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Reduced high-intensity training distance in growing horses had no effect on IGF-1 concentrations, but training onset interrupted time-dependent IGF-1 decline

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RESEARCH ARTICLE

Abstract

This study investigated plasma insulin like growth factor (IGF)-1 concentrations in 16 young Standardbred horses introduced to systematic high-intensity training at two different levels of intensity. Growth and locomotion asymmetry and correlations between these and plasma IGF-1 concentrations were also examined. From September as 1-year olds to March as 2-year olds (Period 1), all horses were subjected to the same submaximal training program. In March (start of Period 2), the horses were divided into two groups (n=8) and one group was introduced to regular high-intensity training. The other group was introduced to a program where the high-intensity exercise distances were reduced by 30%. These two training programs were maintained for the remaining 21 months of the study (Periods 2, 3, and 4). There was no effect of training group on plasma IGF-1 concentrations. A continuous decline in IGF-1 levels was observed throughout the study ($P<0.0001$), with one notable interruption in Period 2 when the IGF-1 concentration remained at the level seen at the start of Period 1. Growth rate of body length was equally high in Periods 1 and 2 ($P>0.05$). Front and hind limb asymmetry was elevated in Period 2 compared with Period 1. There were positive correlations between IGF-1 concentrations and changes in body condition score, and a negative correlation between IGF-1 concentration and weight. These results indicate that introduction to high-intensity training induces IGF-1 release in horses, but that a 30% difference in the distances used in high-intensity training does not affect IGF-1 levels. The temporary interruption in decline in IGF-1 release with the onset of high-intensity training may influence growth pattern and locomotion asymmetry, but further studies are needed to assess causality.

Keywords: locomotion symmetry, growth, body length, exercise

1. Introduction

It is generally accepted that exercise can act as a stimulus for the hypothalamus to produce growth hormone-releasing hormone (GH-RH), which stimulates the release of growth hormone (GH) and, secondarily, the production of insulin-like growth factor 1 (IGF-1) by several tissues in humans and other species (De Graaf-Roelfsema *et al.,* 2007; Kraemer and Ratamess, 2005). However, this is not very well documented in horses. In humans, it is well known that high-intensity exercise increases plasma concentrations of growth hormone (Felsing *et al.*, 1992; Jenkins, 2001; Pritzlaff *et al.*, 1985). Introduction to high-intensity training could therefore also be expected to stimulate growth hormone and IGF-1 release in horses. While GH has a short half-life in plasma (20 min) (Faria *et al.*, 1989), elimination of IGF-1 is slow (half-life 20 h) (Fortier *et al.*, 2005), so analysis of IGF-1 could be more relevant when monitoring long-term growth stimuli responses. There are a few studies on the effect of training on plasma IGF-1 levels in horses, but the results are somewhat contradictory. For example, Jackson *et al.* (2003) observed a difference in the relative change in plasma IGF-1 concentrations between horses subjected to two different 20 weeks training programs, while Noble *et al.* (2007) observed no differences in horses subjected to different training programs. Interestingly,

in the study by Jackson *et al.* (2003), horses subjected to the lightest training program (only walk) had the highest (positive) IGF-1 changes. However, nutrient intake and energy balance were not controlled for in those studies, and it is generally known, including from studies in horses (Salazar-Ortiz *et al.,* 2014; Sticker *et al.,* 1995), that protein intake and energy balance can significantly affect IGF-1 plasma concentrations. To better understand the effect of high intensity training, studies with improved dietary control is therefore warranted and the aim of this study was to study long term IGF-1 concentrations in growing horses introduced to two different high intensity programs while fed the same controlled diet.

GH and IGF-1 target a number of cell types in various tissues, such as cartilage, bone, and skeletal muscle (Ballesteros *et al.*, 2000; Verwilghen *et al.*, 2009). Studies on GH-deficient animals and humans treated with IGF-1 have shown that the hormone stimulates longitudinal bone growth (Yakar and Isaksson, 2016). Fortier *et al.* (2005) analysed serum levels of IGF-1 and an IGF-1 carrier protein (IGFBP-3) in growing horses and concluded that concentrations peak at around 225 days of age, defining the onset of puberty, and then decline to steady-state levels at around 450 days, signalling the end of puberty. Those authors also observed correlations between structural changes (e.g. disappearance of cartilage canals) in articular-epiphyseal cartilage complex and IGF-1 and IGFBP-3 levels. In a study on horses representing a wider range of ages, IGF-1 concentrations showed a gradual decrease in mares and geldings from the age of one to 19 years (Noble *et al.*, 2007).

An increase in GH and IGF-1 release due to increased exercise intensity would stimulate growth, and thereby possibly also alter conformation and muscle growth in growing horses. One possible effect of such changes is alterations in the locomotion pattern. Anecdotal observations by horse trainers support the suggestion that young Standardbred horses in training may show uneven growth (e.g. more rapid growth at the croup than at the withers) and periodically show flaccid and stumbling locomotion patterns, even at slow velocities.

The aims of this study were to: (1) determine the concentrations of plasma IGF-1 in response to high-intensity training in young Standardbred horses (kept under controlled dietary conditions); (2) compare plasma IGF-1 concentrations in horses subjected to two different levels of high-intensity training for 21 months; and (3) assess whether plasma IGF-1 concentrations are correlated to growth rates and locomotion asymmetry. The hypotheses tested were that: introduction to high-intensity training increases plasma concentrations of IGF-1; horses under a reduced high-intensity training program show an attenuated increase; and there are correlations between IGF-1 concentration, growth and locomotor patterns.

2. Material and methods

The study was performed at the Swedish National Centre for Trotting Education at Wången, where the horses were cared for and trained by high school students under the supervision of professional trainers. The protocol was approved by Umeå Local Ethics Committee (A90-10, 2010-09-14).

Horses and management

Sixteen Standardbred colt yearlings from four Swedish breeders were used. The horses had mainly an American pedigree, but eight horses also had some (<27%) French ancestors. These eight horses were all tested by the SychroGait gene test (Capilet Genetics, Västerås, Sweden) and were all homozygous for the stop codon in the DMRT3 gene, which has been shown to negatively affect the ability for a balanced trot at high speed if heterozygous (Andersson *et al.*, 2012). They entered the study in September 2010 as 1-year-olds and the study ended in December 2012 as 3-year-olds. The colts were all castrated in December 2010-January 2011. They were stabled individually (box stalls, ~9 m^2) for approximately 14 h per day Monday to Thursday/Friday, and spent the rest of the time in a paddock with access to shelter. The horses had *ad libitum* access to water and haylage, both in the boxes and in the paddock. The haylage was analysed for energy and nutrient content, and the diet was supplemented with pelleted lucerne (Krafft AB, Malmö, Sweden), a commercial mineral supplement (Krafft AB, Malmö, Sweden) and NaCl to meet nutrient requirements (NRC, 2007). Mean daily energy and crude protein intake of the horses are summarised below (Table 1). The results of all haylage analyses and full data on feed, energy, and nutrient intake can be found in Ringmark *et al.* (2013, 2017). Hoof trimming and shoeing were performed every 5-6 weeks, while during wintertime (October/November-March) permanently studs were fitted on the shoes (four 8-mm high studs per shoe).

Training

From September 2010 as 1-year olds to March 2011 as 2-year olds, all horses were subjected to the same training program, which involved only occasional exercise at heart rate >180 beats/min. The goal was to train the horses to a level where they could trot with ease for 5-7 km at a velocity of 5.6 m/s (3 min/km). In March 2011 as 2-year olds, when regular twice-weekly high-intensity training was about to start, the horses were divided into two groups. These groups were balanced with respect to breeder and parameters known to affect performance, such as genetic potential (sire and mean pedigree index estimated with the Best Linear Unbiased Prediction (BLUP) method), percentage of French ancestry, inbreeding coefficient, age in days, abnormal radiographic findings, conformation, height at

withers, and proportion of type IIA/type IIB muscle fibres (Ringmark *et al.*, 2015). Mean age of the horses in March 2011 was 657±31 days (range 595-713 days) and there was no difference between the training groups (P>0.05). High-intensity exercise was defined as training expected to cause a heart rate >180 beats per minute (heat training, interval training, and uphill interval training). One group was allocated to a control training program (group C) and the other to a reduced training program (group R) where the high-intensity exercise distances was reduced by 30%. Thus, for the remainder of the study, horses in group C performed heat training over 1,600 m, whereas horses in group R performed heat training over 1,100 m, and when horses in group C performed six intervals, horses in group R performed only four. The same velocity was aimed for with both groups. Full details about training distances and number of training sessions of these horses can be found in Ringmark *et al.* (2015).

Growth and feed intake recording

All growth measurement were performed by the same person. Body weight (BW) was recorded with a scale (weight indicator U-137, UNI Systems and Vågspecialisten, Skara, Sweden). Height at withers and height at croup was measured with a ruler with a precision of 0.5 cm. If the horses had spikes in the shoes 0.8 cm were subtracted. Body length was measured from the point of the shoulder to the point of the buttock using a folding ruler (positions identified by palpation of the tip of humerus (*humeral tubercles*) and *tuber ischii*). Circumference right below carpus (at cannon and splint bone tops) was measured with a tape measure on both left and right front limb and a mean was calculated. Thickness of *m. longissimus dorsi* and subcutaneous fat at croup was measured with a DP-6600 Vet ultrasound system (Mindray Medical International, Shenzhen, China P.R.) using 7.5 MHz, 38 mm linear probe. The measurement of *m. longissimus dorsi* were taken above the 18th rib by the first lumbar vertebra. The thickness of fat at croup was measured 5 cm from the middle line at the croup. Both thickness of *m. longissimus dorsi* and subcutaneous fat at croup were measured three times and a mean was calculated. To evaluate the body condition score (BCS, adopted from Henneke *et al.*, 1983) the body were divided in to four sections; neck and shoulders, back, ribs and tailhead, and given a score with 0.5 precision (Ringmark *et al.,* 2013). BCS was then calculated as the mean of the different sections. In the present study, the change in these observations (i.e. growth rate) was calculated for four periods: Period 1: October 2010 to March 2011 (before high-intensity training); Period 2: March 2011 to August 2011 (first 6 months with high-intensity training); Period 3: August 2011 to December 2011; and Period 4: December 2011 to December 2012 (longer than previous periods since low growth rate was expected). Mean growth rate was calculated as the difference between the last observed measurement in the period and the first measurement, divided by the number of months in the period.

Individual feed intake was measured for three consecutive days on 12 occasions and daily metabolisable energy (ME) and crude protein (CP) intake were calculated based on feed analyses (Ringmark *et al.*, 2017). The mean values obtained for the four periods are shown in Table 1. The dietary CP/ME ratio was planned to decrease, as horses reach maturity at the age of 3 years (NRC, 2007). There were no differences in energy and CP intake between training groups C and R (Ringmark *et al.*, 2017).

Locomotion asymmetry

Locomotion asymmetry in front and hind limbs was measured on 17 occasions, approximately in alternate months (there were four months between measurements in one case), starting in September as 1-year-olds and ending in December as 3-year-olds. Locomotion asymmetry was evaluated during trot at hand for ~100 m on a hard surface (packed gravel), using a sensor-based system (Lameness Locator; Equinosis LLC, Columbia, MO, USA) similar to that described by Keegan *et al.* (2011). The difference in left versus right maximal and minimum position of head was used to calculate the vector sums (VS): VS = √(maximum difference^2 + minimum difference^2) for head (front limbs, VSf). For hind limbs, pelvis differences was divided into pushoff (max difference of pelvis) and impact (minimum difference of pelvis) differences. Individual mean of VSf, pushoff and impact for Periods 1-4 were then calculated.

Table 1. Daily metabolisable energy (ME) intake and crude protein (CP) intake (per 100 kg body weight) and dietary CP/ME ratio in 16 Standardbred horses in training in Periods 1-4 (least squares mean ± standard error).[1]

	Period 1	Period 2	Period 3	Period 4
ME, MJ/day	22±0.4	25±0.4***	26±0.4***	23±0.4*
CP, g/day	290±5	370±5***	350±5***	280±5*
CP/ME	13±0.1	15±0.1***	14±0.1***	12±0.1***

[1] Significant differences compared with Period 1 are indicated by * (P<0.05) or *** (P<0.001).

Blood sample collection

On six occasions (November 2010, March 2011, May 2011, December 2011, May 2012, and December 2012), each within three days of a locomotion asymmetry evaluation, blood was collected early in the morning (05:00-06:00 h) before any activity had started in the stables. These blood samples were collected from the jugular vein in lithium heparin tubes (10 ml), using the vacutainer technique. The samples were directly centrifuged at room temperature (10 min, 2,700 rpm, 920×*g*) and the plasma was frozen (-20 °C) for later analysis of IGF-1. For correlation analysis, the IGF-1 concentration observed in the beginning of each period was used, and for periods with more observations (Periods 1 and 4) the mean IGF-1 concentration was calculated.

Insulin-like growth factor-1 ELISA

IGF-1 levels were determined using an ELISA kit (Immunodiagnostic Systems, Boldon, UK) manufactured for human plasma, but validated for horse plasma (Baskerville *et al.*, 2017). The homology of IGF-1 protein sequence for humans and horses is 100% (Otte *et al.*, 1996). The analysis was performed according to the manufacturer's instructions. All samples from the same horse were run on the same plate and horses from both training groups were included on all plates. The samples were run in duplicate and the intra-assay coefficient of variation (CV) for the ELISA plates was 4, 5, 5 and 7%, respectively, and the inter-assay CV was 8%. The detection range was 10-1,200 ng/ml.

Data and statistical analyses

All analyses except correlation analysis were carried out in R (v4.0.3, R Core Team, 2020) using the packages *nlme* (v3.1-149) and *emmeans* (v1.5.3). Normal distribution of data was verified by residual plots and if the data deviated from normality, they were log-transformed except for hind limb pushoff and impact differences in locomotion asymmetries that were rot square-transformed. Differences were considered statistically significant at $P<0.05$. Results are presented as least square means ± standard error unless otherwise stated. The IGF-1 values were log-transformed and analysed using a mixed model that included effect of date, training group, and horse, which was considered random and repeated. Changes in body measurements, VSf, hind limb pushoff and impact asymmetries were analysed with a mixed model where period and training group were included and where horse was considered random and repeated. Correlation analysis was performed on individual mean values per period using PROC CORR (Pearson's correlation coefficient) to calculate correlations (Statistical Analysis System package, version 9.4, SAS Inst. Inc., Cary, NC, USA).

3. Results

Insulin-like growth factor-1 concentration

There was no significant effect of training group on plasma IGF-1 concentrations, and therefore data from the two groups were pooled (Figure 1). There was a significant decrease in IGF-1 level over time ($P<0.0001$) except for a notable exception in May 2011 (Period 2), when the IGF-1 concentration was not different from the start level in Period 1 (Figure 1). That temporary disruption in decline in plasma IGF-1 concentration coincided with the introduction of systematic high-intensity exercise (Figure 1).

Growth rate and changes in body measurement

There were no significant differences in growth parameters and body measurements between the training groups, and therefore the data were pooled (Table 2). Compared with Period 1, growth rate in height at withers decreased and was lower in all remaining periods (Table 2). The growth rate in height at croup and change in BW showed a similar pattern except in Period 3, when the changes were not significantly different from those in Period 1. Growth in body length and front limb circumference showed a different response, in that the changes were equally high in Periods 1 and 2 (no significant difference), while lower rates were first observed in Period 3. The growth rate of *m. longissimus* increased in Periods 2 and 3 compared with Period 1. The change in fat thickness at croup was greater in all periods compared with Period 1, but no differences were observed in changes in BCS between Period 1 and 2, while after that the change was lower than in Period 1 (Table 2).

Locomotion asymmetry

As reported by Ringmark *et al.* (2016), there were no significant differences in locomotion asymmetry pattern between the two training groups, and therefore the data were pooled. Compared with Period 1, hind limb asymmetry for both pushoff and impact was elevated in Period 2 ($P=0.02$ and $P=0.0008$, respectively), but not in Period 3 (Table 3). Hind limb asymmetry increased again in Period 4 for pushoff and impact ($P=0.0008$ and $P=0.004$, respectively), but there were no differences between Periods 2, 3, and 4 ($P>0.05$). Front limb asymmetry was significantly elevated in all periods compared with Period 1 ($P<0.0001$, $P=0.0003$, and $P<0.0001$ for Period 2, 3, and 4, respectively), but there were no differences between Periods 2, 3, and 4 (Table 3).

Correlations

There was a positive correlation between IGF-1 concentrations and BCS and a tendency for a negative correlation between IGF-1 and body length, but IGF-1

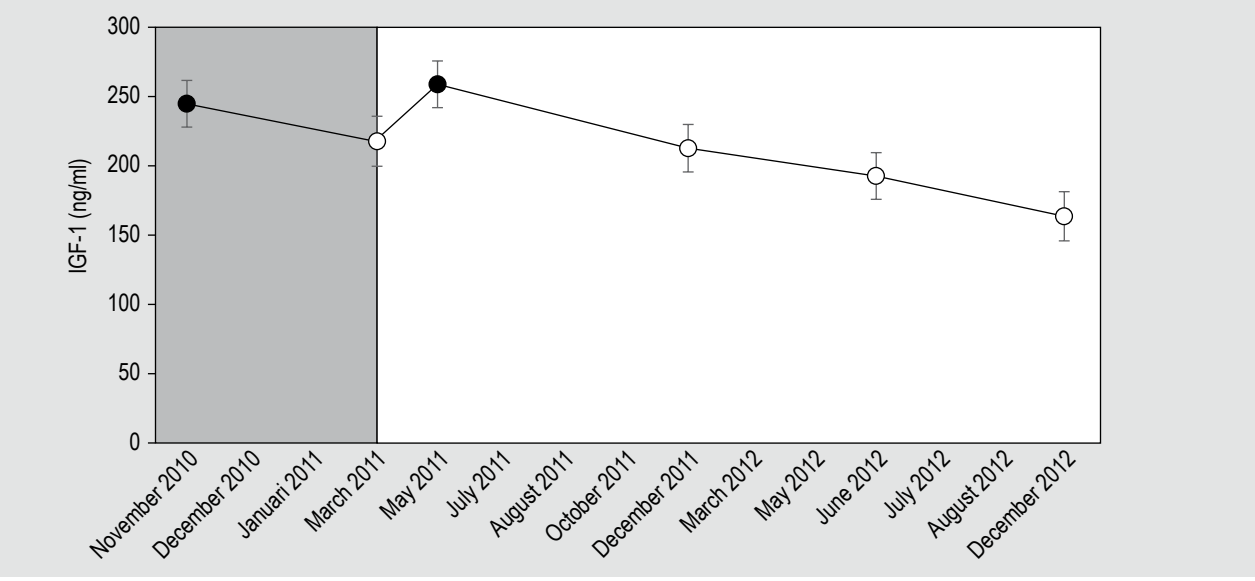

Figure 1. Insulin-like growth factor 1 (IGF-1) in 16 Standardbred horses in training from November 2010 as one-year olds to December 2012 at three years of age (least-squares mean ± standard error). Unfilled markers are significantly different *(P*<0.05) from the first observation in November 2010. Dark grey background: training intensity below <180 bpm, White background: regular high intensity exercise at >180 bpm.

Table 2. Changes in body weight, height at withers and croup, body length, front limb circumference, depth of *m. longissimus dorsi*, subcutaneous fat thickness at croup, and body condition score (scale 1-9) in 16 growing Standardbred horses in training during Periods 1-4 (least squares mean ± standard error).[1]

	Period 1 (Oct-Mar) 1-2 yrs	Period 2 (Mar-Aug) 2 yrs	Period 3 (Aug-Dec) 2 yrs	Period 4 (Dec-Dec) 3 yrs
Height at withers (cm/month)	1.0±0.04	0.2±0.04***	0.6±0.04***	0.1±0.05***
Height at croup (cm/month)	0.7±0.06	-0.1±0.06***	0.6±0.06	0.1±0.07***
Body length (cm/month)	1.1±0.2	0.7±0.2	-0.1±0.2***	0.3±0.2*
Front limb circ. (cm/month)	0.1±0.02	0.1±0.02	-0.1±0.02*	-0.1±0.02
m. longissimus (mm/month)	-1.4±0.5	0.9±0.5**	0.4±0.5*	-0.1±0.5
Fat at croup (mm/month)	-0.2±0.04	0.2±0.04***	-0.1±0.04*	0.1±0.04***
Body condition score[2]	0.1±0.03	0.1±0.03	-0.1±0.03*	-0.1±0.03**
Weight (kg/month)	5.8±0.6	2.4±0.6***	6.1±0.6	0.5±0.6***

[1] Significant differences from Period 1 are indicated by * (*P*<0.05), ** (*P*<0.01) or *** (*P*<0.001).
[2] Scale from Henneke *et al.* (1983).

Table 3. Mean hind and front limb asymmetry in 16 growing Standardbred horses in training during Periods 1-4 (least squares mean ± standard error).1

	Period 1 (Oct-Mar) 1-2 yrs (n=89)[2]	Period 2 (Mar-Aug) 2 yrs (n=48)	Period 3 (Aug-Dec) 2 yrs (n=32)	Period 4 (Dec-Dec) 3 yrs (n=94)
Hind limb pushoff asymmetry (mm)	3.4±0.5[a]	4.6±0.6[b]	4.7±0.7[a]	4.8±0.5[b]
Hind limb impact asymmetry (mm)	3.2±0.5[a]	5.4±0.6[b]	3.9±0.6[a]	4.6±0.5[b]
Front limb asymmetry (vector sum, mm)	10.0±1.0[a]	19.3±1.3[b]	15.4±1.6[b]	17.0±1.0[b]

[1] Values in a row with different superscript letters are significantly different (*P*<0.05).
[2] n = number of observations during the period.

concentrations were not correlated to height at withers or croup, depth of *m. longissimus dorsi*, front limbs circumference or fat thickness at croup (Table 4). There was a negative correlation between IGF-1 concentration and weight (Table 4). There was also a negative correlation between IGF-1 concentration and hind limb pushoff asymmetry and a tendency for a negative correlation for hind limb impact asymmetry, but no correlation with front limb asymmetry (Table 4). IGF-1 concentrations showed no correlation to ME or CP intake, but a positive correlation to CP/ME intake ratio (Table 4).

4. Discussion

The main aims of this study were to describe and compare plasma concentrations of IGF-1 in growing Standardbred horses when introduced to two high-intensity training programs for 21 months. The starting hypothesis was that high-intensity training would elevate IGF-1 concentrations. The results showed that there was a significant interruption of the decline in IGF-1 levels at the time when high-intensity exercise training was introduced. This could indicate that high-intensity training stimulates IGF-1 release in horses. To our knowledge, this is the first study to describe this is in growing horses subjected to high-intensity training. However, the results are somewhat in contradiction to observations made by Noble *et al.* (2007), who observed no acute changes in plasma IGF-1 concentrations after race-like exercise in adult Thoroughbred horses (10±2 years). The reason for this discrepancy is unclear, but it could be due to the response differing depending on age of the horse (growing or not) or to recurrent high-intensity exercise bouts being required to stimulate IGF-1 production (e.g. twice weekly as in the present study). Although the interruption of the decline in IGF-1 concentration coincided with the introduction of high intensity training it cannot be excluded that it was due to other reasons. A seasonal effect seems however, unlikely since there was no second interruption at the same time next year (May 2012).

Table 4. Correlations between insulin-like growth factor 1 (IGF-1) and front and hind asymmetries, body weight (BW), height at withers and croup, body length, front limb circumference, depth of *m. longissimus dorsi*, subcutaneous fat thickness at croup, body condition score (scale 1-9), daily metabolisable energy (ME) and crude protein (CP) intake/100 kg BW and dietary CP/ME ratio in 16 growing Standardbred horses in training. Correlation analysis performed on data from Periods 1-4.

	Correlation	*P*-value
Body length	-0.23	0.074
Depth *m. longissimus*	0.040	0.75
Weight	-0.28	0.029
Height at withers	-0.18	0.16
Height at croup	-0.12	0.37
Front limb circ.	-0.013	0.92
Fat at croup	-0.14	0.26
Body condition score	0.34	0.006
Front limb asymmetry	0.010	0.94
Hind limb pushoff asymmetry	-0.25	0.045
Hind limb impact asymmetry	-0.23	0.067
CP intake	0.14	0.26
ME intake	-0.074	0.56
CP/ME	0.34	0.007

Although IGF-1 release may have been stimulated when high-intensity exercise was introduced, there was no difference between the two training intensity levels (R and C). This indicates that IGF-1 release does not display an incremental response to increasing exercise intensity, or perhaps that the peak response was achieved already at the intensity of the group R horses. Another explanation could be that the difference in high-intensity training evaluated (30% shorter distance) was not enough to stimulate clear differences in IGF-1 release.

As mentioned, IGF-1 levels showed a continuous decline throughout the study apart from the temporary elevation when high-intensity exercise was introduced. This observation is in accordance with previous studies which have linked IGF-1 levels to age (Malinowski *et al.*, 1996; Noble *et al.*, 2007; Popot *et al.*, 2001). It is also in accordance with Fortier *et al.* (2005), who observed a peak at the age of 225 days, which is before our horses entered the study.

An additional aim of this study was to investigate whether changes in plasma IGF-1 concentrations are linked or correlated to growth pattern and possibly changes in locomotion patterns. Our horses entered the study at the age of 15.5±1 months, by which time horses generally have reached 75% of their adult body weight and >90% of their adult height at withers (Martin-Rosset, 2004; NRC, 2007). At this age, daily growth rates can be expected to be less than 400 g day and show a continuous decline as horses get older (NRC, 2007). A decline in growth rate compared with Period 1 (i.e. before high-intensity training was introduced) was observed for height at withers and BW, and for height at croup for all periods except Period 3. However, growth rate of body length and front limb circumference showed a different response, with the rates being equally high in Periods 1 and 2 (no significant difference). Altogether, this provides some support for the suggestion that IGF-1 release triggered growth in some bones. IGF-1 is the major regulator of growth and controls elongation of long bones (such as humerus, radius/ulna, femur, and tibia/fibula (but likely also the vertebral column (Adem *et al.*, 1994)) by promoting chondrocyte proliferation and hypertrophy (Racine and Serrat, 2020). Growth was possible in these

bones since fusion is not completed in horses until around 3 years of age (Strand *et al.*, 2007). The uneven growth can be seen by comparing the relationship between length and height during Periods 1 and 2. In Period 1, body length was 100% of height at withers whereas in Period 2 it was 102%, i.e. the horses were 3 cm longer than their height (Ringmark *et al.*, 2013, 2017). This relationship (longer than tall) was present also at the age of 3 years in these horses (Ringmark *et al.*, 2017). Accordingly, our data show that the body proportions of the horses changed following the point when high-intensity exercise was introduced and that these changes may have been IGF-1-stimulated. However, further studies are needed to confirm the importance of high-intensity exercise for alterations in IGF-1 release and growth patterns in growing horses.

Interestingly, the growth rate of *m. longissimus* increased in Periods 2 and 3 compared with Period 1. However, it is unlikely that IGF-1 was the long-term trigger for this growth, since the elevated growth continued many months after the peak in IGF-1 was observed. There was also a small, but significant, change (positive compared with Period 1) in body fat thickness at the croup throughout the study, but this pattern was not reflected in the body condition scoring. We have no explanation for this contradiction, but the changes were very small and may not have had any biological impact. We concluded that growth rates did not differ between the training groups (C, R), which was as expected since there was no difference in IGF-1 levels.

Changes in front limb circumference were small and clearly within the range of error of the method (tape measure with 1 mm precision). Growth was expected to be small, but front limb circumference is also highly affected by several other factors, e.g. the size of the cannon bone and tendons, the thickness of the horse's coat, and possible swelling.

The correlations observed between IGF-1 and BW and BCS most likely reflect the long-term parallel processes of decreasing IGF-1 levels, increased BW and decrease in BCS as horses approach race fitness by the end of the study. If horses go through a period with uneven growth, this could be expected to affect their locomotion pattern. In this study, locomotion pattern was altered when high-intensity training was introduced, with both hind and front limb asymmetry increasing in Period 2. For hind limb asymmetry, the elevation was temporary and during the subsequent six-month period (Period 3) no elevation was observed. In contrast, mean front limb asymmetry remained elevated for Period 3 and 4. However, front limb asymmetry showed large variation between recording occasions, as previously reported by Ringmark *et al.* (2016), with the highest peak observed in April of Period 2. Front limb asymmetry then declined in Period 3 and on one occasion was not different from that observed in Period 1. In Period 4 there was another peak, but by the end of that period the level did not differ from that observed in Period 1. However, when the data were pooled into longer periods (each including several recording occasions), this pattern was only observed as numerical differences. It is possible that the temporary increase in IGF-1 and the growth pattern observed during Period 2 contributed to the changes in locomotion pattern during that period, but other factors most likely had a greater influence, e.g. true lameness or muscle soreness. The horses also showed an elevated response to flexion tests during Period 2 (Ringmark *et al.*, 2016). One possible explanation that has been offered previously is that unaccustomed exercise elevates aspartate aminotransferase (AST) levels, indicating muscle damage (Mack *et al.*, 2014) and possibly soreness. To better understand the effect of growth on changes in locomotion pattern further studies are needed, preferably with more detailed objective locomotion asymmetry measures.

In the present study, the horses were fed a standardised diet *ad libitum* and it was interesting to observe that daily energy intake increased when high-intensity exercise was introduced, while body fat and body condition remained stable. Since IGF-1 levels can be influenced by energy and protein intake, it is important to control and monitor feed intake. If the increased requirement for energy and protein with increasing exercise is not met, the body will end up in a catabolic condition, which will have significant lowering effects on IGF-1 levels (Chelikani *et al.*, 2004; Kiani, 2013; Salazar-Ortiz *et al.*, 2014; Sticker *et al.*, 1995). In the present study, the variations in CP intake were due to variations in the CP/ME ratio of the different haylage batches used throughout the study.

In conclusion, the results of this study indicate that introduction to high-intensity training induces IGF-1 release in horses, but that a 30% difference in the volume of high-intensity training does not affect IGF-1 levels. The temporary interruption in decline in IGF-1 release with the onset of high-intensity training may have influenced growth pattern in the horses, but further studies are needed to assess causality.

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Conflict of interest

All authors declare that they have no conflict of interest.

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