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Physiology

Effects of different winter climates in Japan on body composition of young Thoroughbreds in training

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ABSTRACT. Changes in the body composition of 50 Thoroughbreds colts and fillies, born between 2004 and 2010, were compared between those reared at the Hidaka Training and Research Center (Hidaka), Hokkaido, which is extremely cold in winter, and those reared at the Miyazaki Yearling Training Farm (Miyazaki), Kyushu, which is mildly cold in winter. The horses were divided into two sex groups and reared and trained in Hidaka or Miyazaki for 7 months from October of one year of age to April of two years of age. Body weight (BW), rump fat thickness (RFT), fat-free mass (FFM), and percentage of fat (%F) were used as parameters of body composition. This study revealed that BW and FFM were higher, and %F was lower in colts than in fillies at both training sites. Among colts, Miyazaki colts tended to have higher FFM values than Hidaka colts, and %F was significantly lower in Miyazaki colts than in Hidaka colts. Furthermore, from October to April, Miyazaki horses had a higher rate of increase in BW than Hidaka horses in both sexes and a higher rate of increase in FFM in colts. The higher rate of increase in FFM in Miyazaki colts suggests that training young Thoroughbreds in winter under mildly cold climate is more effective, than severely cold climate, particularly in colts.

KEYWORDS: body composition, body weight, fat-free mass, rump fat thickness, Thoroughbred

The rearing period of Thoroughbred racehorses comprises three stages [50]: the foal stage, weanling to yearling stage, and pre-training stage. The "foal stage" is from birth to weaning, and the "weanling to yearling stage" is from weaning in the autumn of the current year to the start of breaking at yearling in the next autumn. They mainly consist of pasture management at the turned out paddock. The "pre-training stage" is from breaking in autumn at yearling to the time when the horse is ready to race in the next spring or summer of the third year [50]. More than 97% of Thoroughbreds produced in Japan are born in Hokkaido [45], and most spend approximately 2 years in the rearing period in there. Southern Kyushu, currently produces only about 50 Thoroughbreds [45], but in the 1970s there were more than 500 stud farms [15], especially breeding Anglo-Arabian racehorses, and used to be a major breeding region in Japan. Japan Racing Association (JRA) has training and research facilities in Hidaka, Hokkaido in north and Miyazaki, Kyushu in south. Their aims are to develop climate-matched breeding and rearing methods with scientific basis and to disseminate the methods to breeders or pre-training trainers in order to produce "world-class Thoroughbreds" in Japan. Japan has four seasons, but the climate differs somewhat between Hokkaido and Kyushu. Hokkaido has cool summers but extremely cold winters. The other side, Southern Kyushu has very hot and humid summers but mild winters. Decades ago, Sothern Kyushu has been considered as a suitable place for training young Thoroughbreds in "pre-training stage", because of its year-round green pasture and mild climate in winter. On the other hand, it was difficult to train in Hokkaido during winter due to snowfall and icy conditions on the training courses. But after 2000s, indoor training facilities have been developed in Hokkaido, making it possible to train horses for fast work even in winter, regardless of weather conditions. In contrast, it has been reported that Thoroughbreds in Hokkaido at the "pre-training

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stage" have their growth suppressed during winters due to cold sensitization and other factors [27, 34, 49]. Training Thoroughbreds adequately during "the pre-training stage" is considered important for them to show their racing performance from the early 2 years of age [38, 44]. However, if the horse is immature, the training load can cause musculoskeletal injuries [9, 36, 46] and delay their debut as racehorses. Therefore, developing management techniques that promote the growth of young horses during winter in Hokkaido is an important issue at the " pre-training stage".

Our previous studies have shown that Thoroughbred yearlings and juveniles trained in Miyazaki, Kyushu where winters are mildly cold, start secreting gonadal, testicular and ovarian hormones earlier than horses trained in Hidaka, Hokkaido where winters are severely cold [31, 42]. Miyazaki horses also show greater body weight (BW), height at withers (height), girth circumference (girth), and cannon circumference (cannon) than Hidaka horses [27, 31, 42, 43]. However, providing extended photoperiod method [33] to the colts and fillies in Hidaka, it was clearly demonstrated that gonadal, testicular and ovarian function was accelerated as same as in Miyazaki [21, 42], and the growth of BW, height, girth and cannon were also accelerated [42]. Our studies on body composition changes in Hidaka shows that fat-free mass (FFM) increased with growth under natural light condition in both colts and fillies [28]. It was also reported that FFM was higher in colts and rump fat thickness (RFT) was lower in colts than in fillies at the pre-training stage [28]. Moreover, if we provide extended photoperiod to colts in Hidaka, increased FFM and decreased RFT was reported [33]. But the comparisons of body composition between Hidaka and Miyazaki are unknown.

A high skeletal muscle characterizes Thoroughbreds to BW ratio of 53% [13]. Therefore, FFM indicates muscle mass [1, 12, 18, 22]. FFM and percentage of fat (%F) are estimated from BW, and RFT [16, 19, 47]. This study was aimed to clarify the effects of different winter climates on the body composition, including BW, FFM, and %F, of Thoroughbreds raised and trained at Hidaka Training and Research Center (Hidaka), Urakawa, Hokkaido (42°17'N, 142°72'E), and at the Miyazaki Yearling Training Farm (Miyazaki), Miyazaki City, Southern Kyushu (31°90'N, 139°42'E).

MATERIALS AND METHODS

Animals

For body composition analysis, 50 Thoroughbreds (25 colts and 25 fillies) born in 2004 and 2010 were used. All horses were purchased by JRA on the yearling auctions in July and August, and their height were 150 cm or taller at the sales. In allocating the yearlings into Hidaka or Miyazaki, we tried to generally equalize their purchase price and stallions, thereby reducing the differences in the qualities and body shape of the horses. They were transported to Hidaka or Miyazaki a week after sales, and managed by 17 hr of day-and-night grazing in the paddock until the start of the experiment. They were randomly divided into two sex groups and raised and trained in Hidaka (16 colts and 16 fillies) or Miyazaki (9 colts and 9 fillies) for 7 months from October at the 1 year of age to April at the 2 years of age. The horses were 19–22 months old at the time of experiment initiation. The results were compared between Thoroughbreds reared in Hidaka and those reared in Miyazaki.

Feed and exercise

Feed and exercise were standardized as much as possible to match the managing conditions in Hidaka and Miyazaki. All horses in Hidaka and Miyazaki were fed Oats, pelleted compound feed (Nosan Corporation, Yokohama, Japan), grass and hay as their basic diet. The energy content was adjusted according to the Japanese Feeding Standard for Horses (2004 edition) [10] (Table 1). The horses' feedings were at 6:00 hr, 12:00 hr, 16:00 hr, and 20:00 hr. All horses were allowed to be turned out in the paddock for 1–2 hr and to eat hay or fresh grass freely. For the training of the horses in Hidaka and Miyazaki, riding exercises were performed for approximately 1 hr. In Hidaka, indoor 800 m track course and indoor 1,000 m uphill course were used, and in Miyazaki, 500 m track course and 1,600 m track course was used for daily exercise. Although the facilities in Hidaka and Miyazaki differ due to climatic differences, adjustments were made with reference to heart rate (HR) and blood lactate concentration (lactate) measurements to ensure that the training load is almost the same. On work days, the load was applied so that the lactate was greater than 4 mmol/L and the HR was greater than 200 beats/min. Riders generally weighed between 50 and 65 kg and had sufficient experience of racehorses. The training intensity was gradually increased systematically, and by the end of April, cantering training was performed 6 days a week (Monday through Saturday) at a distance of 2,000–3,200 m based on a speed of 600–660 m/min. Additionally, in April at the 2 years of age, speed training was performed once or twice a week, running the last 400 m at a speed of approximately 800–1,000 m/min (Table 2). All procedures complied with the guidelines for the use of horses established by the Hidaka Training and Research Center of Japan Racing Association (JRA).

Table 1. Daily feed for 1–2 years old horses

	Oats (kg)	Compound feed (kg)	Timothy hay (kg)	Alfalfa hay (kg)	Digestible energy (Mcal)
Oct.	1.0	2.5	7.0	0	21.0
Nov.	1.0	3.5	7.0	0	24.1
Dec.	1.0	4.0	7.0	1.0	27.5
Jan.	1.0	4.0	7.5	1.0	28.3
Feb.	1.0	4.0	7.5	1.0	28.3
Mar.	1.0	4.0	7.5	2.0	30.1
Apr.	1.0	4.5	7.5	2.0	31.6

Body composition measurement

BW of the horses were measured by scale (TRU-TEST S3, Datamars, Auckland, New Zealand) on the 15th of each month from October at the 1 year of age to April at the 2 years of age. RFT of the horses were measured on the same day of weighing, using B-mode ultrasonography (LOGIQB Book XP Basic, GE Health Care, Hino, Japan). The probe was placed over the rump approximately 5cm lateral from the midline at the center of the pelvic bone [47] (Fig. 1). %F, and FFM was calculated using the following formula [16, 19]:

%F=2.47 + 5.47 × RFT (cm)

FM (kg)=BW (kg) × %F/100

FFM (kg)=BW (kg) × (1 – %F/100)

Statistical analysis

All results are presented as means ± standard errors of the mean (SEM). Statistical analyses were performed by use of statistical software JMP (Ver 16.0). The one-way ANOVA were used to detect significant differences between months in BW, FFM and %F in the same facility or the same sex. When the analysis was significant, difference between specific months were analyzed by Tukey-Kramer test. Statistical comparisons between the two groups by sex or the facilities on BW, FFM, and %F were performed by student's *t*-test when uniformity of variance was confirmed by the *F*-test. When the variance was not uniform, an unpaired *t*-test with Welch's correction was used. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Comparison of sex differences in Hidaka horses

Figure 2 shows changes in BW, FFM, and %F in Hidaka horses from October at the 1 year of age to April at the 2 years of age.

BW increased slowly with growth in both colts and fillies. BW from February to April was significantly higher than that in October ($P<0.05$) (Fig. 2A). BW tended to be higher in colts than in fillies throughout the period from October to April, however, there were no significant differences between the two sexes.

Table 2. Training program for 1-2 years old horses (Sunday off [a])

	Walk (m)	Trot (m)	Canter (m)	:Speed (m/min)
Oct.	1,400	1,000	1,600	:500–600
Nov.	1,600	800	2,000–2,400	:600
Dec.	2,400	800	2,000–3,200	:600–660
Jan.	2,400	800	2,000–3,200	:600–660
Feb.	2,400	800	2,000–3,200	:600–660
			Workdays[b)]	*:750 (twice/week)*
Mar.	2,400	800	2,000–3,200	:600–660
			Workdays[b)]	*:800 (twice/week)*
Apr.	2,400	800	2,000–3,200	:600–660
			Workdays[b)]	*:800–1,000 (twice/week)*

a) Training was performed 6 days a week. Monday through Saturday. b) Speed in the last 400 m of work.

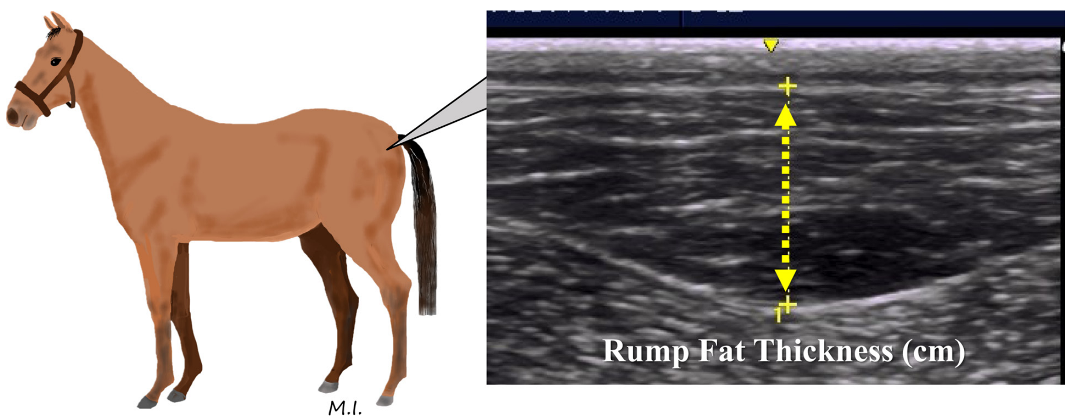

Fig. 1. Measurement of rump fat thickness in the Thoroughbred colts and fillies. Rump fat thickness (RFT) of the horses were measured using B-mode ultrasonography (LOGIQB Book XP Basic, GE Health Care, Hino, Japan). The probe was placed over the rump approximately 5 cm lateral from the midline at the center of the pelvic bone. BW of the horses were measured by scale (TRU-TEST S3, Datamars, Auckland, New Zealand) on the 15th of each month from October at the 1 year of age to April at the 2 years of age.

FFM increased slowly with growth in both colts and fillies, with fillies showing significantly higher FFM in March and April than in October (P<0.05) (Fig. 2B). In contrast, no significant differences in FFM were observed between October and November to April in colts. Moreover, colts tended to have higher FFM values than fillies, with a significant difference in November (P<0.05) (Fig. 2B).

%F remained at a nearly constant level for both colts and fillies. Fillies tended to have a higher %F than colts, with a significant difference in November (P<0.05) (Fig. 2C).

Comparison of sex differences in Miyazaki horses

Figure 3 shows changes in BW, FFM, and %F of Miyazaki horses from October at the 1 year of age to April at the 2 years of age. BW increased with age in both colts and fillies. In colts, the BW values in January, February, March, and April were significantly higher than that in October (P<0.05) (Fig. 3A). In fillies, the BW values in February, March, and April were significantly higher than

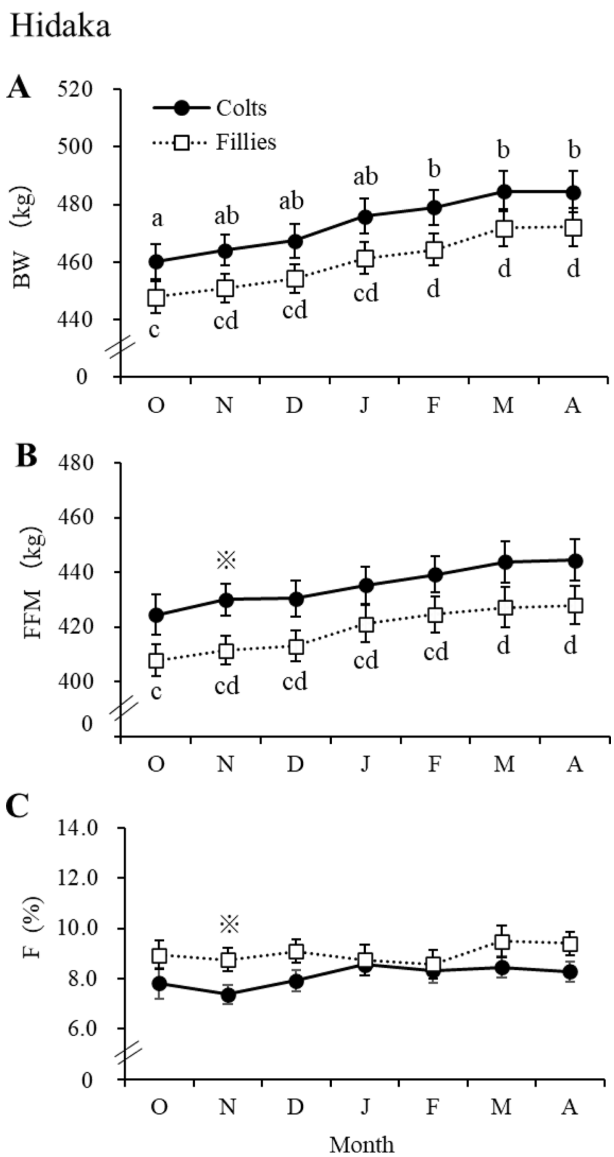

Fig. 2. Monthly changes in the body weight (BW) (**A**), fat-free mass (FFM) (**B**), and percentage of fat (%F) (**C**) from October at the 1 year of age to April at the 2 years of age in Thoroughbred colts (●; n=16) and fillies (□; n=16) reared at the Hidaka Training and Research Center of Japan Racing Association (JRA). Results are expressed as means ± SEM. Months are indicated by their initial letters. *Significant differences between the sex on the same month at P<0.05 (Student's t-test), and different alphabets on the same sex represent significant differences at P<0.05 (Tukey-Kramer test).

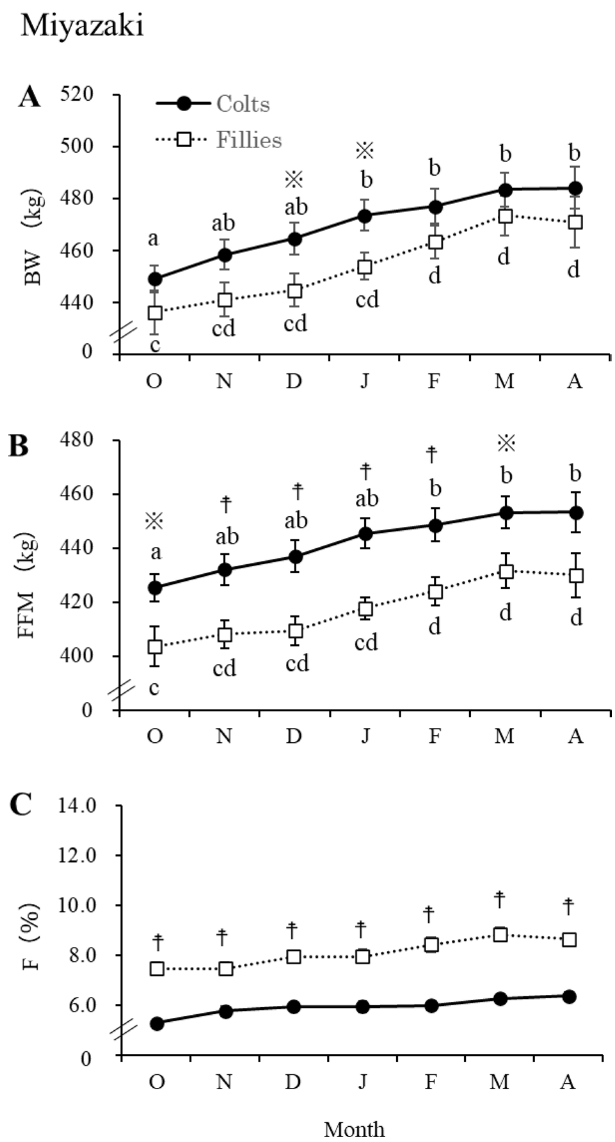

Fig. 3. Monthly changes in the body weight (BW) (**A**), fat-free mass (FFM) (**B**), and percentage of fat (%F) (**C**) from October at the 1 year of age to April at the 2 years of age in Thoroughbred colts (●; n=9) and fillies (□; n=9) reared at the Miyazaki Yearling Training Farm of JRA. Results are expressed as means ± SEM. Months are indicated by their initial letters. *Significant differences between the sex on the same month at P<0.05 (Student's t-test), *Significant differences between the sex on the same month at P<0.01 (Student's t-test), and different alphabets on the same sex represent significant differences at P<0.05 (Tukey-Kramer test).

that in October ($P<0.05$) (Fig. 3A). BW tended to be higher in colts than in fillies. In December and January, the BW values were significantly higher in colts than in fillies ($P<0.05$) (Fig. 3A).

FFM increased with age in both colts and fillies. The FFM values in February, March, and April in both colts and fillies were significantly higher than that in October ($P<0.05$). The FFM values from October to March were also significantly higher in colts than in fillies (October and March, $P<0.05$; November to February, $P<0.01$) (Fig. 3B).

%F did not vary significantly from October to April for both colts and fillies but was significantly lower in colts than in fillies in all months ($P<0.01$) (Fig. 3C).

Comparison of Hidaka and Miyazaki horses

The changes in BW, FFM, and %F in Hidaka and Miyazaki horses from October at the 1 year of age to April at the 2 years of age were compared. The results for colts are shown in Fig. 4, while those for fillies are shown in Fig. 5.

For colts, BW increased with age from October to March for both groups. However, there was no significant difference between the two groups (Fig. 4A). FFM increased with age from December to April for both groups, with the FFM values of Miyazaki colts showing a tendency to be higher than those of Hidaka colts, but there was no significant difference between the two groups (Fig. 4B). %F generally remained constant from October to April for both groups, with Hidaka colts showing significantly higher values than Miyazaki colts in all months ($P<0.01$) (Fig. 4C).

For fillies, BW increased with age from October to March for both groups. However, there was no significant difference between the two groups (Fig. 5A). FFM increased with age from October to March for both groups, but there was no significant difference

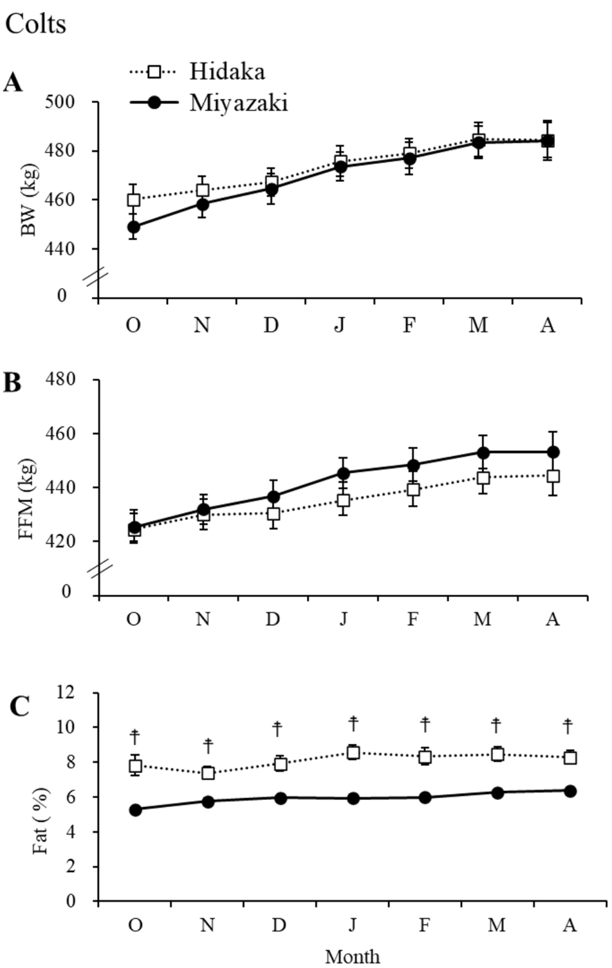

Fig. 4. Monthly changes in the body weight (BW) (**A**), fat-free mass (FFM) (**B**), and percentage of fat (%F) (**C**) from October at the 1 year of age to April at the 2 years of age in Thoroughbred colts reared at the Hidaka Training and Research Center of JRA (□; n=16) and the Miyazaki Yearling Training Farm of JRA (●; n=9). Results are expressed as means ± SEM. Months are indicated by their initial letters. *Significant differences between Hidaka and Miyazaki on the same month at $P<0.01$ (Student's *t*-test).

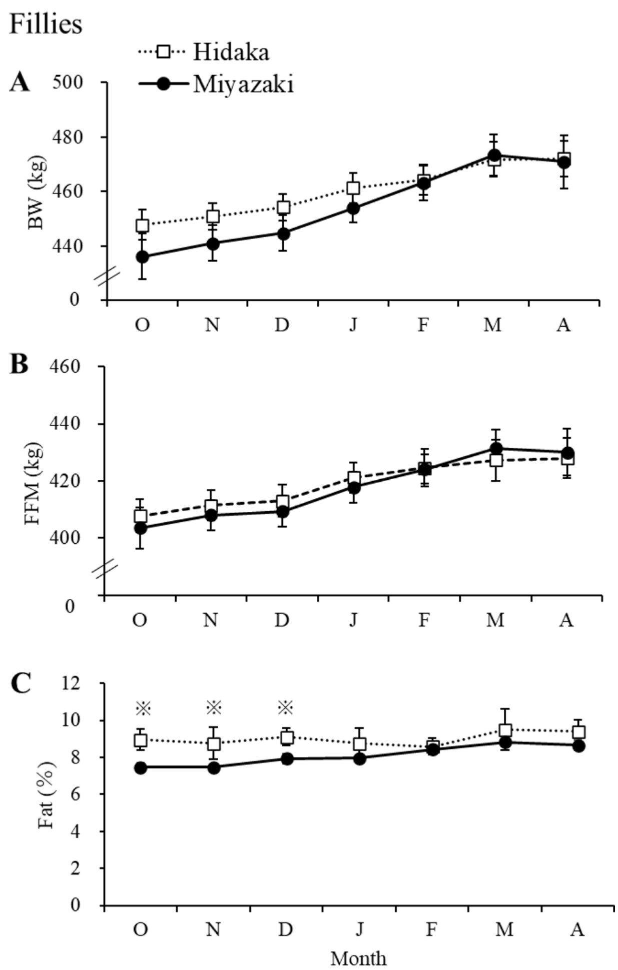

Fig. 5. Monthly changes in the body weight (BW) (**A**), fat-free mass (FFM) (**B**), and percentage of fat (%F) (**C**) from October at the 1 year of age to April at the 2 years of age in Thoroughbred fillies reared at the Hidaka Training and Research Center of JRA (□; n=16) and the Miyazaki Yearling Training Farm of JRA (●; n=9). Results are expressed as means ± SEM. Months are indicated by their initial letters. *Significant differences between Hidaka and Miyazaki on the same month at $P<0.05$. (Student's *t*-test).

between the two groups (Fig. 5B). For %F, Hidaka fillies showed significantly higher values than Miyazaki fillies from October to December ($P<0.05$) (Fig. 5C).

The results for the mean rates of increase in BW and FFM between horses reared and trained at the Hidaka Training and Research Center and Miyazaki Yearling Training Farm are shown in Figs. 6 and 7. In addition, the monthly rates of increase in BW and FFM between horses reared and trained at the Hidaka Training and Research Center and Miyazaki Yearling Training Farm from October to April are shown in Fig. 8. Miyazaki horses tended to have a higher rate of increase in BW from October to April in both colts and fillies than Hidaka horses (Fig. 6A). Significant differences in the rate of increase in BW were observed from October to March in colts ($P<0.05$) (Fig. 6B) and from October to March ($P<0.05$) (Fig. 6B) and from December to March ($P<0.05$) (Fig. 6C) in fillies. Regarding the FFM values from October to April, Miyazaki horses tended to have a higher rate of increase in FFM than Hidaka horses in both colts and fillies (Fig. 7A), and in colts, significant differences in the rate of increase in FFM were observed from October to February ($P<0.05$) (Fig. 7B).

Figure 8 compares the monthly rate of increase in BW and FFM from October to April in Hidaka and Miyazaki horses according to sex. No significant difference in the monthly rate of increase in BW was observed between Miyazaki and Hidaka colts (Fig. 8A), whereas Miyazaki fillies showed significantly higher values than Hidaka fillies in February ($P<0.05$) (Fig. 8B). Miyazaki colts tended to have a higher monthly rate of increase in FFM in December and January than Hidaka colts, however, the difference was no significant (Fig. 8C). Moreover, Miyazaki fillies tended to have a higher monthly rate of increase in FFM in March than Hidaka fillies, the difference was no significant (Fig. 8D).

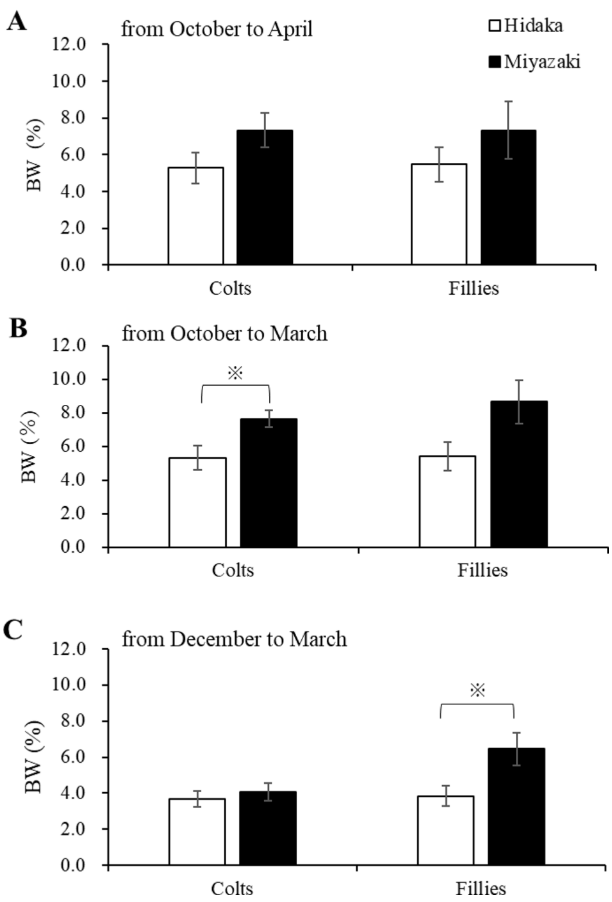

Fig. 6. Comparison of the mean rates of increase in body weight (BW) between horses reared and trained at the Hidaka Training and Research Center (□) and Miyazaki Yearling Training Farm (■) of JRA, from October at the 1 year of age to April at the 2 years of age (**A**; Hidaka n=16, Miyazaki n=9), from October to March (**B**; Hidaka n=16, Miyazaki n=9), and from December to March (C; Hidaka n=16, Miyazaki n=9) in coltsand fillies. Results are expressed as means ± SEM. *Significant differences between Hidaka and Miyazaki at $P<0.05$ (Student's *t*-test).

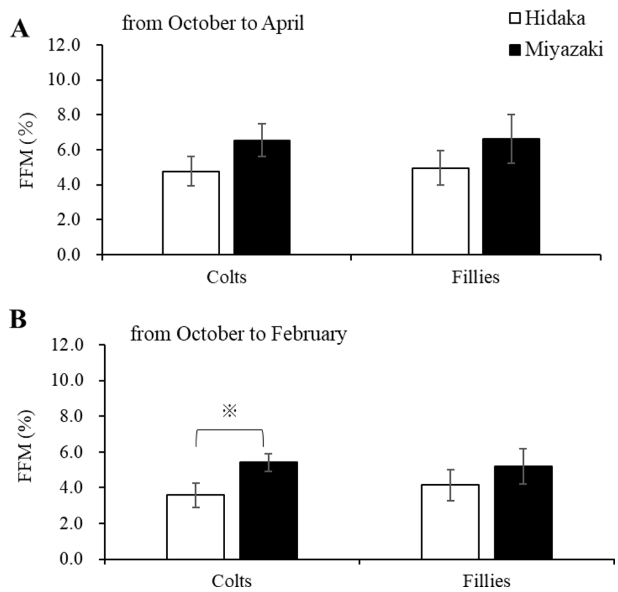

Fig. 7. Comparison of the mean rates of increase in fat-free mass (FFM) between horses reared and trained at the Hidaka Training and Research Center (□) and Miyazaki Yearling Training Farm (■) of JRA, from October at the 1 year of age to April at the 2 years of age (**A**; Hidaka n=16, Miyazaki n=9), from October to February (**B**; Hidaka n=16, Miyazaki n=9) in colts and fillies. Results are expressed as means ± SEM. *Significant differences between Hidaka and Miyazaki at $P<0.05$ (Student's *t*-test).

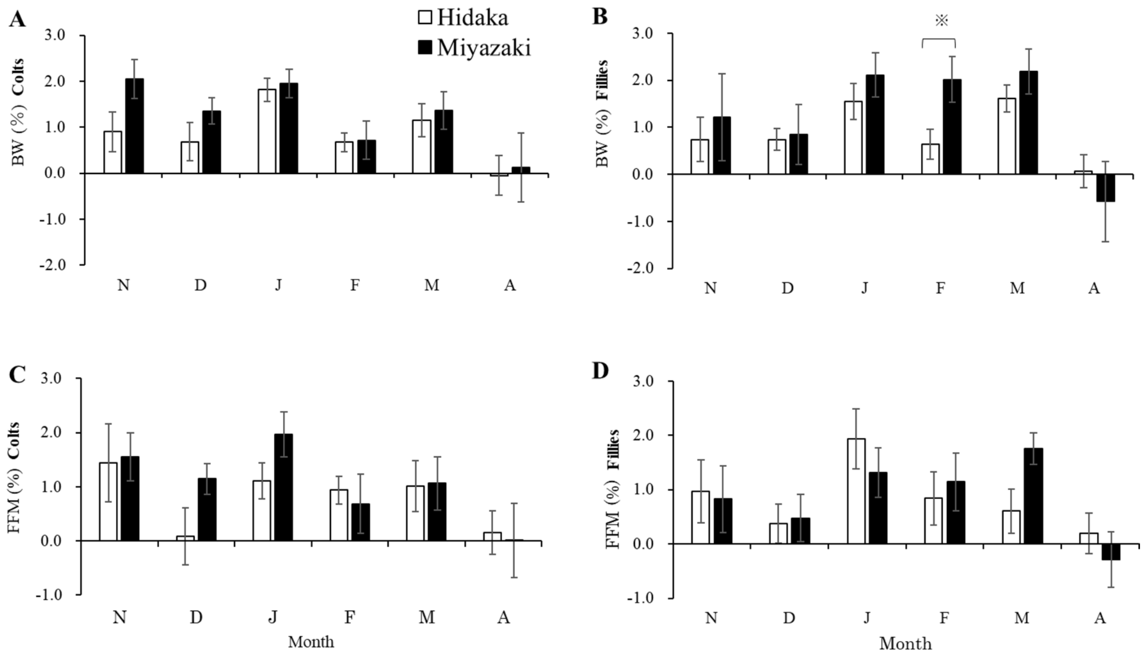

Fig. 8. Comparison of the monthly rates of increase in body weight (BW; A, B) and fat-free mass (FFM; C, D) between horses reared and trained at the Hidaka Training and Research Center (□) and Miyazaki Yearling Training Farm (■) of JRA from October at the 1 year of age to April at the 2 years of age in colts (**A**; Hidaka n=16, Miyazaki n=9) and in fillies (**B**; Hidaka n=16, Miyazaki n=9). Comparison of the monthly rates of increase in fat-free mass (FFM) between horses reared and trained at the Hidaka Training and Research Center (□) and Miyazaki Yearling Training Farm (■) of JRA, from October to April in colts (**C**; Hidaka n=16, Miyazaki n=9) and in fillies (D; Hidaka n=16, Miyazaki n=9). Results are expressed as means ± SEM. Months are indicated by their initial letters. *Significant differences between Hidaka and Miyazaki at $P<0.05$ (Student's *t*-test).

DISCUSSION

This study was aimed to clarify the effects of different winter climates in Japan on body composition of young Thoroughbreds in training. Thoroughbred colts and fillies were raised and trained for 7 months from October at the 1 year of age to April at the 2 years of age at the Hidaka Training and Research Center, Hokkaido, and at the Miyazaki Yearling Training Farm, Kyushu, and the changes in body composition were analyzed in this study.

BW increased with age in both colts and fillies. In Miyazaki horses, colts showed higher BW values in December and January than fillies. FFM increased with age in Hidaka fillies only and in both Miyazaki colts and fillies. FFM values of Hidaka colts were higher than those of Hidaka fillies in November, whereas FFM values from October to March of Miyazaki colts were higher than those of Miyazaki fillies. %F generally remained constant for both Hidaka and Miyazaki horses. Hidaka colts only showed lower %F values than their fillies counterparts in November, whereas Miyazaki colts showed %F values lower than their fillies counterparts in all months.

Comparing Hidaka horses with Miyazaki horses, no significant differences in BW and FFM values were observed between them for both sexes. %F was higher in Hidaka horses than in Miyazaki horses from October to December for fillies, whereas, for colts, Hidaka horses showed lower %F than Miyazaki horses in all months. Regarding the rate of increase in BW, Miyazaki horses showed a higher rate of increase in BW than Hidaka horses for both sexes, whereas, regarding the rate of increase in FFM, Miyazaki colts showed a higher rate of increase in FFM than Hidaka colts.

Among Thoroughbreds and Standardbreds, colts have higher BW [12, 14, 28, 35], higher FFM [12, 28], and lower %F and RFT [19, 28] than filles. In this study, colts had higher BW and FFM and lower %F than fillies, and these sex differences were more pronounced in Miyazaki horses than in Hidaka horses.

In the previous study, it was clearly demonstrated that Miyazaki colts secrete testosterone and estradiol-17β from their testes earlier than Hidaka colts, and the plasma concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secreted by the pituitary gland have also been reported to be high in the pre-training stage [31]. Insulin-like growth factor (IGF-1) is secreted from the liver in response to growth hormone and directly acts on the growth of the animal. Mizukami *et al*. [31] reported that Miyazaki colts have higher circulating IGF-1 levels than Hidaka colts and that IGF-1 levels in Miyazaki colts were similar to those reported in mature horses [6–8]. Moreover, Dhakal *et al*. [8] demonstrated that the circulating IGF-1 is higher in stallions during breeding than during non-breeding seasons. The intensity of the immune response to IGF-1 and its receptor labeling in stallion Leydig cells was shown to be age-dependent and increased in the post-pubertal stage [51].

According to the data from the Japan Meteorological Agency (Fig. 9A), the average and minimum temperatures from 2005 to 2012 are about 10°C higher throughout year in Miyazaki than in Hidaka. And also, according to the data from Japan Coast Guard (Fig. 9B), daylight of Miyazaki in winter is about one hour longer than Hidaka. These climatic differences may have affected early growth

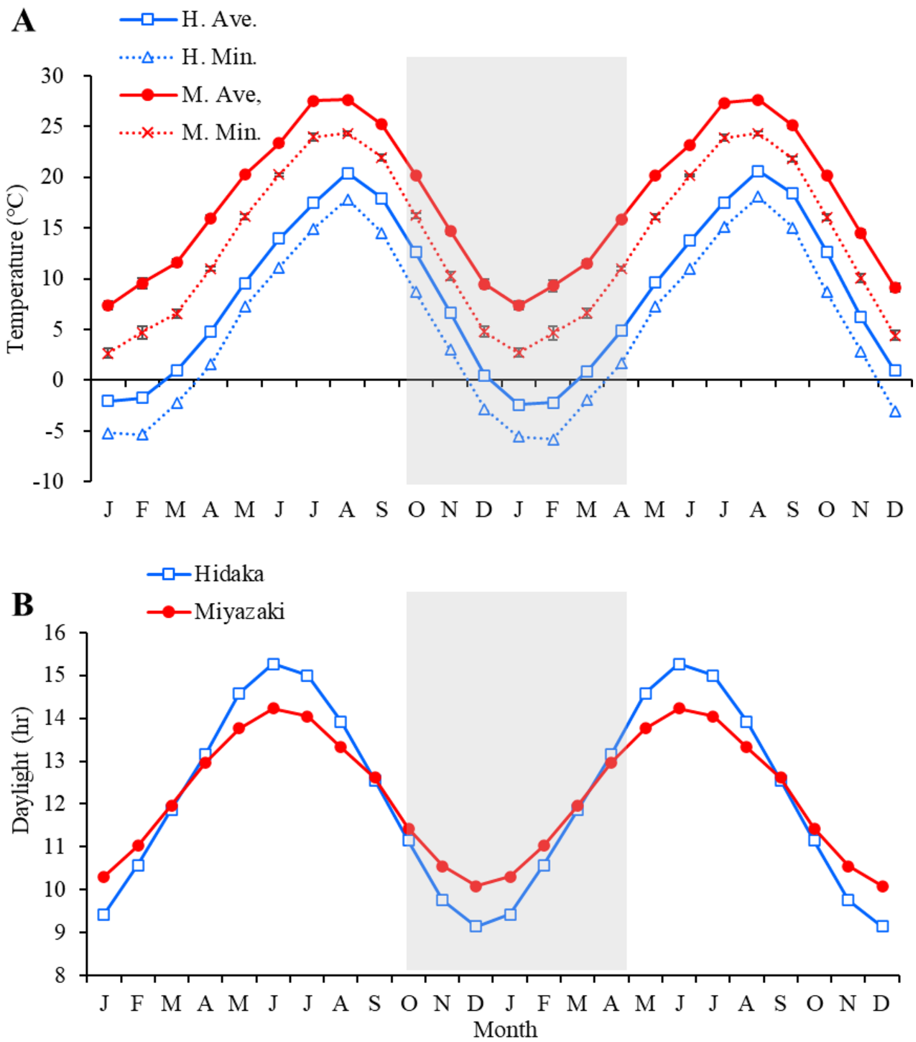

Fig. 9. Monthly changes in the pattern of temperature (**A**) from 2005 to 2012 in Hidaka average temperature (H.Ave.;□), Hidaka average minimum temperature (H.Min.;Δ), Miyazaki average temperature (M.Ave.;●), and Miyazaki average minimum temperature (M.Min.;×). Monthly changes in the pattern of daylight (**B**) from 2005 to 2012 in Hidaka (□) and Miyazaki (●) in Japan. Values are represented by means. Months are indicated by their initial letters.

and gonadal function in young Thoroughbreds in Miyazaki. Since horses are long-day seasonal breeders, the results of our previous study suggest that the gonadotropin-releasing hormone–LH/FSH and growth hormone–IGF-1 systems are more active in Miyazaki colts than in their Hidaka counterparts [31, 43].

Testosterone is an anabolic hormone that stimulates protein synthesis, which helps increase muscle mass [4, 5, 11, 32], inhibits fat catabolism, and triglyceride accumulation, and decreases body fat accumulation in fat metabolism [4]. IGF-1 also acts directly on satellite cells of skeletal muscles, promoting protein synthesis *via* Akt and mTORC1, known as serine/threonine kinases [2, 39–41], and protein degradation *via* the inactivation of FoxO, which induces hypertrophy [17, 37, 39]. Testosterone is also thought to promote protein synthesis by indirectly activating Akt and mTORC1 in satellite cells, similar to IGF-1 [48]. In contrast, myostatin, which is produced mainly in skeletal muscles, is thought to contribute to maintaining muscle homeostasis by suppressing cell proliferation and differentiation in muscle tissue by inactivating Akt and mTORC1 and activating FoxO [3, 23, 24]. The inhibition of myostatin function has also been reported to reduce adipose tissue [26, 29, 30]. The FFM values of Miyazaki colts increased due to the effects of IGF-1 and testosterone; moreover, they had lower average monthly values of %F than fillies.

Studies have reported that younger Thoroughbreds reared and trained in Miyazaki have higher rates of BW, height, girth circumference, and cannon circumference in both sexes than Thoroughbreds reared and trained in Hidaka [27, 31, 43]. However, this study's comparison of body composition changes showed no clear differences between Hidaka and Miyazaki fillies. It has been reported that Miyazaki fillies had higher plasma levels of estradiol-17β secreted from the granulosa cells of mature follicles in the ovaries than Hidaka fillies and had earlier ovulation timing [31]. In contrast, it has also been reported that there were no clear differences in IGF-1 or gonadotropic hormone concentrations between Hidaka and Miyazaki fillies [31, 43], which was suggested as a reason why changes in body composition in Miyazaki fillies in this study were unclear. Further investigations are needed.

FFM is considered as an indicator of muscle mass [1, 12, 18, 22] and increases with growth [28] and training [12]. Elite Thoroughbred or Standardbred racehorses and individuals with higher athletic performance have high FFM values [12,18–20, 25]. Previous studies reported that FFM is positively correlated with one-mile performance in Standardbreds and VO_{2max} (maximal oxygen uptake) values in treadmill exercise tests [19, 20]. Furthermore, it was also reported that %F is negatively correlated with V_{LA4} (velocity of 4 mmol/ blood lactate concentration) in treadmill exercise [25]. Based on these facts, FFM and %F are considered helpful indices for determining competitive performance and training effects.

In conclusion, the higher rate of increase in FFM of Miyazaki colts suggests that training young Thoroughbreds in winter under mildly cold climate is more likely to mature earlier than severely cold climate [27, 34, 49], particularly in colts. The increased FFM in the Miyazaki colts suggests that the training may have been effective [12]. It suggests that horses in Miyazaki may be able to undergo more high-intensity training than in Hidaka during the winter season, and also there are possibilities to perform their abilities in the early stage of 2 years old racing. In contrast, Hidaka colts tended to have a lower rate of increase in FFM than Miyazaki colts. Therefore, in Hokkaido, training should be conducted carefully to avoid overloading immature individuals and to prevent musculoskeletal injuries [9, 36, 46]. Since Hokkaido is a major region for breeding and training Thoroughbreds in Japan, further investigations are needed to improve the management of horses that promote proper growth under cold winter climate in Hokkaido such as adaptation of extended photoperiod method [33].

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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