

## Myosin heavy chain profiles and body composition are different in old versus young Standardbred mares

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### Abstract

There are limited data on age-related changes in body composition or skeletal muscle in the horse. Therefore, the purpose of this study was to investigate any differences in muscle myosin heavy chain (MHC) and body composition associated with aging. Twenty-three young (4–8 years) and eight old (20+ years) unfit Standardbred mares were evaluated. Rump fat thickness was measured using B-mode ultrasound and *per cent* body fat (% fat) was calculated. Needle muscle biopsies were obtained from right *gluteus medius* muscle. MHC composition was determined via sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Three MHC isoforms were subsequently identified as type I, type IIA, and type IIX and quantified using a scanning and densometric system. There were no significant differences ( $p > 0.05$ ) between old and young mares in fat (%) ( $19.0 \pm 6.4$  vs  $20.5 \pm 5.4$ ), fat mass (kg) ( $102.3 \pm 39.9$  vs  $106.9 \pm 37.1$ ), or body weight (kg) ( $529.4 \pm 34.9$  vs  $512.7 \pm 57.7$ ). However, the old mares had significantly ( $p < 0.05$ ) greater lean body mass than the young mares ( $427.1 \pm 24.5$  vs  $405.7 \pm 37.9$ ). Aged mares had significantly ( $p < 0.05$ ) less type I ( $7.8 \pm 2.9\%$  vs  $12.1 \pm 4.4\%$ ) and IIA ( $27.8 \pm 7.1\%$  vs  $36.1 \pm 9.5\%$ ) fibres than the young group but more type IIX ( $64.6 \pm 4.7\%$  vs  $51.8 \pm 11.1\%$ ). The MHC data are consistent with the age-related changes seen in other species.

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### 1. Introduction

Recent surveys have indicated that up to 15% of the equine population is over 20 years of age with many of these horses continuing to perform in athletic activities (McKeever and Malinowski, 1997). More horses are in their late teens and are in the prime of their performance careers, competing in trail rides, dressage, driving, three-day eventing, and other athletic competitions. Age appears to affect cardiopulmonary function in the horse (McKeever and Malinowski, 1997; McKeever et al., 1998). It has also been shown that older horses may be at increased risk of hyperthermia due to changes in fluid

and electrolyte status leading to the impairment of cardiovascular function and heat dissipation mechanisms (McKeever et al., 2000). While those data have documented age-related changes in central factors affecting exercise capacity in the horse, few studies have examined peripheral changes associated with aging.

Many workers have examined muscle fibre type in the horse (Essen-Gustavsson and Lindholm, 1985; Lovell and Rose, 1991; Rivero, 1996a,b; Rivero and Serrano, 1999; Rivero et al., 1993a,b; Rivero et al., 1997; Snow et al., 1981; Snow and Guy, 1980); however, only two papers to date have attempted to present limited data grouped by age (Essen et al., 1980; Rivero et al., 1993a,b). Unfortunately, the mean age of the oldest age group in one study was 15 years (Rivero et al., 1993a,b) while the other study grouped together the horses from 10 to 28 years. This intermediate age group (10–16

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years) is an age where many horses are still in their prime athletically and are considered to be physiologically analogous to 40+ year old humans (Masoro, 1995).

Fibre type distribution is one set of measures of muscle function that may be altered by aging. Other measures that may have a more important bearing on the ability to perform exercise are body composition and, more importantly, total fat-free mass (FFM). The predominant component of a horse's FFM is muscle mass and a recent study has documented the strong correlation between FFM and performance in elite Standardbred horses (Kearns et al., 2002a). We are unaware of any experiments that have examined these parameters in older horses, analogous to geriatric humans. As mentioned, many horses continue to perform athletic activities beyond 20 years of age, a period when changes in body composition and changes in fibre type profile may limit the ability to perform exercise. Therefore, the object of this study was to test the hypothesis that aging induces differences in body composition (fat mass and muscle mass) and muscle fibre profile in the horse.

## 2. Materials and methods

### 2.1. Animals

Thirty-one Standardbred mares were used in this study. Twenty-three were four to eight years old and classified as "young". Eight were 20 years or older and classified as "old". All the horses were housed as a group on pasture. They were fed 6 kg/day alfalfa and grass hay, and 3 kg/day of a commercially available grain twice per day, morning and evening. All of the horses were unfit and had been housed at Rutgers under similar conditions and had not received any exercise training for several years. None of the younger horses had made it to the track prior to arriving at Rutgers. In the case of the old mares, they were brood mares prior to donation to the study and any training they may have received occurred almost 20 years prior to the experiment. Water was provided ad libitum. All of the methods and procedures used in this study were reviewed and approved for use by the Rutgers University Institutional Animal Care Review Board.

### 2.2. Body composition

Per cent body fat was estimated using the equation from Kane et al. (1987) where:

$$\% \text{ fat} = 2.47 + 5.47 \times (\text{rump fat thickness in cm}).$$

Rump fat thickness was measured using B-mode ultrasonography. The measurement site was determined by placing the probe over the rump at approximately 5 cm lateral from the midline, at the centre of the pelvic bone.

The region was scanned and the position of maximal fat thickness was used as the measured site. The calculated average coefficient of variation (CV) based on six animals for this rump fat thickness determination was  $3.6 \pm 0.7\%$ . Fat weight (kg) was calculated by multiplying total body weight (kg) by the percentage of body fat. FFM (kg) was then calculated by subtracting the fat weight from the total body weight.

### 2.3. Muscle biopsies

Muscle biopsies were obtained from each horse at a depth of 2 cm into the *gluteus medialis*. A Bergstrom biopsy needle was used to obtain each tissue sample and the muscle sample was immediately frozen to  $-80^\circ\text{C}$  for later analysis.

### 2.4. Myofibrillar isolation

Myosin fibrils were isolated from each tissue sample using methods previously described by LaFramboise et al. (1992). Briefly, the samples were removed from the  $-80^\circ\text{C}$  freezer and powdered under liquid nitrogen. The powder was then placed in glass-on-glass homogenization tubes and homogenized in a buffer solution of 300 mM NaCl, 100 mM  $\text{NaH}_2\text{PO}_4$ , 50 mM  $\text{Na}_2\text{HPO}_4$ , 1 mM  $\text{MgCl}_2$ , 10 mM  $\text{Na}_4\text{P}_2\text{O}_7$ , and 10 mM EDTA. Extracts were then centrifuged at 13,000g for 30 min at  $4^\circ\text{C}$ . The supernatant was recovered and diluted in a 9× volume precipitation buffer of 1 mM EDTA and 0.1%  $\beta$ -mercaptoethanol. These diluted extracts were then stored overnight at  $4^\circ\text{C}$  to allow precipitation of the myosin filaments. The solution was subsequently centrifuged at 13,000g for 30 min at  $4^\circ\text{C}$  and the resulting pellet was re-suspended (1:1) in a 0.5 M NaCl and 10 mM  $\text{NaH}_2\text{PO}_4$  buffer. This suspension was then diluted 1:100 in a SDS buffer consisting of 62.5 mM Tris-HCl, 2% SDS, 10% glycerol, 5%  $\beta$ -mercaptoethanol, and 0.001% bromophenol blue at a pH of 6.8. The samples were then boiled for 3 min and stored at  $-80^\circ\text{C}$ .

### 2.5. Myosin heavy chains

Myosin heavy chain (MHC) composition was determined using one-dimensional SDS-polyacrylamide gel electrophoresis as described by Talmadge and Roy (1993). Briefly, 1–3  $\mu\text{g}$  of protein were loaded onto a 20 cm long vertical gel and electrophoresed for 24 h at  $4^\circ\text{C}$  using a 14% bis-acrylamide stacking and 8% bis-acrylamide separating gel. This gel was stained with Coomassie blue R-250 and destained with a 10%/10% methanol and acetic acid solution. The relative concentration of MHC was determined by scanning the gel using a NIH computerized image analysis system (Scion Image for Windows, Scion Corporation). The coefficient of variation for this measure in our laboratory was less than 5%.

2.6. Statistical analysis

An ANOVA was used to determine any significant difference ( $p \leq 0.05$ ) between group means. Post-hoc differences were determined using the Tukey test and correlation coefficients were derived using the Pearson product moment (Sigma Stat 2.0).

3. Results

There were no differences ( $p > 0.05$ ) between old and young mares for total body weight, rump fat thickness,

per cent body fat, or fat weight. The old mares, however, had significantly ( $p < 0.05$ ) greater lean body weight than the young mares (Table 1).

The old group could be clearly divided in two by appearance. The animals were either very lean ( $n = 5$ ) (“skinny”), or very fat ( $n = 3$ ) (“fat”). The skinny old mares had significantly smaller rump fat thickness, lower per cent body fat, and less fat weight than both the fat old mares and the young mares. The skinny old mares had significantly less body weight than the fat old mares but not compared to the young mares. The skinny old mares also had greater ( $p < 0.05$ ) lean body weight than the young mares but there was no significant

Table 1  
Body compositional characteristics of all mares

Group	n	Rump fat (cm)	% Fat	Body mass (kg)	Fat mass (kg)	Fat-free mass (kg)
Old	8	3.0 ± 1.2	19.0 ± 6.4	529.4 ± 34.9	102.3 ± 39.9	427.1 ± 24.5*
“Skinny”	5	2.1 ± 0.2 <sup>a</sup>	14.2 ± 1.0 <sup>a</sup>	507.8 ± 27.0 <sup>a</sup>	71.8 ± 3.2 <sup>a</sup>	436.0 ± 27.5
“Fat”	3	4.4 ± 0.4 <sup>b</sup>	26.6 ± 2.4 <sup>b</sup>	563.1 ± 9.2 <sup>b</sup>	150.0 ± 16.0 <sup>b</sup>	413.1 ± 7.0 <sup>a,b</sup>
Young	23	3.4 ± 1.0 <sup>c</sup>	20.5 ± 5.4 <sup>c</sup>	512.7 ± 57.7 <sup>a</sup>	107.0 ± 37.1 <sup>c</sup>	405.8 ± 37.9 <sup>b</sup>

Means (Young, “Skinny” and “Fat” mares) with different letters <sup>a,b,c</sup> are different ( $p < 0.05$ ) from each other.  
\* Old mares (all) different ( $p < 0.05$ ) from Young mares.

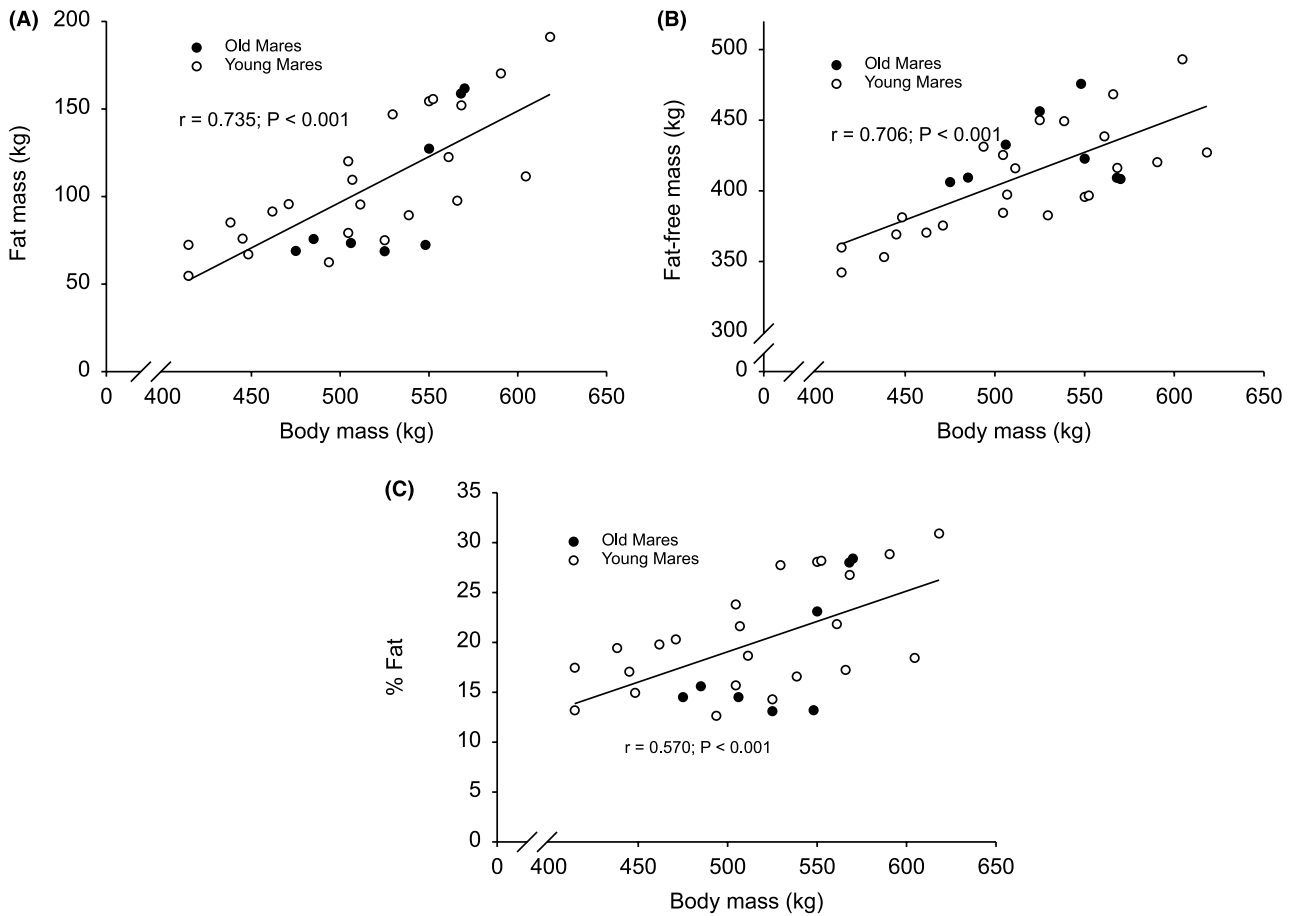


Fig. 1. Scatter diagrams and linear regression lines relating (A) fat mass to body mass; (B) fat-free mass to body mass and (C) per cent body fat to body mass.

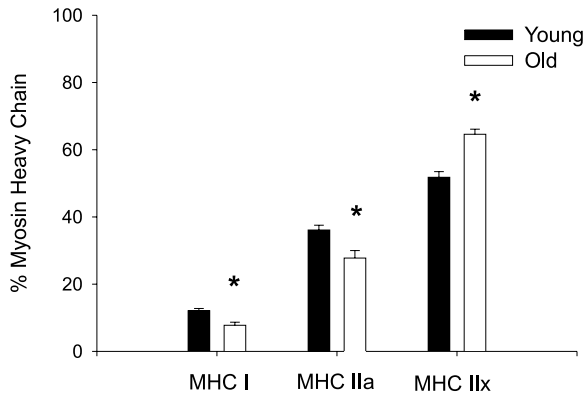


Fig. 2. Percentage distribution of the myosin heavy chain (MHC) isoforms measured in old and young mares. An asterisk (\*) denotes a significant difference between the young and old groups.

difference in this parameter when compare to the old fat mares. In turn, the old fat group had larger ( $p < 0.05$ ) rump fat thickness, *per cent* body fat, and fat mass when compared to the young mares. These comparisons are shown in Table 1.

Plots of individual values for body mass against fat mass and FFM are illustrated in Fig. 1. Significant relationships between body mass and both fat mass ( $r = 0.735$ ;  $p < 0.001$ ; Fig. 1A) and FFM ( $r = 0.706$ ;  $p < 0.001$ ; Fig. 1B) were found. Also, there was a significant relationship between *per cent* fat ( $r = 0.570$ ;  $P < 0.001$ ; Fig. 1C) and body mass.

The results of the gel electrophoresis demonstrated that the old mares had a smaller proportion ( $p < 0.05$ ) of type I MHC ( $7.8 \pm 2.9\%$  vs  $12.1 \pm 4.4\%$ ) and IIA MHC ( $27.8 \pm 7.1\%$  vs  $36.1 \pm 9.5\%$ ) than the young mares, respectively. Conversely, the old mares had a larger proportion ( $p < 0.05$ ) of type IIX MHC ( $64.6 \pm 4.7\%$  vs  $51.8 \pm 11.1\%$ ) than young mares. These mean values  $\pm$  SE are presented in Fig. 2.

#### 4. Discussion

The significant finding of this study was that older horses had a change in myosin heavy chain (MHC) distribution when compared to young horses. On a functional level it appears that the older horse has a shift in MHC population away from that which would be conducive to endurance exercise. These data may, in part, explain the decrease in maximal aerobic capacity documented in other studies (McKeever et al., 1998). One also could argue that the differences in muscle fibre profile and body composition seen in the present study may have been influenced by prior training status; however, as noted in Section 2 the mares used in the present study were broodmares and had not been trained for the several years they were housed at the university research facility. However, it has been

suggested that an age-related decrease in the type II to type I MHC isoform ratio and muscle atrophy in humans is associated with a reduction in the maximal aerobic capacity regardless of training status (Proctor and Joiner, 1997). Such may be the case in the horse.

The prevalent belief of age-related atrophy seen in human skeletal muscle is primarily due to a reduction in the number and size of muscle fibres (Kirkendall and Garrett, 1998; Lexell, 1995; Lexell et al., 1988). However, data from rats (Eddinger et al., 1985) have raised questions as to whether this shift in fibre profile is due to aging or other factors. Due to the large variation in activity and/or hormonal levels common in humans, interpretation of data gathered from muscle biopsy is difficult (Staron, 1997). Data from rats have demonstrated that type IID/X, the presumed “fast-twitch” fibres, made up a significant portion of rat muscle mass (Delp and Duan, 1996) and the percentage of type IID and IIC did not change with aging (Eddinger et al., 1985). These findings are similar to data seen in senescent beagles (Haidet and Parsons, 1991). *Per cent* fibre type distribution was not different in the *triceps*, *semitendinosus*, and *gastrocnemius* of young or old dogs, although there was a reduction in type II fibre area in the old (Haidet and Parsons, 1991). In the *vastus medialis* muscle of swine, there was a reduction of slow fibres in older animals (Cappello et al., 2000). These data, along with findings from the present study, suggest that the reduction of type II fibres seen in older humans may not be associated with aging alone. Aging itself may not cause a loss of type II fibres. In fact, data from a 12-year longitudinal study in humans (Frontera et al., 2000) reported a reduction in the percentage of type I fibres with a concomitant increase in the percentage of type IIB fibres. The authors of that study speculated that since 20–53% of muscle from older subjects co-express two or three MHC isoforms (Andersen et al., 1999), histochemical techniques used in previous studies could misclassify fibres that co-express “slow” and “fast” isoforms (Frontera et al., 2000).

##### 4.1. Muscle fibre typing

Fibre typing in the horse has been widely performed using the muscle biopsy technique. The traditional staining method has been used to determine the percentage of type I, type IIA, and type IIB fibres in different muscle groups (Snow and Guy, 1980; van den Hoven et al., 1985), as well as at different sampling depths (Kline et al., 1987). Fibre types have also been profiled for different breeds of horse (Snow and Guy, 1980; Strull and Albert, 1981). There are a number of ways to differentiate muscle fibre type, including metabolic enzymes, MHC composition, and myosin ATPase (mATPase) activity. It is now clear that metabolic enzymes do not correlate with MHC or mATPase

activities (Pette and Staron, 2000). MHC composition and mATPase activities correlate reasonably well, but it has been recently shown that a single muscle fibre can contain several MHCs at once (termed hybrid fibres) which are not identified by mATPase staining or even the gel electrophoresis technique used in this paper (Pette and Staron, 2000). These hybrid fibres are important in that they likely happen during fibre type transitions (Pette and Staron, 2000).

A limited number of studies have been performed in the horse utilizing the myosin content to describe muscle fibre composition (Barrey et al., 1999; Linnane et al., 1999; Rivero and Serrano, 1999; Snow et al., 1981). To date, there have been no studies to determine MHC composition as it relates to age. Therefore, the observed shift in the fibre profile with aging seen in the horses of the present study is new information that may shed additional light on the special needs of the older equine athlete. On a functional level it appears that the older horse has a shift in fibre type population away from that which would be conducive to endurance exercise. This shift in fibre profile is similar to that seen in disuse atrophy (Serrano et al., 2000) and aging models and may explain some of the decrease in maximal aerobic capacity seen in the older horse (McKeever et al., 1998).

Age has been studied as a factor of fibre type distribution in various breeds including Thoroughbreds, Standardbreds, Quarter Horses, Andalusians and Arabians (Bechtel and Kline, 1987; Gunn, 1991; Gunn, 1995; Rivero et al., 1993a,b; Roneus and Lindholm, 1991). These studies have employed staining techniques to determine fibre composition and have spanned ages pre-natal to beyond 20 years. While some of these studies have included horses older than 20 years in their analysis (Essen et al., 1980; Rivero et al., 1993a,b), the aged horse was not the focus of the study by Rivero and co-workers (1993a,b). Essen et al. (1980) grouped horses as either Foals (two months) or Adults (1–28 years). In that study, horses 10–28 years of age had a larger ratio of type I/II than foals. While this is different from the results presented here, the aged horses of the present study had an average age of 27 years. Horses of less than 20 years of age may be more similar to younger horses in terms of body composition and metabolism than they are of horses older than 20 years (Malinowski et al., 2002). It is possible that addition adaptations occur beyond 20 years of age in horses. On the other hand, when the issue of aging is discussed, it has been in reference to younger horses between the ages of one to six years of age (Bechtel and Kline, 1987; Roneus and Lindholm, 1991). These studies have been reported documenting an increase in type I percentage and a decrease in type IIB percentage with aging in young horses that occur in both mares and stallions (Roneus and Lindholm, 1991; Rivero et al., 1993a,b).

Typically, muscle biopsies have been used to study the effects of training in horses. Pre-and post-test designs have been utilized in both endurance and high intensity interval training regimes to determine effects on fibre composition. Results of these studies have shown no changes in respective fibre type numbers, but increases in area percentage do occur in specific fibre types with specific training regimes (endurance vs high intensity interval) (Beekley et al., 2003; Essen-Gustavsson and Lindholm, 1985; Hodgson and Rose, 1987; Hodgson et al., 1986; Lopez-Rivero et al., 1992; Rivero, 1996a,b; Rivero et al., 1995; Roneus and Lindholm, 1991). Fibre adaptations to detraining have also been studied in different breeds, again with no changes seen in fibre composition (Guy and Snow, 1977; Sinha et al., 1993). Recently it has been demonstrated that long-term endurance training induces a reversible transition of MHC from MHC IIX to MHC I (Serrano et al., 2000). Race performance is yet another parameter that has been investigated as a function of muscle fibre type. It has been shown that a high percentage of type I and type IIA fibres exists in high performing racehorses (Rivero et al., 1993a,b; Rivero, 1996a,b). To date, there have been no published data regarding the potential effect of exercise on the MHC profile of older horses. Therefore, it is unknown whether older horses would demonstrate similar plasticity as do younger horses in either their MHC profile or *per cent* area of these fibres.

#### 4.2. Body composition

A larger body mass was associated with a larger fat mass and FFM in both young and old mares. However, *per cent* body fat significantly increased with body mass signifying that these mares are accumulating excess fat. Excess body fat accumulation, or obesity, is linked to insulin resistance in humans (Arita et al., 1999). These findings are in agreement with recent data in older horses (Malinowski et al., 2002). Older horses appear to be insulin resistant because they required more insulin than younger horses to successfully manage an acute glucose load (Malinowski et al., 2002). This insulin resistance/hyperinsulinaemia can increase the risk of laminitis (Field and Jeffcott, 1989; Pass et al., 1998), exertional rhabdomyolysis (Valentine et al., 1998), osteochondritis dissecans (Pagan et al., 2001; Ralston, 1995), colic and other serious metabolic problems (Cohen et al., 1999).

Another significant finding of the present study was that older horses had a change in body composition when compared to young horses. Morphometrically, the old mares had two aged phenotypes, the “skinny mares” and the “fat mares”. These data are similar to another study in older horses that also saw two distinct phenotypes (Ralston and Breuer, 1996). Using a body condition score (BCS) system (Henneke et al., 1983), horses

from that study were determined to have a BCS of either <3 (thin to emaciated) or >3 (moderately thin to extremely fat) (Ralston et al., 1987). Aged horses have poor dentation and may have reduced absorptive and/or digestive function in their large intestine (Ralston et al., 1989) and it is thought that these complications leads to a lowered plane of nutrition and poor body composition. Alternatively, the lower *per cent* fat in the one group of aged mares might be representative of differences in activity and/or genetics. Since neither dental wear nor digestion was measured in any of these horses, no definitive statements can be made regarding the aetiology of this phenotype. However, all horses received regular dental care and were considered good eaters. This suggests that the body condition of the skinny older mares of the present study may be more related to digestive function than dental wear.

The second phenotype of the aged horse, on the other hand, follows a similar trend as older humans. Body composition differences may be under some of the same influences in the aged horse when compared to aged humans. Aging is associated with an increase in fat mass and a concomitant decrease in FFM. A study (Lesser et al., 1971) that compared weight-matched (~73 kg) young and old individuals found that the older individuals averaged 26.3% fat while the young individuals averaged only 18.3% fat. Those weight-matched old individuals had approximately 40% more fat than their younger counterparts (Lesser et al., 1971). The differences in relative fat are compounded by the fact that aging is also associated with an increase in body weight and changes in regional distribution. Old horses in the “fat” phenotype had a larger fat mass and body mass than their younger counterparts. It is believed that the increase in body fat with advancing age may be due to a decrease in glucose tolerance (Kolterman et al., 1980) and/or a reduction in resting metabolic rate (RMR) (Poehlman et al., 1997). A study by Poehlman and co-workers (1997) reported a significant decrease in both RMR and FFM in healthy women older than 50 years. While no study has looked at changes in RMR during aging in horses it has been shown that insulin resistance is increased in older mares (Malinowski et al., 2002). Unfortunately, there are only limited published data regarding normative values for *per cent* fat in horses (Kearns et al., 2002b,c). It is unknown at what *per cent* fat horses would be considered obese and at risk for other health problems; thus, more research is warranted.

## 5. Conclusions

Data from the present study have demonstrated that the older horse has a shift in fibre type population away from that which would be conducive to endurance exercise. This shift in fibre profile may explain some of the

lower exercise capacity seen in the older horse (McKeever et al., 1998). In addition, the changes in body composition seen in the older horses of the present study are similar to those changes seen in elderly humans (Lesser et al., 1971). Interestingly, aged horses exhibit similar clinical metabolic and endocrine disorders as those seen in aged humans including hyperinsulinaemia and hyperglycaemia, pituitary and thyroid adenomas, Cushing’s disease, decreased somatotropin concentrations, etc. Aged horses also exhibit decreases in nutrient absorption and utilization and research has come up with new diet formulations to address those age-related alterations in nutrition (Malinowski et al., 1997). Aged horses also have significant changes in other hormonal pathways including the plasma renin-angiotensin-aldosterone cascade, and the responsiveness of hormones involved in blood pressure regulation such as vasopressin and atrial natriuretic peptide (McKeever and Malinowski, 1999). Also, like humans, the horse exhibits an age-related decline in immune function (Horohov et al., 1999). Together, these studies provide information on the systemic effects of aging that may allow for more refined protocols for caring for horses that perform athletically into their older years.

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