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Body weight reduction by exercise increases the urinary 3-methylhistidine excretion level with relatively positive nitrogen, sodium, and potassium balances when compared to dietary restriction

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ABSTRACT

Background: Regarding changes in muscle mass, differences due to types of exercise and/or nutritional interventions, and associations with nutrient balances are still unclear.

Methods: To estimate changes in muscle mass during a body weight loss program using either a diet or exercise regimen, we investigated levels of muscle mass-related indices, and body contents of nitrogen, sodium, and potassium as measured by the balance method. Six healthy young adult male volunteers participated in two 10-day crossover experiments (20 days total). The first 5 days comprised an adjustment period (energy intake, 2656 ± 367 kcal/day (mean ± standard deviation)). During the second 5-day period, the participants either reduced their energy intake to 1770 ± 244 kcal/day (diet regimen) or exercised on a bicycle ergometer to expend 886 ± 122 kcal/day (exercise regimen).

Results: The nitrogen, sodium, and potassium balances were significantly more positive during the exercise regimen than during the diet regimen. The urinary excretion levels of creatinine, 3-methylhistidine (3-MH), aldosterone, and catecholamines, and the 3-MH/creatinine ratio were significantly increased only during the exercise regimen.

Conclusions: The exercise regimen suppresses the decrease in muscle mass-related indices during body weight loss compared to the diet regimen with a relatively positive state of whole-body protein, sodium, and potassium balances, accompanied by an increase in sympathetic/adrenal cortical functions.

Abbreviations: 3-MH, 3-methylhistidine; AI, atherosclerosis index; CK, creatine kinase; TP, total protein; RBP, retinol-binding protein; UN, urea nitrogen; A/G ratio, albumin-globulin ratio.

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1. Introduction

People undergo body weight reduction for various reasons, *e.g.,* to address lifestyle-related diseases associated with an excessive body fat mass and/or dysfunctional glucose and lipid metabolism, for aesthetic reasons, or to improve the competitiveness of athletes. The purpose of achieving a negative energy balance is generally not to simply lose body weight, but specifically to lose fat tissue, and it is thus important to suppress decreases in muscle mass.

We previously performed a crossover trial to compare the effects of body weight reduction induced by exercise or by dietary restriction on lipid metabolism. In that study, the amount of additional energy expenditure by exercise or the decrease in energy intake by dietary restriction was one-third (886 kcal/day on average) of the energy intake during the adjustment period. The degree of the increase in the serum high density lipoprotein-cholesterol (HDL-C) level after 5 days of intervention was larger when the body weight was reduced by exercise than when it was reduced by dietary restriction. Thus, the atherosclerosis index (AI), calculated as (total cholesterol (TC) – HDL-C)/HDL-C, was improved only by exercise. These results are thought to be related to multiple factors, such as muscle mass, the individual's underlying nutritional status, and increased metabolic energy expenditure.

We hypothesized that the improvement of lipid metabolism by 5 days of exercise in the previous study was due in part to suppression of the decrease in muscle mass and increased metabolic energy expenditure. However, we did not measure muscle mass in that study. Therefore, to assess this hypothesis, we measured several indirect indices: urinary excretion levels of creatinine, 3-methylhistidine (3-MH), and nutrient balance values as proxies for suppression of a decrease in muscle mass. Furthermore, to confirm increased metabolic energy expenditure, we measured urinary excretion levels of catecholamines.

Although muscle mass can be measured fairly accurately with body composition analyzers, it is not easy to evaluate changes in muscle mass over a short period of time. Urinary creatinine excretion correlates with muscle mass, but it is useful only in those under a meat-free diet [1,2]. The urinary 3-MH excretion level is dependent on the rate of muscle protein degradation and the amount of muscle mass in the body [3,4]. Therefore, when muscle metabolism is in a steady state under a meat-free diet, the amount of muscle mass can be estimated from the amount of 3-MH excreted in urine. It has been reported that the urinary 3-MH excretion levels did not change after 4 days of starvation [5]. Regarding the effects of exercise, Dohm reported that the urinary 3-MH excretion levels increased after both running and weight-lifting [6], and other studies have also reported an increase in urinary 3-MH excretion levels after aerobic exercise [7], eccentric exercise [8], or resistance exercise [9].

Skeletal muscle is mainly composed of protein and has the high concentration of potassium [10]. Therefore, during muscle hypertrophy, these nutrients are accumulated in the muscle. In addition, positive sodium balances have been reported along with increases in the plasma volume during exercise training [11,12], and the circulating renin-angiotensin-aldosterone axis plays a critical role in regulating the basal body sodium and chloride levels [10]. Catecholamines accelerate energy metabolism and enhance the function of the circulatory system [13], leading to increases in its secretion during exercise.

In the present study, we investigated changes in muscle mass-related indices, body nitrogen, sodium, and potassium contents by the balance method, and sympathetic/adrenal medulla function as an indicator of increased metabolic energy expenditure, including their relationships with lipid metabolism as identified in our previous study.

2. Materials and methods

2.1. Subjects

The subjects were six healthy adult male volunteers (age, 21 ± 2 years (mean ± standard deviation); body mass index, 22.6 ± 3.3 kg/m^2). In this experiment, the subjects were limited to healthy young males to avoid the effects of the menstrual cycle. A subject

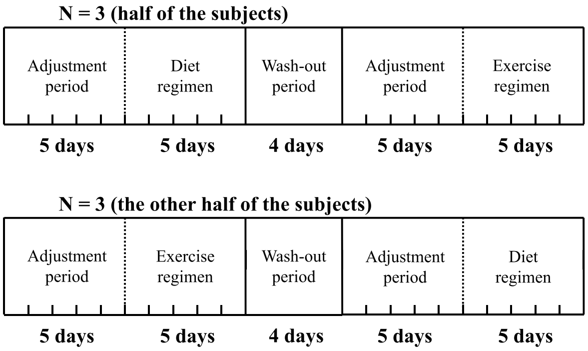

Fig. 1. Experimental protocol. Fasting blood samples were collected in the early morning on the first day of each body weight reduction period (the 6th day of each 10-day experiment) and at the end of each body weight reduction period (the morning after day 10 of each 10-day experiment), and 24-h urine samples were collected daily during the adjustment period and the diet or exercise regimen periods. Whole-body sweat samples were collected from the 4th to 10th days of the 10-day experiments. Feces samples collected on 4 consecutive days during the adjustment period (from the 2nd to 5th days of each 10-day experiment) were pooled, and those collected on 4 consecutive days during the body weight reduction period (from the 6th to 9th days of each 10-day experiment) were also pooled.

briefing session was held to explain the methods of the current study, including the 20-day restriction of usual life activities, stringent dietary restrictions, and exercise load. Consequently, six subjects were enrolled, which was the smallest number needed for the statistical analysis. G*Power 3.1 was used for power analysis [14,15]. That is, the power for detecting differences was calculated using alpha (0.05), sample size (6), and effect size (calculated from means, standard deviations, and Spearman's correlation coefficients between pairs of variables). For the degree of changes in the excretion levels of urinary creatinine and 3-MH (the main parameters in the present study), the power for detecting differences was 90% and more than 99%, respectively. In addition, for the degree of change in HDL-C and AI (the main parameters in the previous study), it was 95% and more than 99%, respectively. The subjects provided written informed consent to participate in all procedures associated with the study, which adhered to the tenets of the Declaration of Helsinki.

2.2. *Experimental procedures*

The Research Ethics Committee of Kanto Gakuin University approved this study.

2.2.1. *Experimental protocol*

The study comprised two 10-day experiments (20 days in total; Fig. 1). All six subjects participated in both experiments. One experiment used only dietary restriction as the intervention (diet regimen), while the other experiment used only exercise as the intervention (exercise regimen). Each experiment consisted of two 5-day periods (10 days total): the first half was a body weight-maintained adjustment period, and the second half was the treatment period. After the end of the first 10-day experiment, there was a 4-day wash-out period during which the subjects had access to an *ad libitum* diet before the second 10-day experiment started. The two experiments were performed with a crossover design, *i.e.,* the diet regimen was followed by the exercise regimen for half of the participants, and the order was reversed for the other half of the participants.

For each experiment, the subjects were gathered at Kanto Gakuin University in the evening of the day before the start of the experiment. The experiment started at 07:00 on the first day. Throughout the experiment, the subjects remained at the university, and stayed in the metabolic experiment building.

During the adjustment period from day 1 to day 5, the subjects woke up at 06:30, performed the final urine collection for the day before at 07:00, then began collecting all urine for the next 24 h from that point on. Breakfast was served between 07:45 and 08:15, lunch was served between 13:15 and 13:45, and dinner was served between 19:00 and 19:30. Other than taking a shower between 20:00 and 22:00, and going to bed at 22:30, they could spend their time freely in the metabolic experiment building doing as they liked, except for eating and additional physical activity.

The experimental periods (the diet regimen or the exercise regimen) lasted from day 6 to day 10. In the diet regimen, the daily schedule remained unchanged from that in the adjustment period, and only the diet was reduced. In the exercise regimen, the daily schedule remained unchanged from that in the adjustment period, except for the addition of the exercise load that was performed from 09:45 in the morning, and the diet was the same as that during the adjustment period.

The room temperature during the experimental period was maintained at a dry bulb temperature of 25 °C–26 °C, and at a wet bulb temperature of 22 °C–23 °C.

2.2.2. *Experimental diet*

For the experimental diet, homogeneous and predominantly meat-free foods (crackers, margarine, jam, cheese, milk, vegetable juice, spaghetti, meat sauce, mashed potatoes, mayonnaise, milled rice, soy protein processed food, soy sauce, miso, *Komachi-fu* (a gluten product), skimmed milk, and soda) were used as ingredients. The experimental diet during the body weight-maintained adjustment period was set to meet the recommended dietary allowance or adequate intake defined in the Dietary Reference Intakes for Japanese [16]. The specific dietary energy intake amount was determined for each participant individually based primarily on their body weight, and the amounts of all foods provided in their diet were adjusted accordingly. For a body weight of 60 kg, the determined dietary energy intake was 2427 kcal/day, the protein:fat:carbohydrate ratio was 13.6:29.6:56.7, and the actual energy intake during the adjustment period of this study was 2656 ± 367 kcal/day. During the diet regimen, the experimental diet consisted of two-thirds of the energy intake consumed during the adjustment period (1770 ± 244 kcal/day). The amounts of all foods were reduced in proportion to match the energy intake levels required for each body weight reduction period.

2.2.3. *Procedures for the exercise*

An exercise tolerance test was performed before the start of this study involving gradually increasing work rate on a bicycle ergometer. Heart rate was recorded by telemetry (DS-3400, Fukuda Denshi Co., Ltd., Tokyo), and oxygen intake was measured by the Douglas bag method (using a dry gas meter (DC-5A; Shinagawa Corp., Tokyo), and an expired gas monitor (1H21B; NEC San-ei Instruments Ltd., Tokyo)).

In the exercise regimen, to match the level of negative energy balance during the diet regimen, the amount of additional energy expenditure induced by exercise was one-third of the energy intake during the adjustment period (886 ± 122 kcal/day). To improve lipid metabolism, aerobic exercise with a bicycle ergometer was used, and the intensity was set to a moderate level (50%–60% of the maximal oxygen intake). Work rate was set at the value achieved at a heart rate of about 130 beats/min. The duration of exercise (164 ± 22 min every morning) was determined by dividing the total additional energy expenditure by the additional energy expenditure (/min) at the work rate. There was a 10-min rest period following each 45 min of exercise.

2.3. Sample collection and measurements

2.3.1. Blood metabolic parameters

Fasting blood samples were collected on the first day of each body weight reduction period (the 6th day of each 10-day experiment) and at the end of each body weight reduction period (the morning after day 10 of each 10-day experiment). The blood samples were sent to Mitsubishi Kagaku Bio-Clinical Labs, Inc. (Tokyo, Japan) to measure the levels of creatine kinase (CK), total protein (TP), retinol-binding protein (RBP), urea nitrogen (UN), and sodium and potassium, and to determine the albumin-globulin ratio (A/G ratio). Each regimen started after blood collection on the first day, and the last bout of exercise during the exercise regimen was completed 18 h before blood collection at the end of the body weight reduction period.

2.3.2. Urinary metabolic parameters

Twenty-four-hour urine samples were collected daily throughout the experiment to measure the urine volume and levels of creatinine [17], 3-MH [18], aldosterone [19], and three types of catecholamines (adrenaline, noradrenaline, and dopamine) [20], the excretion levels of which were then calculated. To determine reference values for the urinary excretion levels, the average from the final 2 days of the adjustment period was used as it would allow subjects to adjust for 3 days during the adjustment period before the reference values were determined. For the calculation of balance data, the averages from 4 days (the 6th to 9th days of each 10-day experiment) were used in both the diet regimen and the exercise regimen as the fecal collection period was also 4 days in length.

2.3.3. Samples for calculating the nutrient balances

As for the experimental diets, breakfast, lunch, and dinner samples were prepared and freeze-dried to obtain homogeneous powders for measurements. Whole-body sweat was collected from the 4th to 10th days of the 10-day experiment during the time period corresponding to exercise performance according to the method of Vellar et al. [21,22]. Fecal samples collected on 4 consecutive days during the adjustment period (from the 2nd to 5th days of each 10-day experiment) were pooled, and those collected on 4 consecutive days during the body weight reduction period (from the 6th to 9th days of each 10-day experiment) were also pooled. The pooled samples were stored in a freezer, and subsequently homogenized.

The nitrogen contents of the experimental diet, urine, sweat, and fecal samples were determined by the Kjeldahl method. The sodium and potassium contents in the samples of drinking water (natural mineral water), urine, sweat, aqueous solutions of the experimental diets, and feces after dry ashing were determined by diluting the samples and measuring the optical density with an atomic absorption spectrometer (AA-6300; Shimadzu Corporation, Kyoto, Japan).

To calculate the balance data, the averages for urine and sweat from the final 2 days of the adjustment period were used, the average for feces from 4 days of the adjustment period (the 2nd to 5th days of each 10-day experiment) was used, and the average values for urine, sweat, and feces from 4 days of each of the diet and exercise regimen periods (the 6th to 9th days of each 10-day experiment) were used.

2.4. Statistical analyses

The results are expressed as the median (range), because there was no guarantee that the data were normally distributed. All statistical analyses were performed using IBM SPSS Statistics 27 (IBM Corporation, Armonk, NY, USA). We applied the Wilcoxon signed-ranks test or Friedman's test to examine differences in the measures of central tendency, and evaluated the relationships

Table 1
Comparison of the blood metabolic parameters.

	Diet regimen			Exercise regimen		
	Before	After	Δ1	Before	After	Δ2
CK (IU/l)	80	81	0	93	234 *#	+150 #
	(72–122)	(72–131)	(-7 to +9)	(65–155)	(91–560)	(+15 to +473)
TP (mg/dl)	7.3	7.3	+0.1	7.3	7.2	-0.1
	(6.6–7.6)	(6.7–7.8)	(-0.1 to +0.2)	(6.7–7.8)	(6.7–7.6)	(-0.4 to +0.1)
A/G ratio	1.9	2.0	+0.1	2.0	2.1	0.0
	(1.7–2.3)	(1.7–2.6)	(-0.1 to +0.3)	(1.7–2.6)	(1.8–2.5)	(-0.1 to +0.2)
RBP (mg/dl)	3.9	3.8	-0.3	4.0	4.0	-0.1
	(3.5–5.4)	(3.1–5.1)	(-0.9 to +0.1)	(3.2–5.3)	(3.2–5.2)	(-0.3 to +0.2)
UN (mg/dl)	13	13	0	14	14 #	+1
	(12–16)	(10–17)	(-2 to +3)	(11–16)	(12–20)	(-1 to +4)
Sodium (mEq/l)	140	141	+1	140	142	+2
	(139–141)	(139–143)	(0 to +3)	(140–143)	(139–145)	(-4 to +5)
Pottasium (mEq/l)	4.1	4.2	0.0	4.2	4.1	-0.2
	(3.7–4.8)	(3.8–4.4)	(-0.4 to +0.3)	(3.9–5.0)	(3.6–4.8)	(-0.5 to +0.2)

N = 6, variables are given as medians (minimum value to maximum value).
Δ1, degree of change after the diet regimen; Δ2, degree of change after the exercise regimen.
*$p < 0.05$ compared to before the diet regimen or the exercise regimen, #$p < 0.05$ compared to the diet regimen.
CK, creatine kinase; TP, total protein; A/G ratio, albumin-globulin ratio; RBP, retinol-binding protein; UN, urea nitrogen.

between pairs of variables using the Spearman’s correlation coefficient. Significance was established at $p < 0.05$ in all analyses.

3. Results

3.1. Blood metabolic parameters

Table 1 shows the changes in the levels of CK, protein metabolism-related parameters, sodium, and potassium. The CK levels remained unchanged after the diet regimen, but significantly increased after the exercise regimen when compared to the adjustment period, and the levels were significantly higher after the exercise regimen than after the diet regimen. Furthermore, the degree of change (Δ) in the CK level (Δ CK) was significantly larger with the exercise regimen than with the diet regimen.

The TP, RBP, and UN levels, and the A/G ratio were almost the same before and after either of the diet and exercise regimens. The sodium and potassium levels also did not significantly change after either of the regimens.

3.2. Balance data for nitrogen, sodium, and potassium

The intake and excretion levels of nitrogen, sodium, and potassium are shown in Table 2, and data on their balances (for 2 days

Table 2
Comparison of the intake and excretion levels of nitrogen, sodium and potassium.

				Excretion			
			Intake	Urine	Feces	Sweat	Total
			(mg/day)				
Nitrogen (mg/day)	Diet regimen	Before	14673 (10950–16644)	13136 (10142–14269)	1626 (640–2207)	48 (15–67)	14836 (10796–16542)
		During	9782 * (7300–11096)	10936 * (8899–12490)	1485 (1178–1831)	36 * (11–64)	12634 * (10250–14005)
		Δ1	- 4891 (- 5548 to −3650)	- 1978 (- 3077 to −659)	- 62 (- 560 to + 700)	- 9 (- 33 to −1)	- 2202 (- 2940 to −546)
	Exercise regimen	Before	14673 (10950–16644)	12813 (9676–14235)	1524 (722–2289)	37 (20–92)	14428 (10418–16616)
		During	14673 # (10950–16644)	14307 *# (11075–15741)	1625 (1332–2310)	463 *# (161–701)	16363 *# (12909–18752)
		Δ2	0 # (0–0)	+ 1453 # (+ 960 to + 2550)	- 22 (- 137 to + 951)	+ 414 # (+ 141 to + 609)	+ 2169 # (+ 1267 to + 2895)
Sodium (mg/day)	Diet regimen	Before	3658 (2737–4150)	3267 (2638–3983)	40 (7–140)	9 (4–18)	3333 (2662–4141)
		During	2446 * (1848–2764)	2504 * (1771–2859)	103 * (10–263)	6 * (3–15)	2546 * (1811–3137)
		Δ1	- 1211 (- 1387 to −889)	- 865 (- 1125 to −653)	+ 62 (+ 4 to + 123)	- 3 (- 4 to −1)	- 840 (- 1018 to −564)
	Exercise regimen	Before	3657 (2755–4155)	3050 (2515–3991)	48 (9–157)	11 (4–33)	3170 (2533–4097)
		During	3689 *# (2772–4202)	2437 * (1811–2826)	34 (8–110)	664 *# (280–1057)	3102 # (2277–3600)
		Δ2	+ 32 # (+ 17 to + 56)	- 729 (- 1427 to −286)	- 8 # (- 49 to + 38)	+ 652 # (+ 276 to + 1044)	- 195 # (- 497 to + 87)
Potassium (mg/day)	Diet regimen	Before	2686 (2005–3047)	2372 (2081–2777)	300 (96–551)	20 (9–38)	2723 (2312–3116)
		During	1791 * (1337–2031)	2150 * (1648–2638)	314 (107–408)	13 (8–24)	2470 * (1924–2948)
		Δ1	- 895 (- 1016 to −668)	- 183 (- 584 to −6)	- 17 (- 160 to + 141)	- 3 (- 25 to + 1)	- 176 (- 597 to −106)
	Exercise regimen	Before	2686 (2005–3047)	2637 (1818–2854)	321 (132–357)	16 (9–36)	2898 (1961–3225)
		During	2687 # (2006–3049)	2327 (1823–2908)	389 # (173–475)	253 *# (159–463)	2974 # (2263–3662)
		Δ2	+ 1 # (0 to + 2)	- 169 (- 622 to + 224)	+ 111 # (- 14 to + 159)	+ 232 # (+ 150 to + 447)	+ 297 (- 416 to + 603)

N = 6, variables are given as medians (minimum value to maximum value).
Δ1, degree of change after the diet regimen; Δ2, degree of change after the exercise regimen.
*$p < 0.05$ compared to before the diet regimen or the exercise regimen, #$p < 0.05$ compared to the diet regimen.

during the adjustment period, and for 4 days during the diet or exercise regimen) are shown in Fig. 2. The intake levels during the diet regimen were approximately two-thirds of those during the adjustment period, and the intake levels during the exercise regimen were almost the same as those during the adjustment period. The sodium and potassium intakes increased slightly as the intake of natural mineral water increased during the exercise regimen.

The urinary nitrogen excretion levels were significantly decreased during the diet regimen, and significantly increased during the exercise regimen when compared to the adjustment period. There was no significant difference in the fecal excretion levels between the two regimens, and the loss of nitrogen from the dermis was significantly higher during the exercise regimen than during the diet regimen. The results for the total nitrogen excretion levels were similar to those for the urinary excretion levels. As a result, the nitrogen balance data shifted significantly in the negative direction with either regimen in comparison to the adjustment period, but the degree of the negative shift was greater with the diet regimen than with the exercise regimen. The Δ nitrogen balance in the negative direction was also greater with the diet regimen than with the exercise regimen.

The urinary sodium excretion levels were significantly decreased during both the diet and exercise regimens when compared to the adjustment period. The fecal excretion levels did not differ significantly between the two regimens, and the loss of sodium from the dermis was significantly higher during the exercise regimen than during the diet regimen. The total sodium excretion levels decreased significantly during the diet regimen, but did not change significantly during the exercise regimen when compared to the adjustment period. As a result, the sodium balance data shifted significantly in the negative direction with the diet regimen when compared to the adjustment period, but no such change was seen between the exercise regimen and the adjustment period, and the shift was significantly more negative with the diet regimen than with the exercise regimen. The Δ sodium balance was also more negative with the diet regimen than with the exercise regimen.

The urinary potassium excretion levels were significantly decreased during the diet regimen, and did not change significantly during the exercise regimen when compared to the adjustment period. The fecal excretion levels were significantly higher during the exercise regimen than during the diet regimen, and the loss of potassium from the dermis was significantly higher during the exercise regimen than during the diet regimen. The total potassium excretion levels decreased significantly during the diet regimen, and did not change significantly during the exercise regimen when compared to the adjustment period. As a result, similar to the results for sodium, the potassium balance data shifted significantly in the negative direction with the diet regimen when compared to the adjustment period, but no such change was seen between the exercise regimen and the adjustment period, and the shift was significantly more negative with the diet regimen than with the exercise regimen. The Δ potassium balance tended to be more negative (p = 0.063) with the diet regimen than with the exercise regimen.

3.3. Urinary metabolic parameters

The daily changes in the levels of urinary creatinine, 3-MH, aldosterone, and three types of catecholamines (adrenaline, noradrenaline, and dopamine) are shown in Table 3, and the pooled mean data for these parameters (for 2 days during the adjustment period, and for 4 days during the diet or exercise regimen) are shown in Figs. 3 and 4. The urinary creatinine excretion levels decreased significantly during the diet regimen, but increased significantly during the exercise regimen when compared to the adjustment period. The urinary 3-MH excretion levels (both the absolute values and per creatinine values) increased significantly during the exercise regimen when compared to the adjustment period, and were significantly higher during the exercise regimen than during the diet regimen.

The urinary aldosterone excretion levels significantly increased during the exercise regimen when compared to the adjustment period. In comparison to the adjustment period, the urinary adrenaline excretion levels were significantly increased during the exercise regimen, the noradrenaline excretion levels were significantly decreased during the diet regimen and significantly increased during the exercise regimen, and the dopamine excretion levels were significantly decreased during the diet regimen. In addition, the values of all of these parameters were significantly higher during the exercise regimen than during the diet regimen.

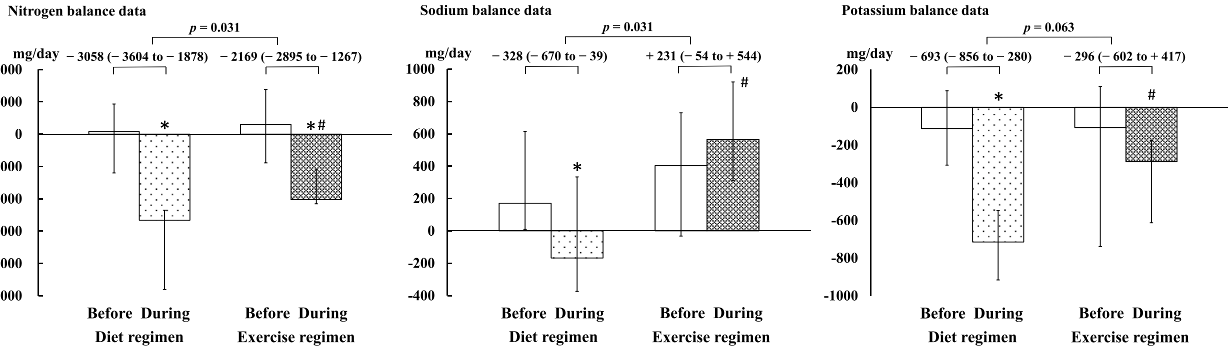

Fig. 2. Comparison of the balance data for nitrogen, sodium, and potassium (for 2 days during the adjustment period, and for 4 days during the diet or exercise regimen). N = 6. Data are shown as the median (range). *$p < 0.05$ compared to before the diet regimen or the exercise regimen. #$p < 0.05$ compared to the diet regimen.

Table 3
Changes in the excretion levels of urinary metabolic parameters.

		Before	Day 1	Day 2	Day 3	Day 4	p-value[a]
Creatinine (mg/day)	Diet regimen	1707 (1101–1821)	1684 (1067–1775)	1617 (1060–1793)	1709 (1076–1846)	1632 (1059–1783)	**0.049**
	Exercise regimen	1645 (1091–1898)	1693 (1117–1872)	1707 (1133–1894)	1734 (1028–1983)	1753 (1109–1882)	0.290
3-MH (µmol/day)	Diet regimen	229 (138–277)	204 (145–268)	228 (147–254)	233 (139–260)	223 (138–253)	0.361
	Exercise regimen	220 (132–249)	235 (138–259)	252 (172–282)	257 (164–307)	256 (158–303)	**< 0.001**
(µmol/g • creatinine/day)	Diet regimen	134.9 (124.9–156.5)	132.3 (116.1–150.7)	137.7 (127.8–159.5)	135.1 (126.0–142.9)	132.7 (127.2–146.6)	0.659
	Exercise regimen	131.0 (121.1–147.5)	129.7 (123.6–151.7)	146.4 (135.4–157.0)	152.9 (136.0–159.1)	144.9 (136.0–161.0)	**< 0.001**
Aldosterone (µg/day)	Diet regimen	10.7 (6.4–14.3)	12.6 (6.4–17.3)	11.7 (6.2–14.5)	9.8 (5.9–15.1)	7.7 (6.0–16.6)	0.133
	Exercise regimen	10.4 (6.5–12.3)	14.9 (9.8–20.5)	15.4 (11.3–25.0)	14.0 (9.8–19.4)	11.9 (9.2–14.2)	**0.012**
Adrenaline (µg/day)	Diet regimen	11.28 (9.13–13.31)	13.92 (10.71–17.82)	11.37 (9.28–19.32)	12.51 (9.40–16.77)	12.81 (9.89–17.16)	0.192
	Exercise regimen	11.39 (8.09–13.12)	23.27 (20.58–39.59)	21.47 (15.70–27.98)	16.51 (13.26–23.86)	17.13 (12.14–20.69)	**< 0.001**
Noradrenaline (µg/day)	Diet regimen	97.0 (44.7–120.3)	91.8 (47.4–127.1)	86.3 (37.1–113.4)	85.2 (43.4–103.0)	79.9 (49.7–94.2)	**0.006**
	Exercise regimen	87.5 (42.6–123.1)	161.2 (78.1–190.9)	172.4 (73.1–198.8)	145.4 (64.8–191.8)	146.3 (73.7–177.9)	**< 0.001**
Dopamine (µg/day)	Diet regimen	962 (647–1135)	776 (560–899)	739 (547–837)	753 (498–858)	757 (527–847)	**0.001**
	Exercise regimen	897 (655–1128)	891 (671–1110)	880 (622–1144)	875 (623–1122)	949 (654–1066)	0.898

N = 6, variables are given as medians (minimum value to maximum value).
3-MH, 3-metylhistidine.

[a] Friedman's test (before to day 4).

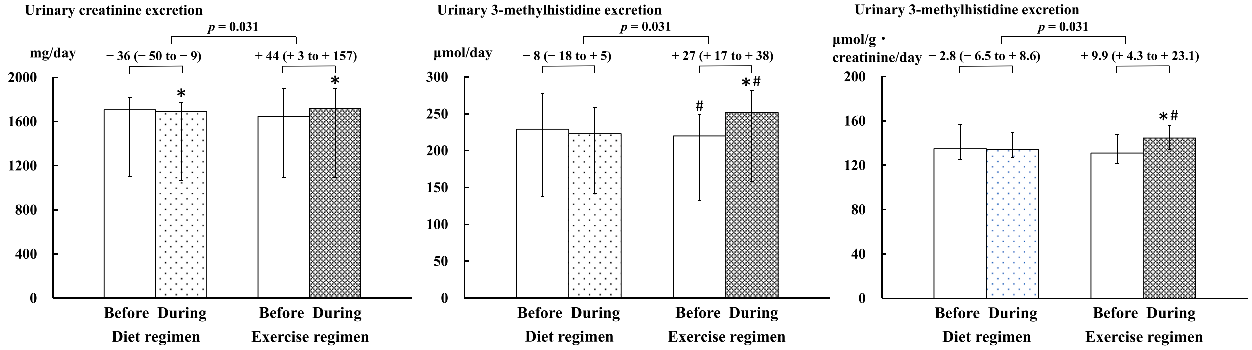

Fig. 3. Comparison of the urinary creatinine and 3-MH excretion levels (for 2 days during the adjustment period, and for 4 days during the diet or exercise regimen). N = 6. Data are shown as the median (range). *$p < 0.05$ compared to before the diet regimen or the exercise regimen. #$p < 0.05$ compared to the diet regimen.

Furthermore, the Δ urinary creatinine, Δ 3-MH, Δ aldosterone, Δ adrenaline, Δ noradrenaline, and Δ dopamine excretion levels were more positive with the exercise regimen than with the diet regimen.

3.4. Correlations among the balance data and urinary metabolic parameters

Spearman's correlation coefficients (the Δ with the two regimens (N = 12)) were calculated to examine the relationships among the balance data (nitrogen, sodium, and potassium balances) and urinary excretion levels (creatinine, 3-MH, aldosterone, and three types of catecholamines (adrenaline, noradrenaline, and dopamine); Table 4). The Δ nitrogen and Δ potassium balances were significantly positively correlated ($r = 0.657$, $p < 0.05$). The Δ sodium balance was significantly positively correlated with the Δ urinary 3-MH, and Δ urinary catecholamine excretion levels ($r = 0.713$ to 0.804, $p < 0.01$). The Δ urinary creatinine and Δ urinary 3-MH excretion levels

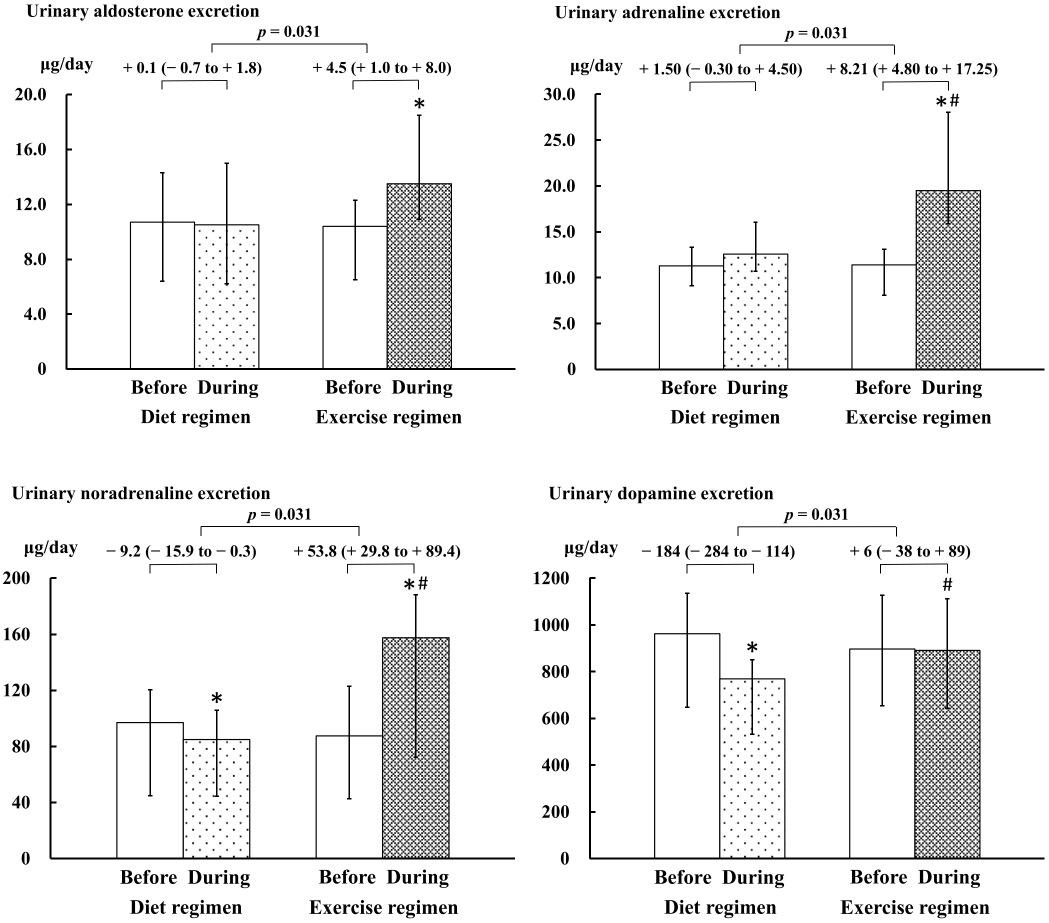

Fig. 4. Comparison of the urinary aldosterone and three types of catecholamines (adrenaline, noradrenaline, and dopamine) excretion levels (for 2 days during the adjustment period, and for 4 days during the diet or exercise regimen). N = 6. Data are shown as the medians (range). $^*p < 0.05$ compared to before the diet regimen or the exercise regimen. $\#p < 0.05$ compared to the diet regimen.

were highly positively correlated with each other ($r = 0.832$, $p < 0.001$), and highly positively correlated with the Δ urinary catecholamine excretion level ($r = 0.804$ ($p < 0.01$) to 0.902 ($p < 0.001$)). The Δ urinary aldosterone excretion level was also significantly positively correlated with the Δ urinary catecholamine excretion level ($r = 0.720$ to 0.797, $p < 0.01$).

3.5. *Correlations between urinary 3-MH excretion and lipid metabolism in the previous study*

The Δ urinary 3-MH excretion level was not significantly correlated with the Δ serum triglyceride and Δ serum remnant-like particle-cholesterol, whereas it was significantly negatively correlated with the Δ AI ($r = -0.599$, $p = 0.040$).

4. Discussion

We previously performed a crossover trial to compare the effects of body weight reduction induced by exercise or by dietary restriction on lipid metabolism. In that study, we concluded that reducing body weight by exercise had more beneficial effects on lipid metabolism than a dietary approach. We hypothesized that these effects of exercise occur as a result of multiple factors, including the underlying nutritional status, maintenance of muscle mass, and increased metabolic energy expenditure. In the present study, we investigated changes in the body nitrogen, sodium, and potassium contents by the balance method, muscle mass-related indices, and sympathetic/adrenal medulla function as an indicator of increased metabolic energy expenditure.

From the results of the CK levels, it appears that the exercise regimen induced substantially more damage to muscles. On the other hand, from the results of the RBP levels, it can be inferred that the protein nutritional status was not greatly affected by the diet or exercise regimen during this short-term body weight loss program.

Although it takes 5–7 days for urinary nitrogen excretion to stabilize after starting a protein-free diet [23], shorter periods are used for adjusting to a normal diet with adequate protein levels. Kido et al. conducted crossover nitrogen balance experiments in which a

Table 4
Spearman's correlation coefficients among variables in terms of change (⊿) during both the diet regime and the exercise regimens (N = 12).

–	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩
①⊿Nitrogen balance (mg/day)	–	0.371	**0.657***	0.510	0.378	0.308	0.252	0.189	0.301	0.406
②⊿Sodium balance (mg/day)		–	0.510	0.573	**0.748****	**0.741****	0.545	**0.769****	**0.804****	**0.713****
③⊿Potassium balance (mg/day)			–	0.420	0.287	0.392	0.056	0.329	0.329	0.552
④⊿urinary Creatinine (mg/day)				–	**0.832*****	0.566	**0.727****	**0.804****	**0.811****	**0.874*****
⑤⊿urinary 3-MH (μmol/day)					–	**0.846*****	**0.776****	**0.818****	**0.902*****	**0.839*****
⑥⊿urinary 3-MH (μmol/g • creatinine/day)						–	0.490	**0.671***	**0.692***	**0.636***
⑦⊿urinary Aldosterone (μg/day)							–	**0.797****	**0.720****	**0.783****
⑧⊿urinary Adrenaline (μg/day)								–	**0.888*****	**0.902*****
⑨⊿urinary Noradrenaline (μg/day)									–	**0.846*****
⑩⊿urinary Dopamine (μg/day)										–

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
3-MH, 3-metylhistidine.

3-day adaptation period was followed by a 10-day exercise or non-exercise period. Taking into account the dermal nitrogen loss, which was measured in the same manner as in the present study, they concluded that it is not necessary to increase protein intake when exercise for health promotion (energy consumption of 200–400 kcal/day) is carried out [24,25].

Despite no changes in the protein intake, the urinary and total nitrogen excretion levels were significantly increased during the exercise regimen, suggesting that exercise acted as a stressor that accelerated protein catabolism [26]. Nevertheless, it was verified that the nitrogen balance values were more negative with the diet regimen than with the exercise regimen. It is well-known that exercise reduces urinary sodium excretion, which involves the activation of the renin-angiotensin-aldosterone system [27,28]. In the present study, exercise increased the urinary aldosterone excretion levels and decreased the urinary sodium excretion levels. As a result, the sodium balance was maintained with the exercise regimen, but was shifted significantly in the negative direction with the diet regimen. Aldosterone causes potassium to be excreted in exchange with the reabsorption of sodium in renal tubules [10]. However, in the present study, exercise therapy did not increase the urinary potassium excretion levels. As a result, similar to the results for sodium, the potassium balance was maintained with the exercise regimen, but was shifted significantly in the negative direction with the diet regimen when compared to the exercise regimen.

The urinary excretion levels of creatinine and 3-MH are muscle mass-related indices [1–4], and when muscle metabolism is in a steady state under a meat-free diet, the amount of muscle mass can be estimated from the amount of 3-MH excreted in urine. In the present study, a significant increase in the urinary excretion levels of creatinine and 3-MH was observed, respectively, and the Δ urinary creatinine, Δ 3-MH, Δ aldosterone, Δ adrenaline, Δ noradrenaline, and Δ dopamine excretion levels were significantly more positive with the exercise regimen than with the diet regimen. The results of the urinary catecholamine excretion levels indicated that muscle metabolism was in a dynamic state during the exercise regimen. Nevertheless, when combined with the results for the nitrogen balance and the urinary creatinine and 3-MH excretion levels, it is possibly considered that muscle mass may have been preserved, at least when compared to the diet regimen.

We calculated the correlation coefficients (the Δ with the two regimens (N = 12)) to examine the relationships among the balance data and urinary excretion levels. Because skeletal muscle has the high concentration of potassium, the total potassium level in the body is closely correlated with the lean body mass, and the cellular nitrogen and potassium levels increase or decrease proportionally [10]. In this study, the balances of nitrogen and potassium showed a significant positive correlation with each other; however, they were not significantly correlated with urinary creatinine or 3-MH excretion. With exercise training, positive sodium balances have been reported along with increases in the plasma volume [11,12]. An increased plasma volume leads to an increased circulating blood volume, which reduces blood viscosity, and increases aerobic endurance [29,30]. In addition, the urinary creatinine, 3-MH, and aldosterone excretion levels showed a highly positive correlation with the urinary catecholamine excretion levels. The fact that the index for estimating muscle mass increased with an increase in catecholamines suggests that muscle turnover was enhanced during exercise. Moreover, it is well known that renin secretion and subsequent activation of the renin-angiotensin-aldosterone system increase with increased sympathetic nerve activity [13].

Positive nitrogen and potassium balances contribute to maintaining and increasing muscle mass, and exercise-induced muscle activity activates lipid metabolism. Although the causal relationship remains unclear, when compared to the diet regimen, it appears that the exercise regimen suppresses muscle mass decreases under a relatively positive state of nitrogen, sodium, and potassium balance, accompanied by increased sympathetic nerve and adrenal medulla activity associated with exercise. In other words, exercise may be expected to suppress decrease in muscle mass when there is an adequate supply of muscle building nutrients and the muscles are actively engaged. Furthermore, it is speculated that these conditions may lead to the improvement of lipid metabolism, since the Δ urinary 3-MH excretion level in this study and Δ AI were significantly negatively correlated with each other.

5. Conclusions

In the early stage of body weight loss, when compared to the diet regimen, the exercise regimen suppresses the decrease in muscle mass-related indices under a relatively positive state of whole-body protein, sodium, and potassium balances, accompanied by an increase in sympathetic/adrenal cortical function. Nonetheless, the present study was a relatively short observational study that examined regimens for only 5 days. Further studies that include examinations of the body composition and indices of body protein anabolic action over a longer intervention period are required to clarify the effects of the diet regimen and/or exercise regimen.

Ethics statement

This study was reviewed and approved by The Research Ethics Committee of Kanto Gakuin University, with the approval number: 2004–02. All participants provided informed consent to participate in the study.

Author contribution statement

Tetsuo Yamada: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Akira Tanaka: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data. Shin-ichi Kurasawa and Masami Matsuzaki: Performed the experiments.

Data availability statement

Data included in article/supp. Material/referenced in article.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19632.

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