



Hatano rats are a suitable metabolic syndrome model for studying feeding behavior, blood pressure levels, and percent body fat

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ABSTRACT. Currently, metabolic syndrome is a worldwide concern. Thus, it is imperative to understand the mechanism of metabolic syndrome by establishing various metabolic syndrome models. In this study, we used Hatano high-avoidance animals (HAA) and low-avoidance animals (LAA), both derived from Sprague–Dawley rats by selective breeding to determine high- or low-avoidance rates in shuttle-box active avoidance tests. HAA and LAA rats have some strain differences related to eating and appetite. Therefore, we determined whether Hatano rats could be used as a metabolic syndrome model. We compared food intake, body weights, blood pressure levels, plasma component levels, and fat contents between HAA and LAA rats. The HAA rats showed more active eating, higher blood pressure, higher percentage fat, and higher triglyceride levels than the LAA rats—these features correspond to some of the risk factors associated with metabolic syndrome. Our study suggests that HAA rats can be considered as a metabolic syndrome model by focusing on their feeding behavior, blood pressure levels, and percent body fat.

KEY WORDS: blood pressure, feeding behavior, Hatano rat, metabolic syndrome, model animal

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Metabolic syndrome has been reported worldwide and is closely related to some of the leading causes of death in the world, such as heart and cerebrovascular diseases [7, 14, 17]. To appropriately resolve this syndrome, the mechanism of the condition needs to be understood. According to the US National Cholesterol Education Programme Adult Treatment Panel III (NCEP-ATP III), metabolic syndrome in humans is diagnosed, when at least three of the following five medical conditions are met: high blood pressure ($X \geq 130/85$ mmHg), high fasting glucose level ($X \geq 110$ mg/dl), high triglyceride level ($X \geq 150$ mg/dl), low high-density lipoprotein cholesterol level (men, $X < 40$ mg/dl; women, $X < 50$ mg/dl), and abdominal obesity (waist circumference: men, $X > 102$ cm; women, $X > 88$ cm) [9]. Unhealthy eating habits and a sedentary lifestyle lead to high blood pressure, high blood sugar, high triglyceride levels, low high-density lipoprotein cholesterol levels, and excess body fat, which constitute metabolic syndrome. The lack of daily exercise and overeating increases the risks of metabolic syndrome. Feeding behavior is regulated by appetite and the neural endocrine system, e.g., corticotropin-releasing hormone (CRH) has an appetite-suppression effect [26]. In contrast, ghrelin and neuropeptide-Y have an appetite-stimulating effect [21, 22, 27, 29]. Previous studies have established metabolic syndrome models [2, 8, 11, 15, 23, 24]. For example, db/db [8] and ob/ob [23] mice showed active feeding together with high body weight and fat levels. For example, db/db mice lack leptin receptors, and ob/ob mice are deficient in leptin. However, because the precise mechanism of metabolic syndrome is yet unclear, establishing various metabolic syndrome models would help understand this mechanism clearly.

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Hatano rats are inbred strains derived from Sprague–Dawley rats at the Hatano Research Institute, Food and Drug Safety Center (Hadano, Japan) [18]. They are selectively bred on the basis of high- and low-avoidance rates in the shuttle-box active avoidance test. Two strains, high-avoidance animals (HAA) and low-avoidance animals (LAA), are maintained at the Hatano Research Institute. Some previous studies have shown that HAA rats are heavier than LAA rats [18]. In addition, under normal conditions, HAA rats show lower CRH levels in the hypothalamic paraventricular nucleus (PVN) than LAA rats [1]. These facts suggest that the strains of Hatano rats differ in their feeding behavior, thereby implying that Hatano rats may be used to study the mechanism of metabolic syndrome. Therefore, in this study, we aimed to investigate if Hatano rats could be used as a model to understand the mechanism of metabolic syndrome. We compared the amount of food intake, body weights, blood pressure levels, plasma component levels, fat weights, and percent body fat between HAA and LAA rats.

MATERIALS AND METHODS

Animals

Male HAA and LAA rats produced at the Hatano Research Institute were used in this study. The rats were maintained under standard ambient lighting (08.00–20.00 hr), room temperature ($22 \pm 1^\circ\text{C}$), and humidity ($50 \pm 20\%$) conditions. Before and after the food-intake recording period (10–12 days habituation plus 2 days measurement period), the rats were grouped in cages with *ad libitum* access to water and normal solid diets (MF, Oriental Yeast Co., Chiba, Japan: Protein 23.1%, Fat 5.1%, Fiber 2.8%, Ash 5.8%, Moisture 7.9%, Carbohydrate 55.3%, 3.59 kcal/g). During the food-intake recording period, including habituation, the rats also had *ad libitum* access to water and food pellets (See below for more information). All the procedures in this study were in accordance with the guidelines of the Animal Care and Use Committee of Musashino University, Meiji University and the Hatano Research Institute of the Food and Drug Safety Center.

Measurement of food intake and body weight

At 9 weeks of age, the rats were moved from group cages into individual cages for the measurement of food intake. In the individual cages, the rats had *ad libitum* access to 45 mg chow pellets (Dustless precision pellets, BioServ Corporation, Flemington, NJ, U.S.A.: Protein 21.3%, Fat 3.8%, Fiber 4.0%, Ash 8.1%, Moisture <10%, Carbohydrate 54%, 3.35 kcal/g) dispensed by a food-intake measuring device (Med Associates Inc., St. Albans, VT, U.S.A.) and *ad libitum* access to water. The device delivered each 45 mg chow pellet in response to the removal of the previous pellet sensed by a pellet-sensing beam.

The rats were allowed to become habituated to their individual cages for 10–12 days while we only checked food intake to determine whether the rats could normally eat the pellets during the habituation. Then, food intake over the next 2 days was measured, and the daily average of the 2-day period was calculated for statistical analysis. On each of the 2 days, food intake was measured by recording the total number of pellets dispensed by the measuring device and that of pellets that had fallen under the cage. The number of fallen pellets was subtracted from the total number of dispensed pellets to calculate the number of pellets consumed by each rat each day. If the ratio of the fallen pellets was greater than 10% of the dispensed pellets, then the data for that rat was excluded. When the measuring device dispensed over 4,000 pellets due to mechanical issues, we excluded these data as extraordinary data. The body weights of the rats were measured before and after they entered the individual cages for the food-intake recording period (10–12 days of habituation plus 2 days of measurement period).

Measurement of heart function

A non-invasive automatic blood pressure measuring device (BP-98A, Softron, Tokyo, Japan) was used to measure blood pressure at 9 and 16 weeks of age using different rats at each age. The levels of systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), and heart rate (HR) were recorded three times on each occasion, and the average for each rat was used for statistical analysis.

Measurement of the amount of abdominal fat and muscle mass

At 17 weeks of age, the rats were intraperitoneally administered pentobarbital (Somnopentyl, Kyoritsu Seiyaku Corp., Tokyo, Japan). Then, X-ray micro computed tomography (CT, Latheta LCT-100, Aloka Co., Ltd., Tokyo, Japan) was used to measure the amounts of abdominal fat and muscle mass. Cross-sections from the chest xiphoid to the front of the pelvis were scanned at intervals of 1.5 mm. From the scanned images, total fat, subcutaneous fat, visceral fat, and muscle were identified and quantified.

Heart blood sampling

After the CT scan, the rats at 17 weeks of age were intraperitoneally administered pentobarbital under deep anesthesia. Blood collection was done from 10.00 to 14.00 hr. A heparinized needle and cylinder (Heparin, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) were used to collect the heart blood. The plasma were multiplied by centrifugation for 20 min at 1,630 g, 4°C and subsequently stored at -30°C until measurement of the levels of triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and glucose.

Measurement of plasma component concentrations

Triglyceride: Each well on the 96-well microplate was filled with 150 μl of buffer solution (Triglyceride E Test Wako, Wako Pure Chemical Industries, Osaka, Japan) and 1 μl of the plasma or 150 μl buffer solution and 1 μl of the standard solution (596.1,

300, 200, or 100 mg/dl). Then, the plate was shaken at 37°C for 5 min, after which the absorbance at 595 nm was measured using an absorption spectrometer (Microplate reader model 680, Bio-Rad Laboratories, Tokyo, Japan). Two measurements were obtained for each sample and averaged.

Total cholesterol: Each well on a 96-well microplate was filled with 150 μ l buffer solution (Cholesterol E Test Wako, Wako Pure Chemical Industries) and 1 μ l of the plasma or 150 μ l buffer solution and 1 μ l of the standard solution (592.2, 397.4, 200, or 100 mg/dl). Absorbance at 595 nm was measured in the same manner as for triglyceride.

HDL cholesterol: Each well on a 96-well microplate was filled with 150 μ l buffer solution (L Type Wako HDL-C.M, Wako Pure Chemical Industries) and 1 μ l of the plasma or 150 μ l buffer solution and 1 μ l of the standard solution (50, 25, or 12.5 mg/dl). Absorbance at 595 nm was measured in the same manner as for triglyceride.

LDL cholesterol: Each well on a 96-well microplate was filled with 150 μ l buffer solution (L Type Wako LDL-C.M, Wako Pure Chemical Industries) and 1 μ l of the plasma or 150 μ l buffer solution and 1 μ l of the standard solution (115, 57.8, or 27.8 mg/dl). Absorbance at 595 nm was measured in the same manner as for triglyceride.

Glucose: Each well on a 96-well microplate was filled with 150 μ l buffer solution (Glucose C2 Test Wako, Wako Pure Chemical Industries, Ltd.) and 1 μ l of the plasma or 150 μ l buffer solution and 1 μ l of the glucose standard solution (500, 400, 300, 200, 100, or 50 mg/dl). Absorbance at 490 nm was measured in the same manner as for triglyceride.

Measurement of fat weight

After the collection of the blood samples, the rats were then dissected, and the weights of mesenteric, perirenal, and gonadal fats were measured.

Statistical analysis

All results are expressed as means \pm standard error of the mean. The Stat View 5.0 software (SAS Institute Inc., Cary, NC, U.S.A.) was used to perform all analyses. For comparing between the HAA and LAA rats, Student's *t*-test was used when *F*-test showed homoscedasticity. Statistical differences were considered significant when the *P*-value was less than 0.05.

RESULTS

Food intake and body weight

Figure 1 shows food intake during the 2-day measurements and body weights before and after the rats entered their individual cages. Food intake was significantly higher in the HAA rats than in the LAA rats (Fig. 1 (a), $t(10)=3.736$, $P=0.0039$). Before the rats were introduced into the individual cages, the HAA rats were significantly heavier than LAA rats (Fig. 1 (b), $t(18)=2.567$, $P=0.0039$). However, just after the food-intake recording period, there was no significant difference in the body weights between the HAA and LAA rats (Fig. 1 (c), $t(18)=-1.881$, $P=0.076$). There was no significant difference in body weight gain rate (HAA: 112.629%, LAA: 115.605%, $t(18)=-1.513$, $P=0.1477$), and the HAA and LAA rats both showed significantly heavier body weights after measurement than before measurement (HAA: $t(9)=-10.771$, $P<0.0001$; LAA: $t(9)=-12.870$, $P<0.0001$).

Heart function

Table 1 shows the heart function of the HAA and LAA rats at the ages of 9 and 16 weeks. At 9 weeks of age, the HAA rats had higher SBP, DBP, and MBP levels than the LAA rats (SBP, $t(14)=2.048$, $P=0.0598$; DBP, $t(14)=3.476$, $P=0.0037$; and MBP,

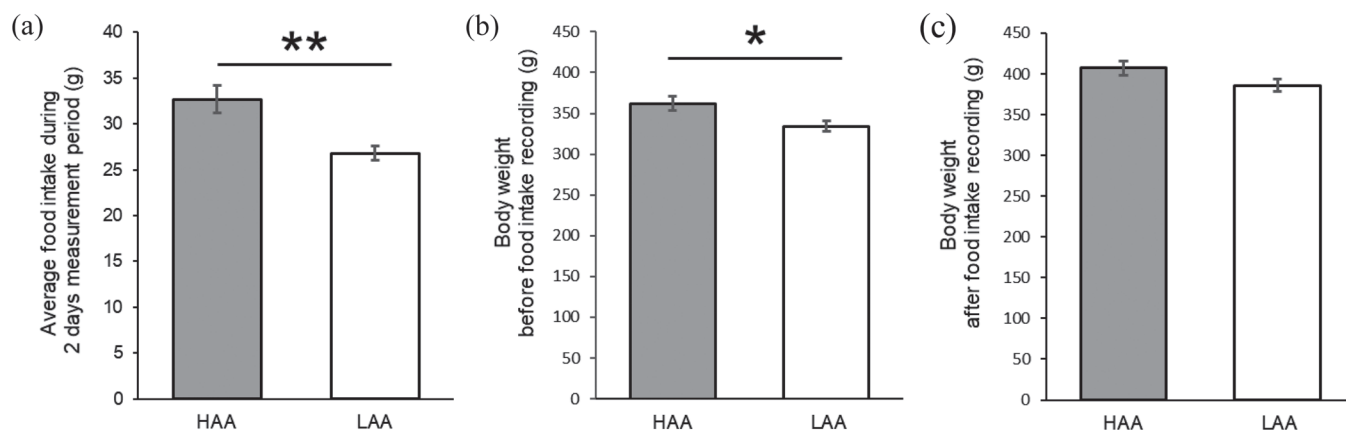


Fig. 1. Comparison of food intake and body weights between the HAA and LAA rats. Bars in the graphs are means \pm standard error of the mean. * and ** indicate significant differences ($*P<0.05$, $**P<0.01$ versus LAA rats). (a) average daily food intake during the 2-day measurement period, HAA: $n=5$, LAA: $n=7$; (b) body weight at 9 weeks of age before entering individual cage, HAA: $n=10$, LAA: $n=10$; and (c) body weight after 12–14 days in the individual cages during food-intake measurement, HAA: $n=10$, LAA: $n=10$.

Table 1. Comparison of heart function parameters between the HAA and LAA rats at the ages of 9 and 16 weeks

Age Strain	9-week-old		16-week-old	
	HAA (n=8)	LAA (n=8)	HAA (n=6)	LAA (n=6)
SBP (mmHg)	152.0 ± 6.3	130.5 ± 8.4	160.6 ± 13.2 ^{a)}	140.5 ± 6.8
DBP (mmHg)	110.1 ± 2.6 ^{b)}	93.2 ± 4.2	131.4 ± 8.9 ^{a)}	113.1 ± 4.7
MBP (mmHg)	124.0 ± 3.2 ^{a)}	105.5 ± 5.5	141.0 ± 10.3 ^{a)}	122.2 ± 5.2
HR (bpm)	359.3 ± 5.1 ^{b)}	413.2 ± 4.4	352.8 ± 25.7 ^{a)}	400.5 ± 48.6

Values are means ± standard error of the mean. HAA, high-avoidance animals; LAA, low-avoidance animals; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; HR, heart rate. a) and b) indicate significant differences (a) $P < 0.05$, b) $P < 0.01$ versus LAA rats.

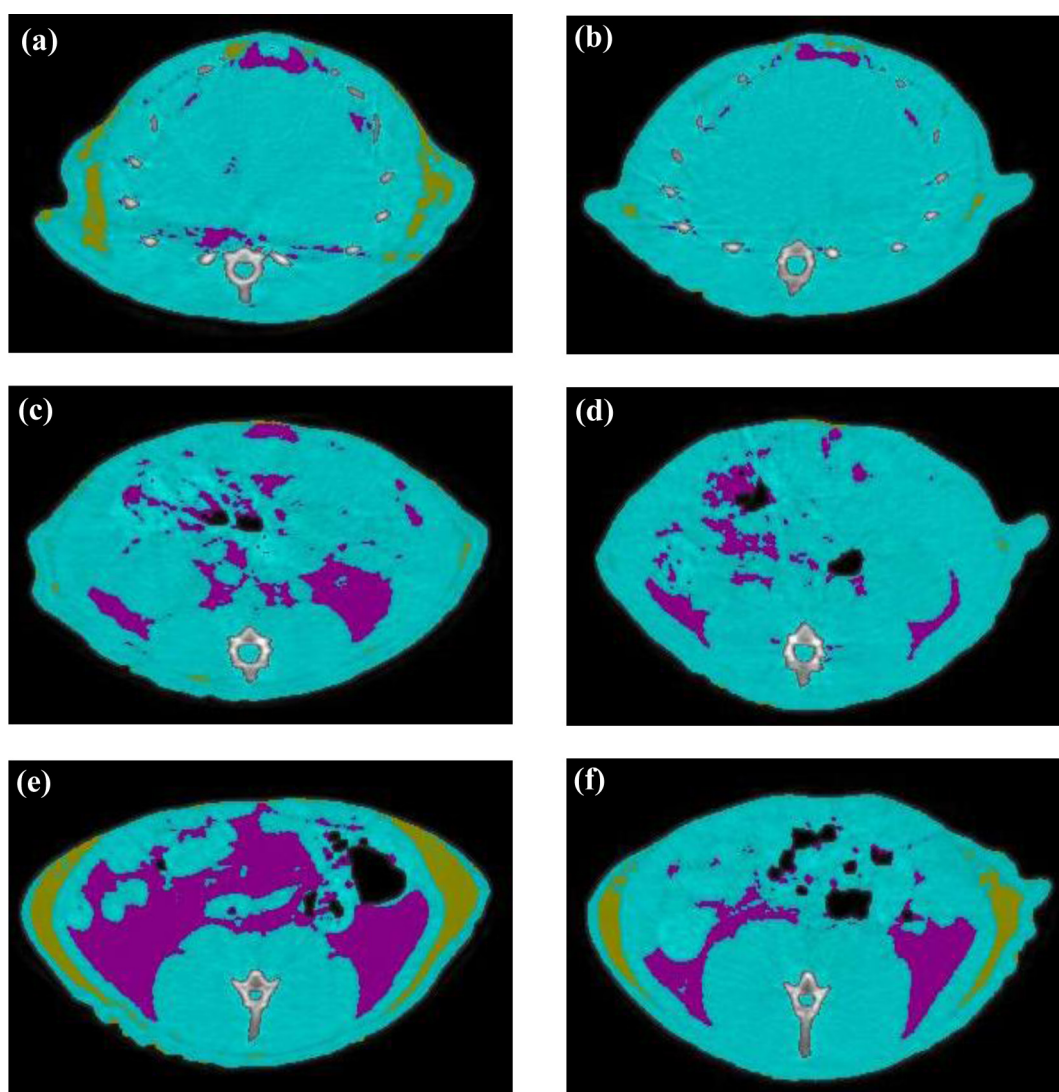


Fig. 2. Representative CT scan of the HAA (a, c, e) and LAA (b, d, f) rats showing subcutaneous fat (yellow), visceral fat (pink), and muscle mass (blue). (a, b) Just below the ribs, (c, d) at the abdomen, and (e, f) in front of the pelvis.

$t(14)=2.930$, $P=0.0110$). However, HR in the HAA rats was significantly lower than that in the LAA rats ($t(14)=-7.990$, $P < 0.0001$). Similar results were obtained at 16 weeks of age, and the difference in SBP was significant only at 16 weeks of age.

Abdominal fat, muscle mass, and fat weights

Figures 2 and 3 show the amount of abdominal fat and muscle mass measured by CT scanning at 17 weeks of age. Figure 2

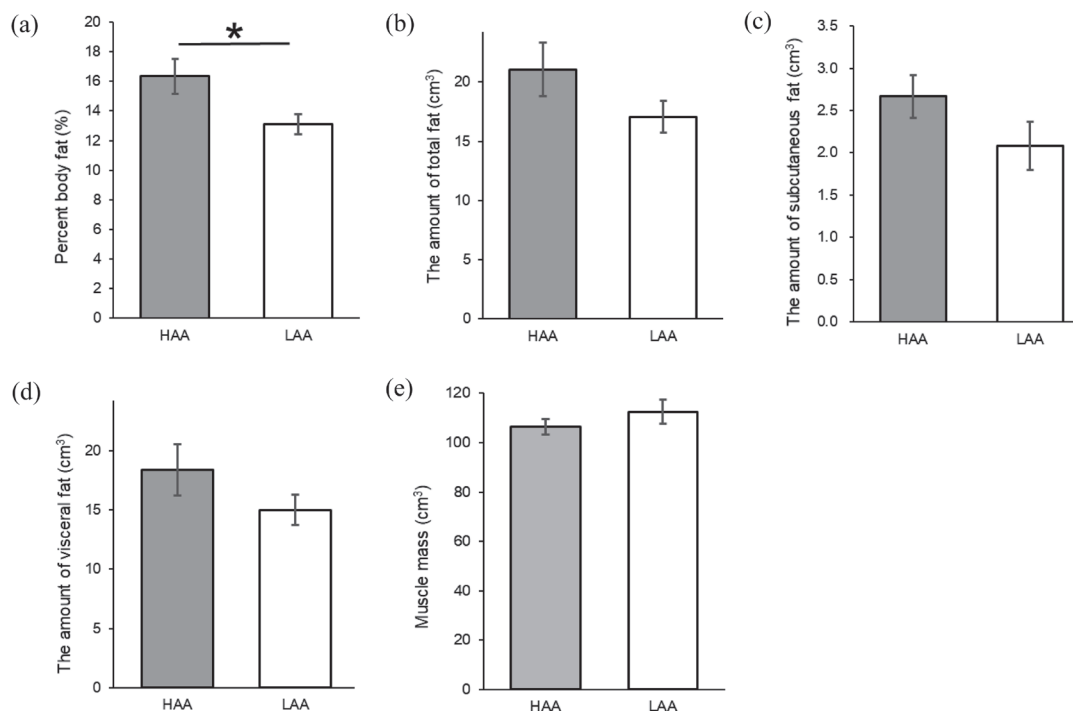


Fig. 3. Comparison of the amounts of abdominal fat and muscle mass between the HAA and LAA rats. Bars are means \pm standard error of the mean. HAA: n=8, LAA: n=8. * indicates significant difference (* P <0.05 versus LAA rats). (a) Percent body fat, (b) amount of total fat, (c) amount of visceral fat, (d) amount of subcutaneous fat, and (e) muscle mass.

Table 2. Comparison of fat weights between the HAA and LAA rats at the age of 17 weeks

Age	17-week-old	
	HAA (n=8)	LAA (n=8)
Mesenteric fat (g)	6.52 \pm 0.56	5.16 \pm 0.30
Perirenal fat (g)	10.85 \pm 0.98	9.43 \pm 0.61
Gonadal fat (g)	7.21 \pm 0.52	6.68 \pm 0.25

Values are means \pm standard error of the mean. HAA, high-avoidance animals; LAA, low-avoidance animals.

Table 3. Comparison in terms of blood plasma component concentrations between the HAA and LAA rats at the age of 17 weeks

Age	17-week-old	
	HAA (n=8)	LAA (n=8)
Triglyceride (mg/dl)	130.3 \pm 16.0 ^{a)}	72.8 \pm 11
Total cholesterol (mg/dl)	34.1 \pm 1.0 ^{b)}	46.5 \pm 2
HDL (mg/dl)	31.2 \pm 1.5 ^{a)}	50.7 \pm 8.6
LDL (mg/dl)	16.1 \pm 2.8 ^{a)}	25.5 \pm 2.6

Values are means \pm standard error of the mean. HAA, high-avoidance animals; LAA, low-avoidance animals; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol. a) and b) indicate significant differences (a) P <0.05, b) P <0.01 vs. LAA rats).

includes the part of HAA and LAA rats' CT images scanned just below the ribs (a, b), at the abdomen (c, d), and in front of the pelvis (e, f). According to these images, HAA rats have more fat than LAA rats. Especially, HAA rats had more visceral fats at the abdomen and in front of the pelvis, and more subcutaneous fats at abdomen and in front of the pelvis. As the results of analysis, the percentage of body fat in the HAA rats was significantly higher than that in the LAA rats (Fig. 3 (a), $t(14)=2.391$, $P=0.0314$). This exactly reflects the CT images: HAA rats have more fat than LAA. Also, the amounts of total, subcutaneous, and visceral fats tended to be higher in the HAA rats than those in the LAA rats; however, the differences were not statistically significant (Fig. 3 (b), total fat, $t(14)=1.722$, $P=0.1072$; Fig. 3 (c), subcutaneous fat, $t(14)=1.659$, $P=0.1193$; and Fig. 3 (d), visceral fat, $t(14)=1.580$, $P=0.1364$). There was no significant difference in terms of muscle mass between the HAA and LAA rats (Fig. 3 (e), $t(14)=-0.273$, $P=0.7888$). At 17 weeks of age, the amounts of mesenteric, perirenal, and gonadal fats were measured (Table 2). The amount of these fats tended to be higher in the HAA rats than those in the LAA rats; however, the differences were not statistically significant (mesenteric fat, $t(14)=2.132$, $P=0.0512$; perirenal fat, $t(14)=1.231$, $P=0.2385$; and gonadal fat, $t(14)=0.924$, $P=0.3712$).

Plasma component levels

Table 3 shows the concentrations of the plasma component at 17 weeks of age. The HAA rats showed significantly higher triglyceride levels than the LAA rats ($t(14)=2.967$, $P=0.0103$). However, they had significantly lower blood levels of total, HDL, and LDL cholesterol than the LAA rats (total cholesterol, $t(14)=-5.488$, $P<0.0001$; HDL, $t(14)=-2.226$, $P=0.0429$; LDL,

$t(14)=-2.429$, $P=0.0292$). There was no significant difference in the glucose levels between the HAA (185.8 ± 7.5 mg/dl) and LAA (187.4 ± 8.1 mg/dl) rats ($t(14)=-0.142$, $P=0.8893$).

DISCUSSION

The purpose of this study was to determine whether Hatano HAA and LAA rats could be used as a metabolic syndrome model. We compared the feeding behavior, body weight, blood pressure levels, amount of abdominal fat, plasma component concentration, and percent body fat between the HAA and LAA rats. Although no studies have specified the conditions that constitute metabolic syndrome in rats, the risk factors for metabolic syndrome in humans are food intake, blood pressure levels, plasma component levels, and fat levels [9], and these risk factors have been studied in previous metabolic syndrome models. We demonstrated that HAA rats show some of the risk factors of metabolic syndrome, such as active eating, high blood pressure, high percent fat, and high triglyceride levels, suggesting that HAA rats could be used as a metabolic syndrome model for studying feeding behavior, blood pressure levels, and percent body fat.

Food intake by the HAA rats was higher than that by the LAA rats. A previous study showed that under normal conditions, HAA rats secrete lesser CRH from the PVN, which suppresses appetite, than LAA rats [1]. Moreover, previous studies have shown that HAA rats are more active than LAA rats [12, 20]. Active locomotion is associated with higher food consumption, suggesting that more active rodents require higher energy intake. In the future, we will also need to consider oxygen consumption and respiratory conversion rate. In contrast, it has been reported that HAA rats show higher plasma testosterone levels, promoting appetite and eating [10], than LAA rats [16]. The strain difference in testosterone may be related to the difference in feeding behavior between HAA and LAA. Therefore, these findings indicate that HAA rats have a more active feeding behavior than LAA rats.

In this study, the HAA rats were heavier than the LAA rats at 9 weeks of age before the rats entered the individual cages for food-intake recording. A previous study [18] also reported that the body weight of HAA rats was greater than that of LAA rats, and the difference was apparent from postnatal day 14. However, in our study, by the end of the food-intake recording period, there was not much difference between the body weights of the HAA and LAA rats. This lack of difference in weights could be attributed to stress because stress has been shown to reduce appetite [30]. Some previous studies have reported that HAA rats have higher stress sensitivity than LAA rats [4, 5, 19]. Therefore, in our study, it is possible that the HAA rats had reduced feeding behavior during the food-intake recording period due to the stress caused by being individually placed in cages, compared with when the rats were placed together in a group. The stress of isolation may have resulted in the HAA rats eating less, thereby resulting in not much of a weight difference from that of the LAA rats. In addition, despite more food intake in HAA rats, there was no strain difference in weight gain rate. The weight gain rate of HAA rats may be suppressed by stress. Thus, the HAA rats had almost similar body weights as the LAA rats at the end of the food-intake recording period.

At 9 and 16 weeks of age, SBP, DBP and MBP blood pressure values in the HAA rats were higher than those in the LAA rats. The HAA rats had similar SBP, MBP, and DBP values as spontaneously hypertensive rats did, which are used as animal models for hypertension [13]. Blood pressure levels and HRs are increased by stress [6, 28], and HAA rats have been shown to have higher stress sensitivity than LAA rats. Immobilization stress, particularly for the HAA rats, may have caused mechanical retention of blood, which subsequently would have resulted in high blood pressure. However, in this study, the HR of the HAA rats was lower than that of the LAA rats. According to the previous study [3], low HR could reduce hypertension by autonomic modulation as baroreflex. Therefore, in our study, the low HR in the HAA rats may have also been caused by the mechanism of homeostasis to reduce hypertension. And, these strain differences may be related to systematic strain differences in acetylcholine. We will need to investigate acetylcholine secretion in Hatano rats next time.

The amounts of abdominal fat and muscle mass were measured by CT at 16 weeks of age. Thereafter, the fat weights were measured at 17 weeks of age. The percent body fat in the HAA rats was significantly higher than that in the LAA rats. Similarly, the amounts of visceral and subcutaneous fats and the weights of mesenteric, perirenal, and gonadal fats in the HAA rats tended to be greater than those in the LAA rats. High amount of body fat is a risk factor for metabolic syndrome [31]. These features support that HAA rats are more likely to metabolic syndrome than LAA rats. The results of body fat in this study suggest that the highly active feeding behavior of the HAA rats could have resulted in their increased percent body fat.

The measurement of plasma component concentrations at 17 weeks of age revealed that the HAA rats had higher triglyceride levels but lower total, HDL, and LDL cholesterol levels than the LAA rats. High levels of plasma triglyceride and low levels of HDL are recognized risk factors of metabolic syndrome [9], suggesting that the HAA rats exhibited higher number of risk factors of metabolic syndrome than the LAA rats. In addition, a high fasting glucose level is one of the symptoms of metabolic syndrome. In this study, there was no strain difference in the glucose level, although it was not at fasting but normal breeding. This result is similar to the previous fasting glucose level data showing no strain difference in the National Bio Resource Project [25]. In the future, it is need to consider the glucose metabolism and insulin resistance by glucose tolerance test or insulin tolerance test in order to clarify the differences between HAA and LAA rats.

In this study, we showed that HAA rats are a suitable model of metabolic syndrome for focusing on the feeding behavior, blood pressure levels, and percent body fat compared with LAA rats. Unpublished studies in our laboratory (Maiko Kawaguchi) have shown that the phenotype of Hatano rats, their anxiety-like behavior, and stress response can be changed by cross-fostering: maternal factors such as nursing behavior during the developing stage. Therefore, the results of our study suggest that the strain differences in this study could be caused and changed not only by genetic factors but also by environmental factors such as cross-fostering. Moreover, Hatano rats can be used as a metabolic syndrome model to understand the influence of environmental factors

on the development and management of metabolic syndrome.

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REFERENCES

1. Akieda-Asai, S., Ohta, R., Shirota, M., Jaroenporn, S., Watanabe, G. and Taya, K. 2011. Endocrinological differences between Hatano high- and low-avoidance rats during early two-way avoidance acquisition. *Exp. Anim.* **60**: 509–516. [Medline] [CrossRef]
2. Amin, F. and Gilani, A. H. 2013. Fiber-free white flour with fructose offers a better model of metabolic syndrome. *Lipids Health Dis.* **12**: 44. [Medline] [CrossRef]
3. Arnold, A. C. and Diz, D. I. 2014. Endogenous leptin contributes to baroreflex suppression within the solitary tract nucleus of aged rats. *Am. J. Physiol. Heart Circ. Physiol.* **307**: H1539–H1546. [Medline] [CrossRef]
4. Asai, S., Ohta, R., Shirota, M., Watanabe, G. and Taya, K. 2004. Differential responses of the hypothalamo-pituitary-adrenocortical axis to acute restraint stress in Hatano high- and low-avoidance rats. *J. Endocrinol.* **181**: 515–520. [Medline] [CrossRef]
5. Asai, S., Ohta, R., Fujikawa, T., Sakai, R. R., Shirota, M., Ogata, M., Watanabe, G. and Taya, K. 2006. Gastric ulceration and expression of prolactin receptor in the brain in Hatano high- and low-avoidance rats. *Endocrine* **30**: 161–166. [Medline] [CrossRef]
6. Ayada, C., Toru, Ü. and Korkut, Y. 2015. The relationship of stress and blood pressure effectors. *Hippokratia* **19**: 99–108. [Medline]
7. Chen, Q., Liu, Y., Yin, Y., Huang, W., Li, G. and Ke, D. 2008. Relationship between metabolic syndrome (MS) and coronary heart disease (CHD) in an aged group. *Arch. Gerontol. Geriatr.* **46**: 107–115. [Medline] [CrossRef]
8. de Cossío, L. F., Fourrier, C., Sauvart, J., Everard, A., Capuron, L., Cani, P. D., Layé, S. and Castanon, N. 2017. Impact of prebiotics on metabolic and behavioral alterations in a mouse model of metabolic syndrome. *Brain Behav. Immun.* **64**: 33–49. [Medline] [CrossRef]
9. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 2001. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA* **285**: 2486–2497. [Medline] [CrossRef]
10. Gentry, R. T. and Wade, G. N. 1976. Androgenic control of food intake and body weight in male rats. *J. Comp. Physiol. Psychol.* **90**: 18–25. [Medline] [CrossRef]
11. Gomez-Smith, M., Karthikeyan, S., Jeffers, M. S., Janik, R., Thomason, L. A., Stefanovic, B. and Corbett, D. 2016. A physiological characterization of the Cafeteria diet model of metabolic syndrome in the rat. *Physiol. Behav.* **167**: 382–391. [Medline] [CrossRef]
12. Horii, Y., Kawaguchi, M., Ohta, R., Hirano, A., Watanabe, G., Kato, N., Himi, T. and Taya, K. 2012. Male Hatano high-avoidance show high anxiety-like behaviors as compared with male low-avoidance rats. *Exp. Anim.* **61**: 517–524. [Medline] [CrossRef]
13. Hoshino Laboratory Animals, Inc. 2012. SHR/Hos Spontaneously hypertensive rats [homepage on the Internet]. Hoshino Laboratory Animals, Ibaraki, Japan. <http://www.hoshino-lab-animals.co.jp/english/products/SHR-en.html> [accessed on July 30, 2018].
14. Janczura, M., Bochenek, G., Nowobilski, R., Dropinski, J., Kotula-Horowitz, K., Laskowicz, B., Stanisz, A., Lelakowski, J. and Domagala, T. 2015. The relationship of metabolic syndrome with stress, coronary heart disease and pulmonary function—An occupational cohort-based study. *PLoS One* **10**: e0133750. [Medline] [CrossRef]
15. Kobori, M., Masumoto, S., Akimoto, Y. and Oike, H. 2011. Chronic dietary intake of quercetin alleviates hepatic fat accumulation associated with consumption of a Western-style diet in C57/BL6J mice. *Mol. Nutr. Food Res.* **55**: 530–540. [Medline] [CrossRef]
16. Nakayama, A., Okawa, H., Zheng, M., Pu, S., Watanabe, G., Ohta, R. and Kawaguchi, M. 2018. Male Hatano low-avoidance rats show more active sexual behavior with lower plasma testosterone than high-avoidance rats. *J. Vet. Med. Sci.* **80**: 1179–1182. [Medline] [CrossRef]
17. Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E. C., Biryukov, S., Abbafati, C., Abera, S. F., Abraham, J. P., Abu-Rmeileh, N. M., Achoki, T., AlBuhairan, F. S., Alemu, Z. A., Alfonso, R., Ali, M. K., Ali, R., Guzman, N. A., Ammar, W., Anwar, P., Banerjee, A., Barquera, S., Basu, S., Bennett, D. A., Bhutta, Z., Blore, J., Cabral, N., Nonato, I. C., Chang, J. C., Chowdhury, R., Courville, K. J., Criqui, M. H., Cundiff, D. K., Dabhadkar, K. C., Dandona, L., Davis, A., Dayama, A., Dharmaratne, S. D., Ding, E. L., Durrani, A. M., Esteghamati, A., Farzadfar, F., Fay, D. F., Feigin, V. L., Flaxman, A., Forouzanfar, M. H., Goto, A., Green, M. A., Gupta, R., Hafezi-Nejad, N., Hankey, G. J., Harewood, H. C., Havmoeller, R., Hay, S., Hernandez, L., Husseini, A., Idrisov, B. T., Ikeda, N., Islami, F., Jahangir, E., Jassal, S. K., Jee, S. H., Jeffreys, M., Jonas, J. B., Kabagambe, E. K., Khalifa, S. E., Kengne, A. P., Khader, Y. S., Khang, Y. H., Kim, D., Kimokoti, R. W., Kinge, J. M., Kokubo, Y., Kosen, S., Kwan, G., Lai, T., Leinsalu, M., Li, Y., Liang, X., Liu, S., Logroscino, G., Lotufo, P. A., Lu, Y., Ma, J., Mainoo, N. K., Mensah, G. A., Merriman, T. R., Mokdad, A. H., Moschandreas, J., Naghavi, M., Naheed, A., Nand, D., Narayan, K. M., Nelson, E. L., Neuhouser, M. L., Nisar, M. I., Ohkubo, T., Oti, S. O., Pedroza, A., Prabhakaran, D., Roy, N., Sampson, U., Seo, H., Sepanlou, S. G., Shibuya, K., Shiri, R., Shiue, I., Singh, G. M., Singh, J. A., Skirbekk, V., Stapelberg, N. J., Sturua, L., Sykes, B. L., Tobias, M., Tran, B. X., Trasande, L., Toyoshima, H., van de Vijver, S., Vasankari, T. J., Veerman, J. L., Velasquez-Melendez, G., Vlassov, V. V., Vollset, S. E., Vos, T., Wang, C., Wang, X., Weiderpass, E., Werdecker, A., Wright, J. L., Yang, Y. C., Yatsuya, H., Yoon, J., Yoon, S. J., Zhao, Y., Zhou, M., Zhu, S., Lopez, A. D., Murray, C. J. and Gakidou, E. 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384**: 766–781. [Medline] [CrossRef]
18. Ohta, R., Matsumoto, A., Nagao, T. and Mizutani, M. 1998. Comparative study of behavioral development between high and low shuttlebox avoidance rats. *Physiol. Behav.* **63**: 545–551. [Medline] [CrossRef]
19. Ohta, R., Shirota, M., Adachi, T., Tohei, A. and Taya, K. 1999. Plasma ACTH levels during early, two-way avoidance acquisition in high- and low-avoidance rats (Hatano strains). *Behav. Genet.* **29**: 137–144. [Medline] [CrossRef]
20. Ohta, R., Shirota, M., Kanazawa, Y., Shindo, T., Furuya, M., Seki, T., Ono, H., Kojima, K., Asai, S., Watanabe, G. and Taya, K. 2009. Effects of transmaternal exposure to genistein in Hatano high- and low-avoidance rats. *Exp. Anim.* **58**: 471–479. [Medline] [CrossRef]
21. Overduin, J., Figlewicz, D. P., Bennett-Jay, J., Kittleson, S. and Cummings, D. E. 2012. Ghrelin increases the motivation to eat, but does not alter food palatability. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **303**: R259–R269. [Medline] [CrossRef]
22. Schick, R. R., Schusdziarra, V., Nussbaumer, C. and Classen, M. 1991. Neuropeptide Y and food intake in fasted rats: effect of naloxone and site of action. *Brain Res.* **552**: 232–239. [Medline] [CrossRef]

23. Taguchi, K., Okada, A., Hamamoto, S., Iwatsuki, S., Naiki, T., Ando, R., Mizuno, K., Tozawa, K., Kohri, K. and Yasui, T. 2015. Proinflammatory and metabolic changes facilitate renal crystal deposition in an obese mouse model of metabolic syndrome. *J. Urol.* **194**: 1787–1796. [[Medline](#)] [[CrossRef](#)]
24. Takahashi, T., Nishida, T., Baba, H., Hatta, H., Imura, J., Sutoh, M., Toyohara, S., Hokao, R., Watanabe, S., Ogawa, H., Uehara, H. and Tsuneyama, K. 2016. Histopathological characteristics of glutamine synthetase-positive hepatic tumor lesions in a mouse model of spontaneous metabolic syndrome (TSOD mouse). *Mol. Clin. Oncol.* **5**: 267–270. [[Medline](#)] [[CrossRef](#)]
25. The National Bio Resource Project for the Rat in Japan. 2002–2012. Phenome project at NBRP-Rat. The National Bio Resource Project for the Rat in Japan, Kyoto, Japan. <http://www.anim.med.kyoto-u.ac.jp/nbr/phenome/> [accessed on July 30, 2018].
26. Tsigos, C. and Chrousos, G. P. 2002. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J. Psychosom. Res.* **53**: 865–871. [[Medline](#)] [[CrossRef](#)]
27. Ushimura, A., Tsuji, T., Tanaka, S., Kogo, M. and Yamamoto, T. 2015. Neuropeptide-Y modulates eating patterns and masticatory muscle activity in rats. *Behav. Brain Res.* **278**: 520–526. [[Medline](#)] [[CrossRef](#)]
28. Vieira, J. O., Duarte, J. O., Costa-Ferreira, W., Morais-Silva, G., Marin, M. T. and Crestani, C. C. 2018. Sex differences in cardiovascular, neuroendocrine and behavioral changes evoked by chronic stressors in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **81**: 426–437. [[Medline](#)] [[CrossRef](#)]
29. Wren, A. M., Small, C. J., Abbott, C. R., Dhillo, W. S., Seal, L. J., Cohen, M. A., Batterham, R. L., Taheri, S., Stanley, S. A., Ghatei, M. A. and Bloom, S. R. 2001. Ghrelin causes hyperphagia and obesity in rats. *Diabetes* **50**: 2540–2547. [[Medline](#)] [[CrossRef](#)]
30. Yau, Y. H. and Potenza, M. N. 2013. Stress and eating behaviors. *Minerva Endocrinol.* **38**: 255–267. [[Medline](#)]
31. Zhu, S., Wang, Z., Shen, W., Heymsfield, S. B. and Heshka, S. 2003. Percentage body fat ranges associated with metabolic syndrome risk: results based on the third National Health and Nutrition Examination Survey (1988–1994). *Am. J. Clin. Nutr.* **78**: 228–235. [[Medline](#)] [[CrossRef](#)]