Relationship between the ratio of increase in lean tissue to body weight gain and energy required to gain body weight in growing rats

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Abbreviations

LT, lean tissue

AT, adipose tissue

BW, body weight

N, normal

HF, high-fat

HP, high-protein

ME, metabolizable energy

ST, stored energy

EX, expended energy

MT, energy for body maintenance

WG, energy for body weight gain.

LT_E, energy stored in the lean tissue

AT_E, energy stored in the adipose tissue

MT, energy for body maintenance

WG, energy for body weight gain

TAG, triacylglycerols

FFM, fat-free mass

FM, fat mass

Abstract

Although the energy stored in the lean tissue (LT) and adipose tissue (AT) is well known, the energy required to synthesize these tissues is obscure. Theoretically, the energy at the point at which ΔLT/Δ body weight (BW) reaches 100% on a regression line, which indicates the relationship between ΔLT/ΔBW and the energy required for BW gain, is considered to be the energy expended to synthesize LT. Therefore, we investigated this relationship in rats. Rats were fed diets with different ratios of protein, fat, and carbohydrates because their ΔLT/ΔBW values were expected to be different. Six-week-old male Sprague-Dawley rats had *ad libitum* access to normal (N, n = 6), high-fat (HF, n = 7), or high-protein (HP, n = 8) diets for 4 weeks. The ΔLT/ΔBW was 0.77 in the N group, 0.70 in the HF, and 0.87 in the HP groups, respectively. The average energy required to gain BW was 8.8 kJ/g in the N group, 7.0 kJ/g in the HF group, and 11.3 kJ/g in the HP group. We observed a positive correlation between ΔLT/ΔBW and energy required for BW gain. The regression line demonstrated that the energy expended to synthesize LT was 13.9 kJ/g and AT was -7.9 kJ/g. Therefore, combined with the energy stored in LT, the energy required to accumulate LT is approximately 19 kJ/g, whereas the energy to accumulate AT could not be elucidated.

Introduction

Although the energy required to synthesize protein and fat has been demonstrated in rats [1] and humans [2, 3], the energy required to synthesize lean tissue (LT; e.g., skeletal muscle, internal organs, and bone) and adipose tissue (AT; e.g., abdominal and subcutaneous adipose tissue) is obscure. Elucidation of the energy required for LT synthesis is important for individuals who aim to increase their skeletal muscle mass, such as athletes. Elucidating the energy required for AT synthesis may have implications for nutritional therapy for individuals such as anorexia nervosa and malnourished. In addition, adipose tissue not only stores fat but also has endocrine and immune functions [4].

Spady *et al.* showed that the energy stored in the body is calculated by measuring the difference between energy intake (metabolizable energy, ME) and the energy expenditure (EX) [5]. EX is the sum of the energy required to maintain the body (MT), for the synthesis of newly accumulated tissues and physical activity (PA). Thus, the energy required to synthesize newly accumulated tissue, that is the energy required for body weight (BW) gain (WG) is calculated by subtracting MT and PA from EX. During BW gain, energy is stored in the LT and AT. The energy stored in LT (ΔLT_E) and AT (ΔAT_E) are calculated by accreted LT (ΔLT) and AT (ΔAT) multiplied by their respective energy densities of 5.23 kJ/g LT and 30.96 kJ/g AT [6]. The sum of the energy stored in these tissues is the energy stored in the body (ST), which is the difference between ME and EX. The relationship of these energies is shown in *Figure 1*.

The sum of the weights of ΔLT and ΔAT is ΔBW. Thus, ΔLT and ΔAT are calculated using the following simultaneous equations [6, 7].

$\Delta LT\ (g) + \Delta AT\ (g) = \Delta BW\ (g)$ —— 1

$\Delta LT\ (g) \times 5.23\ (kJ/g) + \Delta AT\ (g) \times 30.96\ (kJ/g) = ST\ (kcal)$ —— 2

Theoretically, the energy at the point at which $\Delta LT/\Delta BW$ reaches 100% on the regression line, which indicates the relationship between $\Delta LT/\Delta BW$ and WG, is considered

to be the energy expended to synthesize LT. Conversely, the energy at the point at which ΔLT/ΔBW reaches 0% is considered to be the energy expended to synthesize AT.

To determine this relationship, it was necessary to prepare the animals with different ΔLT/ΔBW values. These animals can be prepared by feeding them diets containing different ratios of protein, fat, and carbohydrates [8]. It has been shown that the energy required for protein synthesis is greater than that for fat [1, 2, 3]. Bray *et al.* [9] showed that resting energy expenditure (REE) and body protein (lean body mass) increased with a high-protein diet in humans. This greater REE may be associated with the greater energy expended for protein synthesis. It can be assumed that the energy required to synthesize LT is greater than that required to synthesize AT because the synthesis of proteins requires a large amount of energy. Thus, it is considered that WG differs in animals with different ΔLT/ΔBW values.

Therefore, we investigated the effects of standard, high-protein, and high-fat diets on ΔLT/ΔBW and WG in growing rats to determine the energy required for the synthesis of LT and AT.

Materials and methods

Animals and outline of the procedure

Twenty-seven 5-week-old male Sprague-Dawley rats were obtained from CLEA Japan (Tokyo, Japan). The rats were divided into groups fed a standard diet (N, n = 12), high-protein diet (HP, n = 8), and high-fat diet (HF, n = 7), and were individually housed in metabolic chambers. The rats were fed the respective diets for 7 days prior to the study to acclimatize them to the diets and metabolic chambers. Water and diet were provided *ad libitum*. Six rats in the N group were euthanized after the 7-day acclimatization period to determine the weight of the gastrointestinal contents, which was used to calculate the BW gain, as described below. The remaining rats were used to measure energy expenditure for 4 weeks as described below. Water and diet were provided *ad libitum*. Body weight, food

intake, and energy expenditure were measured daily. The temperature of the animal room was 23 ± 1°C; the dark period was from 8:00 to 20:00, and the light period was from 20:00 to 8:00.

This study was conducted in accordance with the Guidelines for Proper Conduct of Animal Experiments of the Science Council of Japan and was approved by the Experimental Animal Committee of the Research Integrity Committee of Osaka University of Health and Sport Sciences (approval numbers 21-2 and 21-4).

Diet

Table 1 shows the composition of the diets used. A commercial standard diet CE-2 (Clea Japan) was used for the N group.

Not all ingested energy is absorbed or metabolized. In the present study, we used the energy metabolized in the body. Thus, we used metabolizable energy (ME) as energy intake for this study. The ME of the diets used in this study was determined based on reports by MaCraken [10] and Raman *et al.* [11], and the values have been reported previously [12]. Briefly, 5-6 rats were individually housed in metabolic cages and fed each diet for 7 days. For the next 7 days, the rats were fed the same diet *ad libitum*, food intake was measured, and all feces and urine were collected. The energy content of each diet, feces, and urine was measured using bomb calorimetry (Japan Food Research Center, Osaka, Japan). The feces were freeze-dried, and urine was dried in an oven at 60℃ [13] to avoid loss of short-chain fatty acids prior to bomb calorimetry analysis, whereas undried specimens were used for diet analysis. Samples weighing approximately 0.4-0.5 g were used. ME was calculated by subtracting the energy excreted into the feces and urine from the energy intake for the last 7 days. ME was 1,323 kJ/100 g for the N diet, 2,248 kJ/100 g for the HF diet, and 1,675 kJ/100 g for the HP diet.

Measurement of energy expenditure

Energy expenditure was measured using an open-circuit system [14] in rats individually housed in metabolic chambers 22 cm × 34 cm × 14 cm (width × depth × height). The chamber was ventilated at 2,100-4,100 mL/min depending on the rat BW and oxygen consumption. During the experiment, a portion of the ventilated air (150 mL/min) was collected in a 250 L Douglas bag (Yagami, Osaka, Japan) for 23 h and 45 min, and the oxygen concentration was measured using a portable gas monitor (VO2000, S & ME. Tokyo). Oxygen consumption was calculated by multiplying the difference in oxygen concentration between the room air and sampled air by the ventilation rate of the chamber, and the energy expenditure was calculated as 20.08 kJ/L oxygen. The energy expenditure was converted per 24 h.

Sampling of organs and tissues, and whole-body biochemical analyses

The rats were euthanized under isoflurane anesthesia. Internal organs (heart, liver, kidneys, adrenal glands, and intestines), skeletal muscles (flexor hallucis longus, soleus, gastrocnemius, and plantaris), and adipose tissues (perirenal, epididymal, retroperitoneal, and mesenteric) were collected and weighed. After removing the intestinal contents, the intestines were weighed. The collected blood, internal organs, skeletal muscles, and adipose tissues were returned to the abdominal cavity of the carcass and frozen for biochemical analysis.

The carcass was dried in an oven at 60°C [13] to avoid loss of short-chain fatty acids. The dried samples were pulverized into a powder using a mill (Vita-Max Absolute Blender, Osaka Chemical Co., Ltd., Osaka, Japan). The total lipid content was determined using the Folch method. Approximately 1 g of the sample was homogenized in chloroform: methanol (2:1), the chloroform layer was dried, and the weight of the residue was measured. Protein content was calculated as the nitrogen content of the sample, which was determined using the Kjeldahl method multiplied by 6.25. For glycogen, approximately 100 mg of the sample was

decomposed with 30% potassium hydroxide, ethanol was added to precipitate the glycogen, which was then dissolved in an appropriate amount of water and colored using the phenol-sulfuric acid method, and the absorbance was measured [15].

Theoretical distribution of ME

Figure 1 shows the theoretical distribution of ME. Because the ME is expended or stored, the ST was calculated as the difference between the ME and EX.

EX consists of MT, WG, and PA. In the present study, PA was considered minimal because rats were in the chamber; thus, WG was calculated by subtracting only MT from EX. In humans, MT is considered to be 1.5 × basal metabolic rate (BMR) [5]. In the present study, as the rats were in the chamber, their physical activity was assumed to be minimal, as mentioned above. However, no data are available for the appropriate factor to multiply BMR to obtain the energy required to maintain the body in sedentary rats. According to Gleeson *et al.* [16] the lowest energy expenditure of the sedentary rats during the resting period was 1.66 kJ/kg/h which was considered to be their BMR, and the energy expenditure during the rats eating (2.88 kJ/kg/h) was 1.7 × the lowest energy expenditure, and the energy expenditure during the active period while not eating (1.95 kJ/kg/h) was 1.2 × the lowest energy expenditure. The average of 1.7 and 1.2 was 1.45. In the present study, the rats had ad libitum access to food. Therefore, we set the MT as 1.5 × estimated BMR [17].

Calculation for LT and AT deposition

When animals grow, the sum of the increases in the weight of LT (ΔLT) and AT (ΔAT) is the BW gain (ΔBW). In addition, energy is stored in either LT or AT. Therefore, the energy stored in the body (ST) is the sum of the energy stored in LT (LT_E) and AT (AT_E). Thus, ΔLT and ΔAT can be calculated using the following simultaneous equation [6, 7].

ΔLT (g) + ΔAT (g) = ΔBW (g) —— 1

$\Delta LT\ (g) \times 5.23\ (kJ/g) + \Delta AT\ (g) \times 30.96\ (kJ/g) = ST\ (kJ)$ —— 2

Equation 1 indicates that the sum of the increases in LT and AT is the BW gain and Equation 2 indicates that the sum of LT_E and AT_E is the energy stored in the body.

The LT_E and AT_E were calculated by multiplying the energy density of each tissue by the accreted tissue weight obtained using this simultaneous equation.

The BW without gastrointestinal content weight was used to calculate the BW gain in Equation 1 because the gastrointestinal content was measured as BW, but this was not the body. The gastrointestinal content weight used for this calculation was obtained by sampling organs and tissues, as described above. The BW without the gastrointestinal contents of the rats at the start of the study was assumed to be 89.45% of their BW because the gastrointestinal content accounted for 10.55% (SD 0.02) of the BW of the rats that were euthanized before starting the study.

Statistics

The sample size was calculated from a statistical power $(1-\beta)$ of 0.8, α error of 0.05, and a significant minimum effect size (f) of 1.0. As there was no available information regarding changes in the energy required for body weight gain due to differences in diets, we set the effect size to 1 to find a 1 standard deviation difference. This power calculation determined that a minimum sample size of five animals was required to detect a statistically significant difference in the energy required for body weight gain using G*Power 3.1. One-way analysis of variance was used for comparisons among groups, and the Bonferroni test was used as a post hoc test (IBM SPSS Statistics version 27.0.1.0). Pearson's correlation was used to determine the relationship between $\Delta LT/\Delta BW$ and the energy required for BW gain, as the data passed the Shapiro-Wilk test. Statistical significance was set at $P < 0.05$.

Results

Table 2 shows that the BW gain in the HF was the highest, but not significantly differ from the HP group.

Figure 2 and *Table S1* show the distribution of ME. The EX, WG, and LT_E were higher in the HP group than in the N and HF groups. The ST and AT_E were the greatest in the HF group, which did not differ from that of the N group.

The energy density per gram of accumulated tissue in the HF group (12.9 kJ/g [SE 0.8]) was significantly higher than that in the HP group (8.6 kJ kcal/g [SE 0.8], $p = 0.003$, $d = 1.813$), whereas that in the N group (11.3 kJ /g [SE 0.5]) was not different from either the HF ($p = 0.620$, $d = 0.795$) or HP groups ($p = 0.087$, $d = 1.355$).

Table 3 shows the increases in the weights of LT (ΔLT) and AT (ΔAT). ΔLT was significantly greater in the HP group than in the other two groups, whereas ΔAT was the greatest in the HF group, which did not differ from that in the N group. The ratio of ΔLT to the increase in BW (ΔLT/ΔBW) was the highest in the HP group, but was not significantly different from that in the N group. The ratio of ΔAT to the increase in BW (ΔAT/ΔBW) was higher in the HF group than that in the HP group, whereas the ratio in the N group did not differ from that in the HF or HP groups.

Table 4 shows organ and tissue weights. The skeletal muscle weight was significantly greater in the HP group than in the HF group. There was no significant difference in the skeletal muscle weight between the HP and the N group except for FHL. The adipose tissue weight except for perirenal was the highest in the HF group than the other groups, while the weight of retroperitoneal and mesenteric did not significantly differ from the HP group and the weight of epididymal did not differ from the N group. The weights of the kidneys, adrenal, pancreas and intestines were the lowest in the HF group, but the pancreas weight was not significantly different between the N group.

Table 5 shows the organ and tissue weights per 100 g of BW. The skeletal muscle weight was the lowest in the HF group. The weights of the retroperitoneal and epididymal adipose tissues were the highest in the HF group. Retroperitoneal adipose tissue did not differ between the HF and N groups and epididymal adipose tissue did not differ between the HF and H groups. The internal organ weights were lowest in the HF group, but the weights of the heart, spleen, and pancreas did not differ from those in the N group.

Table 6 shows the whole-body protein, total lipid, and glycogen contents. The protein content did not differ among the groups, whereas the total lipid content was the highest in the HF group. The glycogen content was higher in the HP group than in the other groups.

Table 7 shows the whole-body protein, total lipid, and glycogen contents per 100 g of BW. The total lipid content was the highest in the HF group.

Figure 3 shows the positive correlation between ΔLT/ΔBW and the energy required to gain 1 g of BW, which was calculated by dividing ΔBW by WG. The energy required to gain 1 g of BW was significantly higher in the HP group (11.3 kJ/g [SE 0.6]) than in the HF group (7.0 kJ/g [SE 0.8], $p < 0.01$, $d = 2.103$), while there was no difference between the N group (8.8 kJ/g [SE 0.5]) and the HF group ($p = 0.315$, $d = 0.966$) or HP group ($p = 0.074$, $d = 1.442$). The regression line demonstrated that the energy required to gain 1 g of BW at the point of 100% on ΔLT/ΔBW was 13.9 kJ/g, while the energy required to gain 1 g of BW at the point of 0% on ΔLT/ΔBW was -7.9 kJ/g.

Discussion

In this study, no differences in ME were observed between the groups, but BW gain was the highest in the HF group among the groups. Thermic effect of food (TEF) of protein is greater than that of carbohydrates, which is greater than that of fat [18]. The higher BW gain in the HF group was presumably due to the smaller TEF of the HF diet, which resulted in less EX and more ST. This greater ST was thought to be associated with the higher BW gain in the HF

group relative to the N group. In the HP group, it was assumed that the TEF of the HP diet was higher than that of the other diets. Therefore, the ST may be small in the HP group. However, LT accretion in the HP group was greater than in the other two groups. Because the energy density of LT is lower than that of AT, LT can accumulate with less ST. Therefore, it is considered that the increase in the BW of the HP group was not smaller than that of the other groups. The increase in LT was greatest in the HP diet, and the increase in AT was greatest in the HF diet. The AT increase accounted for 13–30% of BW gain, while 47–72% of the energy stored in the body was stored in AT. Thus, the accumulation of LT and AT differed among the groups fed different diets, whereas a large proportion of energy was stored in the AT, even though the weight increase in the AT was not very large.

Energy is required for several metabolic pathways [18]. Major macronutrient fluxes such as gluconeogenesis, *de novo* lipogenesis, triacylglycerol (TAG) synthesis, and protein turnover require energy, and these flux rates can be influenced by both the energy content of the diet and its composition [18]. In the present study, the amount of energy required for BW gain differed depending on diet. It was higher in the HP diet group than in the N- and HF-diet groups. When TAG accumulates in AT, metabolic processes differ depending on the TAG substrate. Regarding carbohydrates, de novo lipogenesis is involved in TAG deposition. In the case of proteins, deamination and urea synthesis are involved, in addition to *de novo* lipogenesis. It is considered that the more metabolic processes involved in accumulating TAG, the more energy is expended. It is suggested that the energy expended to accumulate TAG was greater in the HP diet than in the other diets. Ingested amino acids are utilized for body protein synthesis, and proteins accumulate in the LT, which also requires energy. The amount of amino acids ingested during the study, as estimated by the ME and protein contents of the diets was 140.9 g for the N group, 94.0 g for the HF group, and 175.7 g for the HP group. In addition, the accretion of LT was the greatest in the HP diet group. It is suggested that the energy expended to accumulate protein in the body is greater in the HP diet

than in the other diets. Therefore, it seems reasonable that the energy required for BW gain in the HP diet group was highest among the three groups.

A positive correlation was observed between ΔLT/ΔBW and energy required for BW gain. It is considered that the energy used for weight gain when ΔLT/ΔBW is 100% is the energy required for the synthesis of LT, which was 13.9 kJ/g in this study. In other reports of ours, the energy required to synthesize LT was 12.2 kJ/g [6] and 12.6 kJ/g [7], which are comparable to the energy observed in the present study. To our knowledge, no other studies have reported the energy required to synthesize LT. To increase LT, it is considered rational to add the energy required for LT synthesis and the energy accumulated in the LT. The energy density of LT is 5.2 kJ/g. Therefore, the additional energy intake required to increase LT was estimated to be approximately19 kJ/g. However, the results of the present study could not elucidate the energy required for AT synthesis. Sekiguchi *et al.* [6] reported that the energy required for AT synthesis was 4.6 kJ/g, and that the energy required to accumulate AT, including the energy stored in AT, was approximately 35.6 kJ/g. In their study, the rats with smaller ΔLT/ΔBW were included than the rats in the present study, therefore the regression curve was different from that in the present study, and a positive value was obtained when ΔLT/ΔBW = 0. Rats with smaller ΔLT/ΔBW are necessary to determine the energy required for AT synthesis.

In this study, growing rats were used. Inoue *et al.* [7] reported that in a 2-week study that used rats of 4, 7, 9, and 14 weeks of age, the increase in AT was most of or more than the BW gain at 9 and 14 weeks of age, and some rats of 14 weeks of age showed a decrease in LT. In the present study, it was necessary to examine rats at an age when LT was increasing. Additionally, weight gain was small in 9- and 14-week-old rats. We considered it better to conduct this study during a period of large weight gain for the calculation. Therefore, 6-week-old rats were used in this study.

As discussed below, the values obtained in the present study may vary with age but may not differ significantly among species. When athletes attempt to increase their BW, their aim is to increase muscle mass (which accounts for a large part of the LT) without increasing the AT. To do this, they increase their energy intake and perform resistance exercise training. Garthe *et al.* [19] reported that increasing energy intake by approximately 2,100 kJ per day and adding training to athletes for 8–12 weeks resulted in a 2.7 kg increase in BW with a 1.7 kg increase in fat-free mass (FFM) and a 1.1 kg increase in fat mass (FM). Miyauchi *et al.* [20] showed that when male college American football players increased their daily energy intake by 2,100 to 4,200 kJ and performed power training for one year, they gained 9.7 kg in BW, with an FFM of 5.2 kg and an FM of 4.5. When calculating the energy accumulated in the body from the increase in FFM and FM, and the energy density of these components, approximately 80% of the accumulated energy was stored in the FM. In addition, the energy density of skeletal muscle is 5,200 kJ/kg [6, 7]. Therefore, when 1 kg of skeletal muscle is accumulated, 5,200 kJ/kg of energy should be accumulated. However, skeletal muscle mass does not increase by 1 kg within a few days. Therefore, the added energy intakes in these studies may have been too high.

The present study has several limitations. The energy density of LT in the equation used in this study dose not consider the energy density of the bone. In addition, energy is required for bone synthesis, which is particularly important during the growth period. However, to the best of our knowledge, this energy is not clear, and we are unable to describe this energy from the data of the present study. Rats are known to be coprophagous. In the experiments performed to measure oxygen consumption, we noticed that the amount of feces was small or that fecal matter was sometimes not seen in the chamber when the rats were fasted or fed restricted diets. In the present study, there was a normal amount of feces in the chamber. Therefore, the rats might have eaten their feces, although it was assumed that the amount was not large. Regarding the influence of different animal species, it has been shown

that the energy required for protein synthesis does not markedly different between different species [21, 22, 23]. The basal metabolic rate in species of different sizes is proportional to the body weight raised to the 0.75 power [17]. It has been reported that the contribution of protein turnover to the resting metabolic rate is approximately 20% in an average human [21]. Assuming that this contribution is comparable among species, differences in the energy required for LT synthesis may not be large among species. Regarding sex differences, there are no sex-related differences in the metabolic pathways involved in protein synthesis. Therefore, we presume that there are no sex differences in the energy required for LT synthesis. Therefore, it is inferred that there are few sex differences in the synthetic energy of LT. As animals age, it is assumed that the body needs to synthesize more tissue to gain weight due to increased breakdown compared to synthesis, leading to an increase in the energy required to gain BW. Therefore, the values obtained in this study may have differed according to age. We used the energy of 1.5 × the estimated basal metabolic rate as the energy for maintaining the body (MT). The basal metabolic rate is assumed to be lower in animals with a higher proportion of body fat. In the present study, the total lipid content of the whole body was higher in the HF diet group. Animals with high body fat had less LT. The basal metabolic rate depends on the amount of LT. Therefore, it can be inferred that the basal metabolic rate of the HF group was low. Because WG was calculated by subtracting MT from EX in the present study, WG increased as MT decreased, leading to an increase in the energy required for BW gain.

In conclusion, the energy expended to synthesize LT was 13.9 kJ/g, Therefore, combined with the energy stored in LT, the energy required to accumulate LT is approximately 19 kJ/g in growing rats. However, the energy required for the synthesis of AT has not been elucidated.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this study.

Authorship

KOb, MK, and KOk designed the research; KOb and MK conducted the research; KOb analyzed the data; and KOb, MK, EK, and KOk wrote the paper. KOk was the primary responsibility for the final content. All the authors have read and approved the final manuscript.

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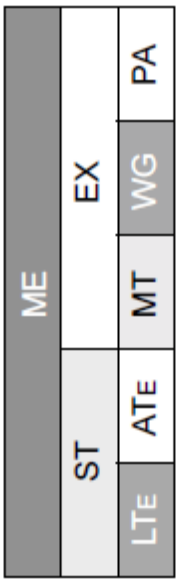

Figure 1. Theoretical distribution of ME. ME, metabolizable energy; ST, stored energy; EX, expended energy; LT_E, energy stored in the lean tissue; AT_E, energy stored in the adipose tissue; MT, energy for body maintenance; WG, energy for body weight gain; PA, physical activity.

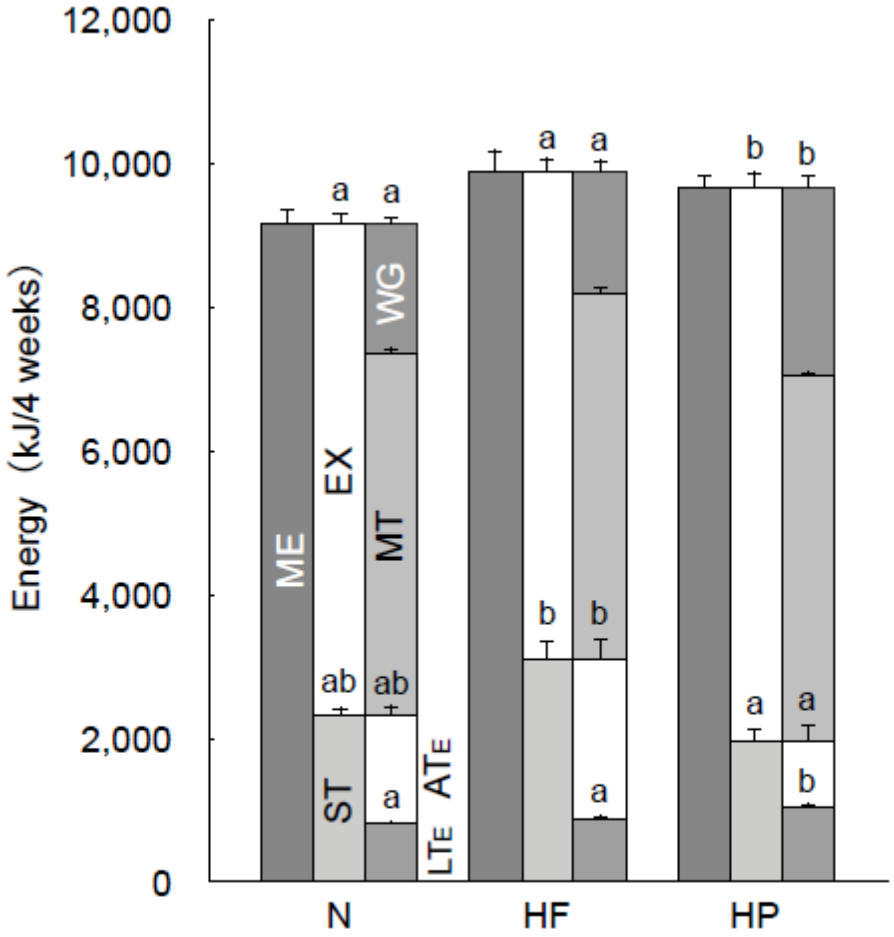

Figure 2. Distribution of ME. Means and SE. Values with different letters differed significantly.

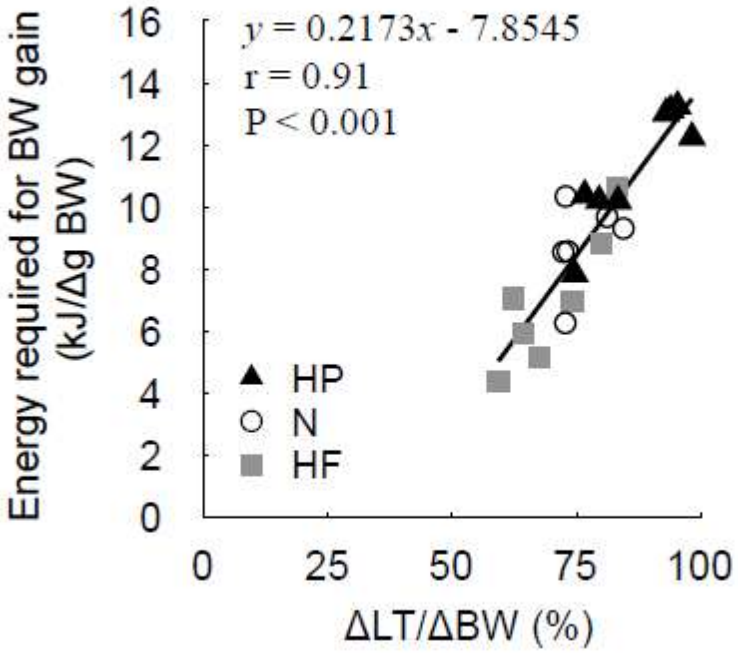

Figure 3. Relationship between ΔLT/ΔBW and the energy required for body weight gain.

Table 1. Dietary composition

	N [1]	HF	HP
Casein (g/kg)		264	379
Corn oil (g/kg)		345.5	70
Corn starch (g/kg)		182.986	289.986
α-corn starch (g/kg)		60	97
Sucrose (g/kg)		46.5	63
Cellulose (g/kg)		50	50
AIN-93G mineral-mix (g/kg)		35	35
AIN-93 vitamin-mix (g/kg)		10	10
L-cystine (g/kg)		3	3
Choline bitartrate (g/kg)		3	3
t-Butylhydroquinone (g/kg)		0.014	0.014
Protein (g/100 g)	25.10	22.78	32.71
Fat (g/100 g)	4.51	35.12	7.84
Carbohydrates (g/100 g)	49.72	27.37	41.45

[1] Information such as the raw materials and content of vitamins and minerals are available on the manufacturer's (CLEA Japan) website (https://www.clea-japan.com/en/products/general_diet/item_d0030).

Table 2. Body weight

	N			HF			HP			One-way ANOVA	Cohen's d		
	Mean	SE		Mean	SE		Mean	SE		P-value	N vs. HF	N vs. HP	HF vs. HP
Initial (g)	144.4	3.6		144.4	2.8		147.1	2.9		0.801	0.001	0.290	0.313
Final (g)	349.3	6.6		385.1	12.5		378.9	6.8		0.054	1.235	1.519	0.219
Δ(g)	204.9	5.8	a	240.7	10.6	b	231.8	4.7	ab	0.020	1.449	1.834	0.387

Values with different letters differ significantly.

Table 3. Accretion of LT and AT

	N			HF			HP			One-way ANOVA	Cohen's d		
	Mean	SE		Mean	SE		Mean	SE		P-value	N vs. HF	N vs. HP	HF vs. HP
ΔLT (g/4 weeks)	156.5	5.8	a	168.7	9.8	a	202.0	8.5	b	0.006	0.525	2.051	1.242
ΔAT (g/4 weeks)	48.4	4.2	ab	72.0	9.1	b	29.8	7.1	a	0.004	1.146	1.036	1.788
ΔLT/ΔBW (%)	76.4	1.97	ab	70.3	3.22	a	87.1	3.09	b	0.004	0.794	1.357	1.814
ΔAT/ΔBW (%)	23.6	1.97	ab	29.7	3.22	b	12.9	3.09	a	0.004	0.794	1.357	1.814

Values with different letters indicate significant differences. The Bonferroni test was used as a post-hoc test.

Table 4. Organ and tissue weight

	N			HF			HP			One-way ANOVA	Cohen's d		
	Mean	SE		Mean	SE		Mean	SE		P-value	N vs. HF	N vs. HP	HF vs. HP
Skeletal muscle													
FHL [(1)] (g)	0.91	0.02	a	0.86	0.04	a	1.05	0.03	b	0.004	0.471	1.712	1.758
Soleus (g)	0.21	0.01	ab	0.17	0.01	a	0.25	0.01	b	0.003	1.131	1.147	1.905
Gastrocnemius (g)	3.80	0.15	ab	3.52	0.09	a	4.04	0.11	b	0.021	0.847	0.695	1.761
Plantaris (g)	0.66	0.08	ab	0.59	0.03	a	0.76	0.03	b	0.005	0.899	1.082	1.935
Adipose tissue													
Perirenal (g)	0.83	0.13		1.20	0.14		1.12	0.08		0.129	1.019	1.009	0.244
Retroperitoneal (g)	2.79	0.35	a	5.57	0.79	b	3.92	0.25	ab	0.011	1.558	1.355	1.003
Epididymal (g)	3.76	0.36	ab	5.22	0.53	b	3.47	0.16	a	0.012	1.128	0.395	1.610
Mesenteric (g)	2.99	0.31	a	4.97	0.70	b	3.47	0.19	ab	0.030	1.247	0.699	1.047
Organ													
Heart (g)	0.94	0.04		0.93	0.04		1.07	0.03		0.050	0.112	1.263	1.255
Liver (g)	13.20	0.50		13.28	0.52		14.41	0.42		0.193	0.063	0.929	0.820
Kidneys (g)	2.82	0.09	b	2.34	0.11	a	2.96	0.10	b	0.002	1.607	0.517	1.998
Adrenal (g)	0.028	0.006	b	0.015	0.004	a	0.028	0.007	b	0.008	1.617	0.252	1.831
Spleen (g)	0.73	0.04		0.63	0.04		0.80	0.05		0.060	0.842	0.518	1.289
Pancreas (g)	1.47	0.10	ab	1.22	0.05	a	1.60	0.10	b	0.023	1.202	0.488	1.623
Intestines (g)	6.10	0.21	b	4.16	0.19	a	5.44	0.25	b	<0.001	3.504	0.960	1.907

Values with different letters indicate significant differences. Bonferroni test was used as a post-hoc test. [(1)] FHL: Flexor hallucis longus.

Table 5. Organ and tissue weight per 100 g of body weight

	N			HF			HP			One-way ANOVA	Cohen's d		
	Mean	SE		Mean	SE		Mean	SE		P-value	N vs. HF	N vs. HP	HF vs. HP
Skeletal muscle													
FHL [(1)] (g)	0.26	0.01	b	0.22	0.01	a	0.28	0.01	b	<0.001	1.456	0.839	2.610
Soleus (g)	0.06	0.00	b	0.04	0.00	a	0.06	0.00	b	<0.001	1.982	0.707	2.504
Gastrocnemius (g)	1.09	0.07	b	0.92	0.03	a	1.07	0.02	b	0.008	1.451	0.219	2.605
Plantaris (g)	0.19	0.01	b	0.15	0.01	a	0.20	0.01	b	0.002	1.489	0.546	2.306
Adipose tissue													
Perirenal (g)	0.23	0.03		0.31	0.03		0.30	0.02		0.195	0.916	0.818	0.215
Retroperitoneal (g)	0.79	0.10	a	1.42	0.19	b	1.03	0.06	ab	0.011	1.549	1.147	1.062
Epididymal (g)	1.08	0.11	ab	1.34	0.11	b	0.91	0.03	a	0.009	0.909	0.840	2.008
Mesenteric (g)	0.85	0.09		1.27	0.17		0.91	0.04		0.034	1.157	0.392	1.127
Organ													

Heart (g)	0.27	0.01	ab	0.24	0.01	a	0.28	0.01	b	0.020	0.912	0.536	1.923
Liver (g)	3.77	0.09	b	3.45	0.09	a	3.80	0.07	b	0.011	1.472	0.132	1.626
Kidneys (g)	0.80	0.02	b	0.61	0.03	a	0.78	0.02	b	<0.001	3.366	0.486	3.037
Adrenal (g)	0.008	0.001	b	0.004	0.000	a	0.007	0.001	b	0.004	1.982	0.463	1.400
Spleen (g)	0.21	0.01	ab	0.16	0.01	a	0.21	0.01	b	0.026	1.334	0.036	1.433
Pancreas (g)	0.42	0.04	ab	0.32	0.02	a	0.42	0.02	b	0.024	1.360	0.027	1.561
Intestines (g)	1.75	0.07	c	1.08	0.05	a	1.43	0.05	b	<0.001	4.501	1.947	2.589

Values with different letters indicate significant differences. Bonferroni test was used as a post-hoc test. [(1)] FHL: Flexor hallucis longus.

Table 6. Protein, total lipid, and glycogen contents in the whole body

	N			HF			HP			One-way ANOVA	Cohen's d		
	Mean	SE		Mean	SE		Mean	SE		P-value	N vs. HF	N vs. HP	HF vs. HP
Protein (g)	71.1	1.4		75.9	3.4		76.2	1.6		0.342	0.618	1.141	—
Total lipid (g)	26.1	1.8	a	50.0	5.5	b	31.7	1.0	a	<0.001	1.985	1.416	1.684
Glycogen (g)	0.07	0.00	a	0.07	0.00	a	0.10	0.01	b	0.002	0.137	1.863	1.583

Values with different letters indicate significant differences. The Bonferroni test was used as a post-hoc test.

Table 7. Weight of protein, total lipid, glycogen in 100g of the whole-body components

	N			HF			HP			One-way ANOVA	Cohen's d		
	Mean	SE		Mean	SE		Mean	SE		P-value	N vs. HF	N vs. HP	HF vs. HP
Protein (g)	20.4	0.2		19.7	0.3		20.1	0.17		0.144	1.017	0.570	0.648
Total lipid (g)	7.4	0.4	a	12.8	1.2	b	8.3	0.2	a	<0.001	2.187	1.138	2.013
Glycogen (g)	0.02	0.00		0.02	0.00		0.03	0.00		0.050	0.512	0.949	1.130

Values with different letters indicate significant differences. Bonferroni test was used as a post-hoc test.