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Blood Plasma Concentrations of Insulin-like Growth Factor-I (IGF-I) in Resting Standardbred Horses

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SUMMARY

A survey of standardbred horses was conducted to build up a normal population profile for insulin like growth factor-I (IGF-I) concentrations in racing standardbreds and to ascertain how age, sex and geographic location affect IGF-I. Blood samples were drawn by jugular venepuncture from 202 racing standardbred horses aged one to eight years located in five different geographic regions of New Zealand. IGF-I concentrations were determined by insulin like growth factor-I binding protein (IGFBP)-blocked radioimmunoassay validated for the horse. As described in other species, age played a significant ($P<0.05$) role in IGF-I concentrations with the highest concentrations occurring in the younger horses. There was a significant ($P<0.05$) sex effect, intact males having significantly higher IGF-I concentrations compared of mares and/or geldings. Geographic location had a significant ($P<0.05$) influence on IGF-I. A significant ($P<0.05$) trainer effect also was noted both within and between geographic locations. We concluded that IGF-I concentrations in racing standardbred horses are affected by age, sex, trainer and geographic location.

KEYWORDS: Insulin-like growth factor-I; standardbred horses; population and horse.

INTRODUCTION

Growth hormone (GH; or somatotropin, ST) is a protein produced by the anterior pituitary gland and is secreted in an episodic nature in horses (DePew *et al.*, 1994; Stewart *et al.*, 1993; Thompson *et al.*, 1992), as occurs in other species (Breier & Sauerwein, 1995; Plouzek & Trenkle, 1991). Growth hormone is a key regulator of somatic growth (Breier & Sauerwein, 1995). The action of GH on tissues is mediated partly by insulin-like growth factor-I (IGF-I) which it is secreted primarily from the liver and other non-hepatic tissues to act in an endocrine-autocrine-paracrine fashion (Le Roith & Roberts, 1991; Ovesen *et al.*, 1996). Plasma concentrations of IGF-I in the peripheral circulation of man and domestic animals are relatively stable due to its long biological half-life with no obvious diurnal rhythm (Breier & Sauerwein, 1995; Gluckman *et al.*, 1987). The measurement of IGF-I is useful in determining the level of activity of the somatotropic axis as GH measurement provides very little information due to its pulsatile secretion.

Various factors such as breed (Eigenmann *et al.*, 1984), sex, age, body weight and nutritional status (Breier & Sauerwein, 1995; Clemmons *et al.*, 1981) are known to influence circulating concentrations of IGF-I in a variety of species. Malinowski *et al.* (1996) studied IGF-I concentrations with respect to age and breed in female horses. Results showed IGF-I concentrations increased continuously from birth to day 14 then remained constant until nine months of age and were significantly decreased in older mares (22 years). The relatively high IGF-I concentrations during the most rapid growth of foals are consistent with reports from other species (Breier *et al.*, 1994; Breier *et al.*, 1988). Studies by Ozawa *et al.* (1995) and

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Malinowski *et al.* (1996) were designed to assess IGF-I concentrations in different breeds with markedly different adult sizes. Both studies found there were significant differences in IGF-I concentrations unrelated to differences in body size but due to age differences.

Data on normal IGF-I concentrations in a number of species are available but there are few reports of IGF-I plasma concentrations in the horse and no studies to date comparing the sexes. Due to the range of IGF-I assays available and their differing abilities to measure total IGF-I, a comparison of published studies is virtually impossible. The assay used in this study is unique as it uses IGF-II displacement to yield total IGF-I (Champion *et al.*, 2000).

The current study aimed at providing a normal population profile for circulating IGF-I concentrations in racing standardbred horses in New Zealand and ascertaining whether age, sex, geographic location or trainer have an effect. Knowledge of normal IGF-I concentrations will enable a greater understanding of somatotropic manipulation for further research studies and possibly aid in establishing methods for the detection of illegal use of GH in racehorses.

MATERIALS AND METHODS

Animals

A total of 202 blood plasma samples were drawn on the same day from racing standardbreds located in five different regions throughout New Zealand. All horses were in full training and aged one to ight years of age.

Experimental Protocol

One summer's morning five veterinarians collected blood samples from racing standardbreds in their designated areas. All samples were drawn between 6 and 7 am. Samples were collected from five distinct geographic areas in New Zealand (1–3 North Island, 4–5 South Island). The numbers in parentheses show the number of samples collected by each veterinarian; site 1 (41), site 2 (41), site 3 (28), site 4 (47) and site 5 (45). Blood samples were taken each horse pre-work and pre-feeding. The conditions of collection, handling and transportation followed a standard protocol. A sample of 10 ml of blood was collected via jugular venepuncture directly into a Lithium/heparin vacutainer (Bectin Dickinson) and stored on ice and transported to the laboratory within six hours of collection. The samples were centrifuged at 1300 rpm and plasma separated and stored at −20°C until IGF-I analysis. All IGF-I analysis was carried out in the one laboratory (The University of Auckland, Research Centre for Developmental Medicine and Biology).

IGFBP-blocked IGF-I Radioimmunoassay

IGF-I was measured using the modified IGFBP-blocked radioimmunoassay described by Champion *et al.* (2000). Recovery of unlabelled IGF-I in blood plasma was 89.5 + 3.1% (Mean + SEM, $n = 32$). Samples were assayed at different dilutions and showed displacement parallel to the standard curve. Correlation with results obtained with acid-ethanol cryoprecipitation was $r = 0.798$, $P = 0.006$ ($n = 10$). The ED-50 was 0.1 ng/tube and the minimal detectable dose was 0.07 ng/tube. Intra- and inter-assay coefficients of variation were 4.5% and 8.6% respectively.

Statistical analysis

IGF-I data were analysed using General Linear Models Analysis of Variance (GLM ANOVA, NCSS), and significant differences assessed using Duncan's Multiple Range test at a significance level of 5%.

RESULTS

There was a significant difference in plasma IGF-I concentrations with age ($P < 0.05$). The highest concentrations of IGF-I occurred in one year olds (299.0 ± 20.3 ng/mL) with a steady decrease to three year olds. There was no significant difference from three to eight years of age (240.84 ± 4.6 ng/mL) (Fig. 1). There was a significant difference ($P < 0.05$) with respect to sex with intact males having a significantly higher resting IGF-I concentration (307.4 ± 26.9 ng/mL) compared to mares (246.7 ± 7.5 ng/mL) and geldings (249.2 ± 5.3 ng/mL). There was no age and sex interaction ($P > 0.05$).

Geographic location had a significant influence ($P < 0.05$) on IGF-I concentrations. The samples collected in the North Island had significantly higher mean IGF-I concentrations than the South Island samples ($P < 0.05$) (Fig. 2). Interestingly, there was a significant effect on IGF-I concentration from trainers within each of the geographic locations (Fig. 3).

DISCUSSION

Based on the results we conclude that age, sex and geographic location affect IGF-I concentrations in the racing standardbred horses.

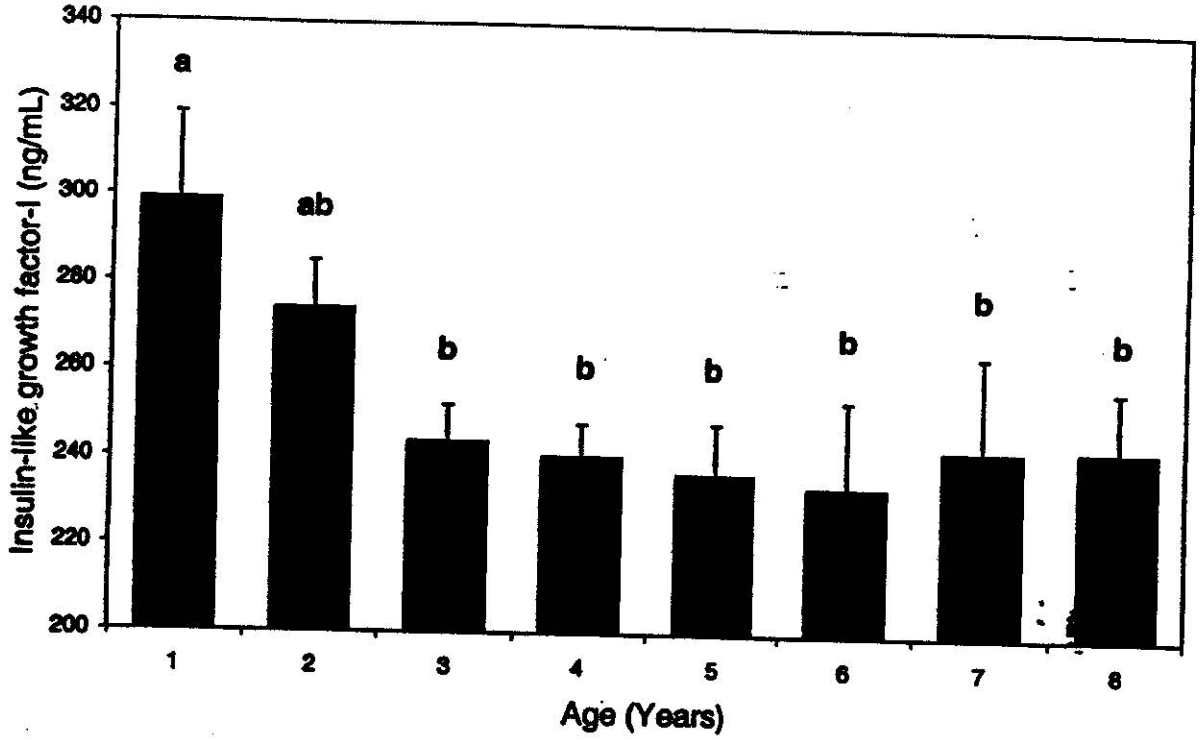

Fig. 1. Insulin-like growth factor-I concentrations in training standardbred horses by age of individual ($n=202$). Mean ± SEM, $^{a,b}P<0.05$.

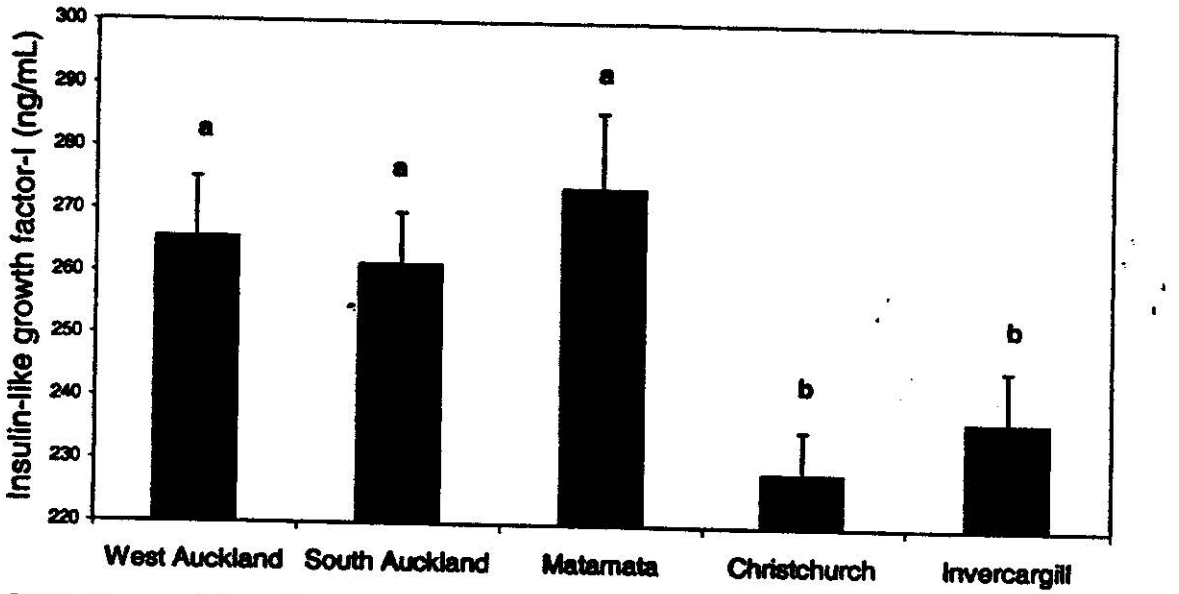

Fig. 2. Insulin-like growth factor-I concentrations in training standardbred horses by geographic location ($n=202$). $^{a,b}P<0.05$ significantly different from the other areas.

A large percentage of IGF-I in the circulation is bound to binding protein-3 (IGFBP-3), forming a high molecular weight tertiary complex (Blum, 1996). The dissociation of IGF-I from its tightly associated binding protein (BP) is important for accurate measurement of total IGF-I concentration. Due to the differing abilities of assays to dissociate these BPs, a comparison of published data is difficult

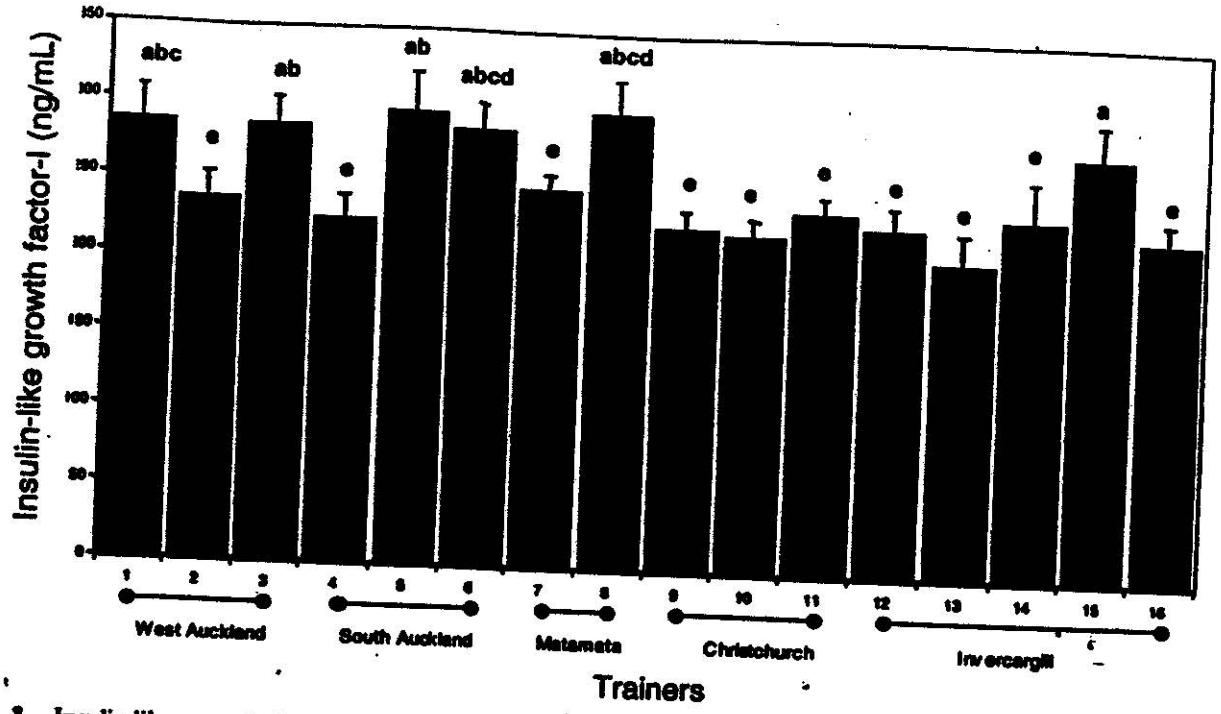

Fig. 3. Insulin-like growth factor-I concentration of training standardbred horses by different trainers ($n=16$). Trainers 1–3 (West Auckland), trainers 4–6 (South Auckland), trainers 7–8 (Matamata), trainers 9–11 (Christchurch) and trainers 12–16 (Invercargill). $^{a,b,c,d,e}P<0.05$.

as IGF-I concentrations vary greatly. This study used the IGFBP-blocked radioimmunoassay modified for the horse. It uses excess IGF-II and a high affinity antibody to remove the last of the bound and free IGFBPs from solution. This IGF-I assay has been validated for the horse (Champion *et al.*, 2000) as each species and each type of sample needs to be validated due to differing concentrations of IGFs and IGFBPs (Bang *et al.*, 1994).

The population database suggests IGF-I concentrations are significantly higher in one year olds, which is consistent with previous findings (Ozawa *et al.*, 1995). There is no literature to date that compares IGF-I concentration over different ages in fillies/mares, geldings and colts/stallions. Blood IGF-I concentration in geldings (Julen Day *et al.*, 1998) and mares (Hess-Dudan *et al.*, 1994; Malinowski *et al.*, 1996) have been measured in separate studies. Values in fillies/mares have been compared with those in intact males (Ozawa *et al.*, 1995; Thomas *et al.*, 1998; Tremblay *et al.*, 1993). Due to the different IGF-I assays utilized by different laboratories, a comparison of IGF-I concentrations is not valid. Results reported by Malinowski *et al.* (1996) have shown that older horses (22 years) have lower plasma IGF-I concentrations compared to younger horses (day of birth to nine months of age). A similar trend is well documented in sheep and cattle (Blum, 1996). The pattern in the horses shows that IGF-I concentrations at birth are low followed by a steep postnatal rise which is associated with an increase in growth hormone receptors in the liver (Breier *et al.*, 1998). The rise in plasma IGF-I postnatally may be related to the increase in sex steroid production (Blum, 1996; Han, 1996). Ageing process is associated with a significant reduction in circulating GH and IGF-I concentration (LeRoith *et al.*, 1996).

Plasma concentrations of GH and IGF-I are clearly dependent on nutritional status (Philips, 1986; Thissen *et al.*, 1994; Breier, 1999). Under restricted nutrition, the ability of GH to maintain plasma IGF-I is impaired and a reduction in the number of growth hormone receptors is observed (Breier, 1999; Breier & Sauerwein, 1995). Dietary protein supply is reported to be the limiting factor for maximal stimulation of IGF-I plasma concentrations (Christensen *et al.*, 1997) in horses. Slicker *et al.* (1995) fed sixteen light horse mares with a diet deficient in protein and energy or in protein alone. They concluded that a diet deficient in protein increased GH secretion whereas energy-restricted diet alone did not. Diets deficient in energy and/or protein reduced the secretion of IGF-I. Christensen *et al.* (1997) studied the effect of feed deprivation for 48 h

and for the 12 h after re-feeding. Feed deprivation caused a decrease in circulating GH but little change occurred in IGF-I concentrations.

No studies have assessed different levels of training on IGF-I concentrations in horses. It is possible that IGF-I concentrations are affected by the fitness or training status of the horse as well as by the environment. One study in women showed a positive correlation of IGF-I concentrations with physical activity (Bonnefoy *et al.*, 1999). A long-term intense training regimen by swimmers showed increased concentrations of total IGF-I, free IGF-I and IGFBP-3 in serum (Koziris *et al.*, 1999).

In the study reported here the differences in IGF-I concentrations between geographic locations could relate to nutritional factors, management or variations in training methods. Further study is needed to investigate these factors. The results show that because age, sex and environmental factors affect basal IGF-I concentrations, this measurement alone may not be a suitable for detecting exogenous GH administration.

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