ANOVA		
	Pre		Post		Pre		Post		Sex	BW reduction	Sex × BW reduction
	mean	SD	mean	SD	mean	SD	mean	SD			
Organ											
Heart	0.22	0.01	0.25	0.02†	0.26	0.04	0.27	0.02	*		
Liver	3.24	0.28	2.43	0.19†	3.20	0.19	2.43	0.09†		*	
Kidneys	0.68	0.04	0.68	0.05	0.68	0.05	0.65	0.04			
Adrenal	0.01	0.00	0.01	0.00	0.02	0.00	0.02	0.00†	*		
Pancreas	0.33	0.06	0.36	0.06	0.51	0.08	0.42	0.05	*		*
Spleen	0.17	0.01	0.16	0.03	0.19	0.02	0.18	0.02			
Intestines	1.39	0.10	1.49	0.12	1.77	0.14	2.11	0.22†	*	*	
Total	6.05	0.21	5.38	0.20†	6.64	0.27	6.07	0.23†	*	*	

*$p<0.05$, Two-way ANOVA. †$p < 0.05$, vs. Pre (t-test).

SD, standard deviation; ANOVA, analysis of variance

Table 2-2. Tissue weight before and after BW reduction (g/100g)

	Male				Female				Two-way ANOVA		
	Pre		Post		Pre		Post		Sex	BW reduction	Sex × BW reduction
	mean	SD	mean	SD	mean	SD	mean	SD			
Skeletal muscle											
Gastrocnemius	1.05	0.03	1.12	0.03†	1.13	0.05	1.15	0.05	*	*	
Soleus	0.06	0.01	0.07	0.01	0.07	0.01	0.07	0.01	*		
Plantaris	0.31	0.30	0.19	0.01	0.21	0.02	0.20	0.02			
Flexor hallucis longus	0.26	0.01	0.27	0.02	0.28	0.02	0.29	0.01	*		
Total	1.68	0.30	1.66	0.04	1.69	0.08	1.71	0.07			
Adipose tissue											
Retroperitoneal	1.81	2.10	0.96	0.19	0.97	0.37	0.72	0.20			
Perirenal	0.28	0.08	0.30	0.07	0.42	0.15	0.24	0.13†			
Epididymal / parametrial	1.21	0.20	1.27	0.20	1.88	0.52	1.58	0.36	*		
Mesenteric	1.05	0.30	0.72	0.27	1.14	0.47	0.73	0.31		*	
Total	4.34	2.32	3.25	0.67	4.40	1.32	3.27	0.92			
Skin	18.0	0.36	19.2	0.84†	15.8	1.20	17.2	0.39	*	*	
Carcass	60.0	0.86	63.6	1.30†	59.8	2.92	63.6	0.82†		*	

*$p<0.05$, Two-way ANOVA. †$p < 0.05$, vs. Pre (t-test). SD, standard deviation; ANOVA, analysis of variance

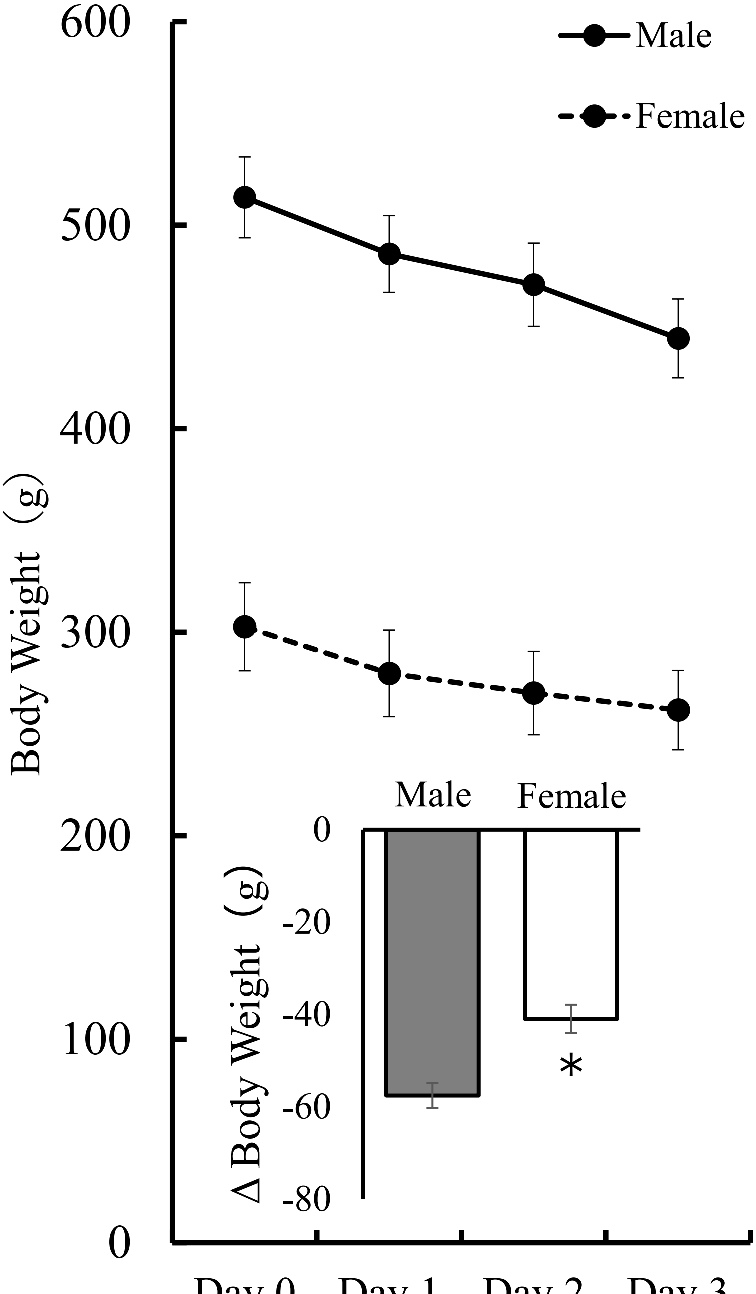
Table 3. Whole- body protein, total lipid, and glycogen content

	Male				Female				Two-way ANOVA		
	Pre		Post		Pre		Post		Sex	BW reduction	Sex × BW reduction
	mean	SD	mean	SD	mean	SD	mean	SD			
Wight (g)											
Protein	86.9	7.64	76.8	4.80 †	54.0	7.23	47.4	3.43	*	*	
Total lipid	58.6	8.21	50.3	5.84	37.0	9.61	27.5	3.68 †	*	*	
Glycogen	0.25	0.02	0.23	0.01 †	0.23	0.05	0.19	0.02	*	*	
Weight BW (%)											
Protein	16.9	1.09	16.9	1.13	18.1	0.91	18.2	0.44	*		
Total lipid	11.4	1.36	11.1	0.91	12.3	2.15	10.6	1.16			
Glycogen	0.05	0.00	0.05	0.00	0.08	0.01	0.07	0.01	*		

*p < 0.05, Two-way ANOVA. †p < 0.05, vs. Pre (t-test).

SD, standard deviation; BW, body weight; ANOVA, analysis of variance

Figure 1



600
500
400
300
200
100
0
Body Weight（g)
Male
Female
Day 0
Day 1
Day 2
Day 3
Male
Female
0
-20
-40
-60
-80
Δ Body Weight（g)
*

Figure 2

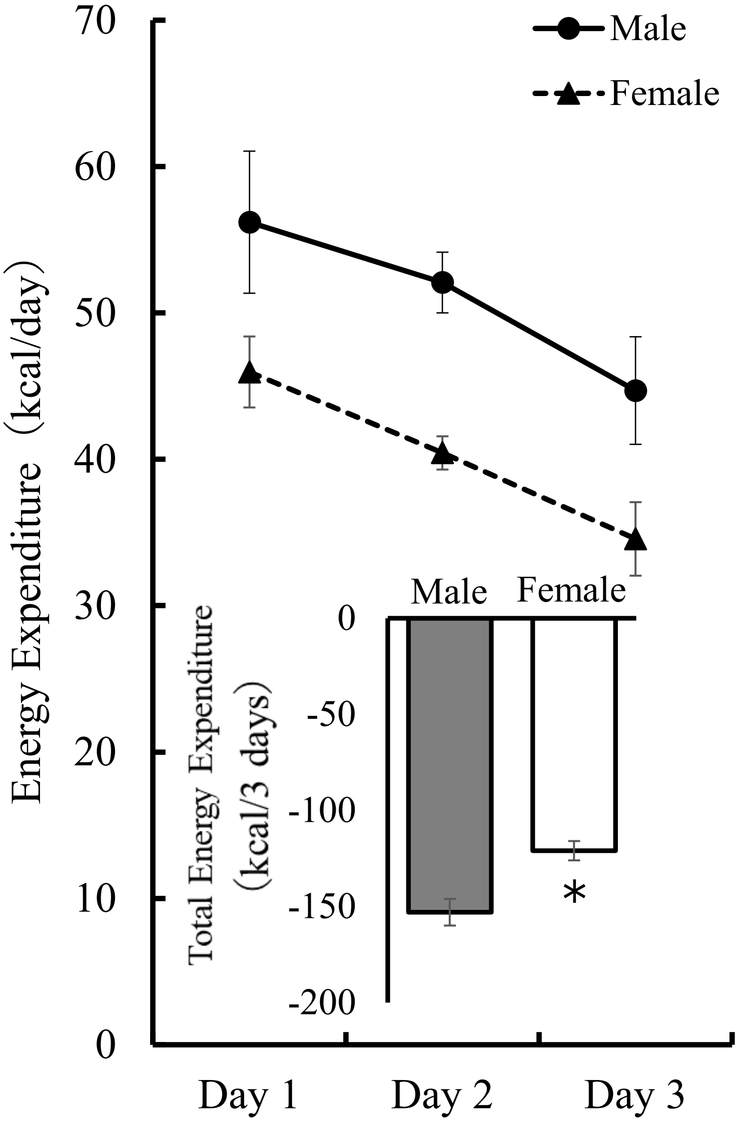
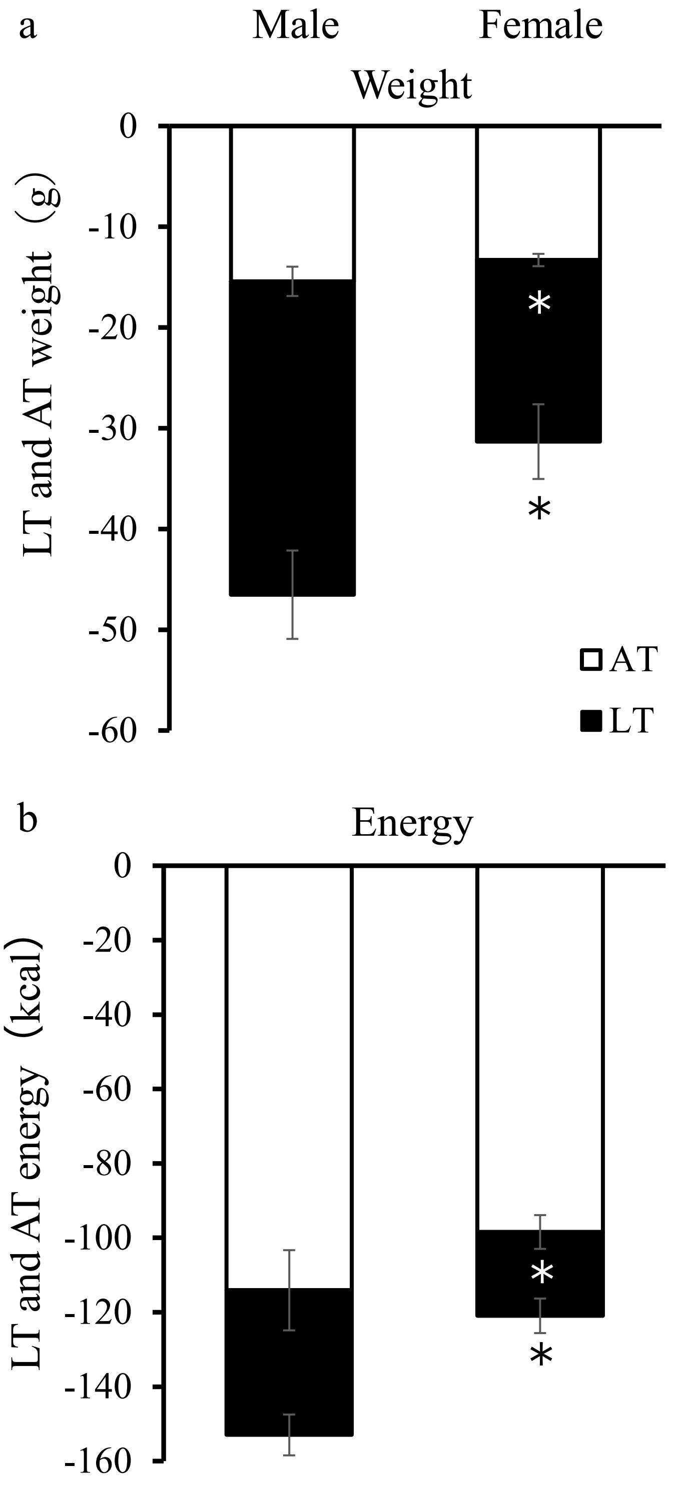

70
60
50
40
30
20
10
0
Energy Expenditure（kcal/day）
Male
Female
Day 1
Day 2
Day 3
Total Energy Expenditure
（kcal/3 days）
Male
Female
0
-50
-100
-150
-200
*

Figure 3


a
Male
Female
Weight
0
-10
-20
-30
-40
-50
-60
LT and AT weight（g）
*
*
AT
LT
b
Energy
0
-20
-40
-60
-80
-100
-120
-140
-160
LT and AT energy（kcal）
*
*