Age related decreases in thermoregulation and cardiovascular function in horses

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Summary
Reason for performing study: Older horses have an increased risk of hyperthermia due to impaired cardiovascular function. While many studies have investigated thermoregulation in horses during exercise, none have investigated the effects of ageing.

Objective: To test the hypothesis that there is a difference in thermoregulation during exercise and plasma volume (PV) in young and old horses.

Methods: Study 1: 6 young (Y, 7.7 ± 0.5 years) and 5 old (O, 26.0 ± 0.8 years) unfit Standardbred mares (507 ± 11 kg, mean ± s.e.) ran on a treadmill (6% grade, velocity calculated to generate a work rate of 1625 watts) until core temperature reached 40°C. Core (CT), skin (ST), rectal temperature (RT) and heart rate (HR) were measured every min until 10 min post exertion. Packed cell volume (HCT), lactate (LA) and plasma protein (TP) were measured in blood samples collected before, at 40°C and every 5 min until 10 min post exercise. Sweat loss was estimated using bodyweight. Study 2: Plasma volume was measured in 26 young (8.2 ± 0.7 years) and 8 old (26.6 ± 0.7 years) Standardbred mares (515 ± 12 kg) using Evans Blue dye. Pre-exercise blood (rBV) and red cell (rRCV) volumes were calculated using PV and HCT. Data analysis utilised repeated measures ANOVA and t tests and data are expressed as mean ± s.e.

Results: Old horses reached 40°C faster (998 ± 113 s vs. 1925 ± 259 s; P<0.05) with a greater HR at 40°C (184 ± 6 vs. 140 ± 5 beats/min; P<0.05) and greater sweat losses (P<0.05). Heart rate did not differ (P>0.05) post exercise. Age did not alter (P>0.05) CT, ST, RT, LA, HCT or TP. Plasma volume was greater in Y vs. O horses (P<0.05, 28.5 ± 1.4 vs. 24.1 ± 1.6 l) as was rBV (41.3 ± 2.0 vs. 35.3 ± 2.3 l) and rRCV (13.3 ± 0.6 vs. 11.1 ± 0.8 l).

Conclusion: Ageing compromises the ability to handle the combined demand of exercise and thermoregulation in part due to decreased absolute pre-exercise PV.

Introduction

Human epidemiological evidence has documented higher mortality rates from hyperthermia among older men and women (Kenney 1995). Furthermore, studies of older, active humans have reported substantial age-related decreases in fluid and electrolyte balance, sweat production, central cardiovascular function and the control of skin blood flow that contribute to a decline in the ability to thermoregulate during acute exercise (Armstrong and Kenney 1993; Kenney and Zappe 1994; Kenney 1995, 1997). Older humans appear to have a state of hypohydration that results in a lower total body water, plasma volume and blood volumes (Kenney 1995, 1997). This not only affects cardiac output, it also affects the reserve of fluid needed to produce enough sweat to facilitate proper evaporative cooling (Kenney 1995, 1997). This ageing-induced disruption in the central and peripheral mechanisms affects the ability to maintain exercise-induced increases in blood flow both to the skin as well as to the working muscles, and ultimately contributes to the reported decline in cardiovascular and thermoregulatory stability in older individuals (Haskell and Phillips 1995; Kenney 1995, 1997; Lakatta 1995; Ho et al. 1997). This is a decline that can negatively impact exercise capacity as well as increase the risk of thermal injury.

From a comparative standpoint, the horse is the only species, other than man, that depends on sweating and evaporative cooling as its primary mechanism for thermoregulation (McConaghy 1994; McKeever 1998). Furthermore, equids exhibit thermoregulatory responses to heat stress that are similar to man (McConaghy 1994). During high work intensities, the rate of heat production of the horse can exceed basal levels by 40- to 60-fold (McConaghy 1994). Failure to dissipate metabolic heat can cause a continuous and excessive rise in internal body temperature (McConaghy 1994). Unfortunately, even though the horse’s athletic capacity can be considered elite among mammalian species, its ability to dissipate heat during exercise is limited due to a relatively small surface area to mass ratio (McConaghy 1994). If the excess metabolic heat generated during exercise is not dissipated, life-threatening elevations in body temperature may develop and the horse’s athletic performance will be adversely affected. The adverse effects of hyperthermia on the health and performance of horses can develop during all exercise intensities and weather conditions (McConaghy 1994). A great deal of information has been published on thermoregulation in the horse and ways to counter perturbations to the ability to cool (McConaghy 1994; Geor et al. 1995; McConaghy et al. 1995; Kingston et al. 1999; McCutcheon...
et al. 1999; Geor et al. 2000; McCutcheon and Geor 2000). Unfortunately, no studies have examined the effects of ageing on thermoregulation during exercise in the horse.

Ageing is an important factor to consider since there is a growing population of older horses (20+ years) still performing exercise with many being used for a range of activities from pleasure rides to endurance competitions (Hintz 1995; McKeever and Malinowski 1997). A larger population of horses in their late teens are in the prime of their performance careers, competing in trail rides, dressage, driving, 3-day eventing and other more athletic competitions (McKeever and Malinowski 1997). As with humans, ageing appears to affect many physiological systems including the cardiovascular, renal and endocrine systems (McKeever 2005). More specifically, age appears to cause reductions in aerobic capacity, maximal heart rate, maximal stroke volume and cardiac output in the horse (McKeever and Malinowski 1997; Betros et al. 2002). It follows that ageing has the potential to alter blood flow and fluid balance and thus alter the ability to thermoregulate during exertion. If heat loss mechanisms are impaired by ageing then the only way that a horse would be able to decrease its body temperature is to decrease the rate of heat gain by decreasing the intensity of the exertion. Therefore, during exercise, older horses may be at increased risk of hyperthermia due to the impairment of cardiovascular function that has the potential to affect heat dissipation mechanisms. While there have been many studies of thermoregulation in young horses during exercise (McConaghy 1994; Geor et al. 1995, 2000; McCutcheon et al. 1995; Kingston et al. 1999; McCutcheon et al. 1999; McCutcheon and Geor 2000), no research to date has investigated the effects of ageing. The object of this study was to test the hypothesis that ageing alters plasma volume (PV), cardiovascular function and thermoregulation during exercise.

Materials and methods

The Rutgers University Institutional Animal Care Review Board approved all methods and procedures used in these experiments.

Experiment 1

Animals: Six young (7.7 ± 0.5 years) and 5 old (26.0 ± 0.8 years) Standardbred mares with an average bodyweight of 507 ± 11 kg were used in this experiment. The young horses were considered to be in early adulthood and the older mares analogous to humans aged 60–78 years (McKeever and Malinowski 1997). All horses were unfit, but accustomed to the laboratory and running on the treadmill (Sato 1999). They were clinically normal and sound and were provided with routine care and examinations by the attending veterinarians. The horses were housed as groups on 3 acre dry lots and fed ~6 kg/day alfalfa and grass hay and 3 kg/day of a commercially available grain twice per day, morning and evening. Water was provided ad libitum.

Experimental design: The experiment was comprised of 2 parts, a familiarisation period and a submaximal exercise test. All exercise tests were conducted in the Rutgers Equine Exercise Physiology Laboratory using a high-speed equine treadmill set at a 6% slope. The familiarisation period was used to refresh the horses to exercise on the treadmill as well as the noises, smells and environment of the equine exercise physiology laboratory. During this acclimatisation period, horses began by walking for 4 min at 1.5–2.0 m/s in order to get used to the treadmill belt moving. The treadmill speed was then increased to 4 m/s for 3 min, then 6 m/s for 2 min, and 8 m/s for 1 min. The horses each had a minimum of 2 acclimatising sessions before performing the submaximal exercise test (SET).

During the SET, horses ran on the treadmill until core temperature reached 40°C. The velocity at which each horse ran was calculated to elicit an absolute work intensity of 1625 watts (Evans and Rose 1987; Jones 1988). The velocity used for each horse was individualised using the weight of the horse and previously published formula for the calculation of work rate in watts (Evans and Rose 1987; Jones 1988):

\[
\text{Watts} = \frac{\text{bwt} \times \text{Speed} \times \text{min} \times \text{sin} \times \text{treadmill angle}}{6.12}
\]

All horses were tested between 08.00 and 10.00 h. The mean room temperature of the laboratory during exercise was 8.2 ± 0.6°C. Before the test, the horses were weighed and catheters (2-Angiocath, 14 gauge) were inserted percutaneously into the left and right jugular veins, respectively, using sterile techniques and local lidocaine anaesthesia. The horses then stood quietly for 30 min in a stall before being walked onto the treadmill where 2 thermistor probes were positioned with one was placed on the shaved skin (SST-1) below the withers and the second (IT-24P) inserted through one of the catheters and positioned in the pulmonary artery (position verified by the representative waveform) for the measurement and recording (Model # Bat-10) of core body temperature. Rectal temperature was measured using a thermister probe (YSI Precision 4000A).

Measurements: Time was recorded from the start of the study through the end of recovery. Heart rate was measured and recorded every minute throughout the trial using an external HR monitor (Polar). Core, rectal and skin temperatures were measured continuously via thermistor probes throughout the exercise trial and recorded at 1 min intervals from rest until 10 min post exercise. Venous blood samples (20 ml) were taken before exercise, at 5 min intervals during exercise, immediately at the end of exercise and at 5 and 10 min post exercise. Blood samples were placed into prechilled tubes containing EDTA (Vacutainer) and immediately placed on ice. The blood samples were used to measure total blood lactate concentration, plasma protein concentration and packed cell volume (HCT). Blood lactate concentrations were measured in triplicate using a lactate analyser (Sport 1500) in microhaematocrit technique and refractometry (McKeever and Malinowski 1997). The horses were weighed before and after exercise to estimate the amount of water lost from the body via all routes (sweat, respiratory losses, etc.). This parameter was recorded only as a rough estimate of water loss. Care was taken to scrape off as much sweat as possible and to correct for faecal losses. Additionally, laboratory temperature was recorded before and after exercise.

Statistical analysis: Results are expressed as mean ± standard error of the mean (s.e.). Data were analysed using ANOVA and the t test for paired comparisons (SigmaStat Version 2.03). Data was found to be normally distributed. The null hypothesis was rejected when P≤0.05.
**Experiment 2**

*Animals:* Twenty-six young mature (8.2 ± 0.7 years) and 8 old (26.6 ± 0.7 years) Standardbred mares with an average bodyweight of 515 ± 11 kg were used in this experiment. The young horses were considered to be in early adulthood. The old mares were analogous to humans aged 60–78 years (McKeever and Malinowski 1997). The horses were clinically normal and sound and provided with routine care and examinations by the attending veterinarians. All the horses were unfit and had not received any exercise training for at least 4 years prior to the experiment. However, they were all accustomed to the laboratory, to all procedures used in the experiment and to running on the treadmill (Sato I). They were housed on pasture and fed the same diets consisting of alfalfa, grass hay and grain. Water was available ad libitum.

*Experimental design:* All tests were conducted in the Rutgers Equine Exercise Physiology Laboratory. Plasma volume (PV) was measured using a modified Evans Blue dye (A16774) dilution technique while the horses stood quietly in stalls. This method has been previously validated for use in the horse with step-by-step methods previously detailed in a prior publication (McKeever et al. 1988). Each horse then performed an incremental exercise test (GXT) which was conducted after completion of the Evans Blue dye determinations of PV so as not to affect the resting plasma volume measurements with exercise-induced fluid shifts. The purpose of the GXT was to obtain the haematocrit used to estimate (see below) total blood volume (tBV) (Persson 1967; Rose and Hodgson 1994). The blood sample collected for packed cell volume (HCT) was obtained at the end of the 8 m/s step of the GXT because all the horses could complete the 8 m/s step of the test and it has been previously documented that the haemodynamic changes and fluid shifts are similar when horses are exercised at the same submaximal intensity of a GXT (McKeever et al. 1993a,b). During the tests, the animals ran on a treadmill (Sato I) up a fixed 6% grade. The test started at an initial speed of 4 m/s for 1 min, after which speed was increased to 6 m/s for 1 min, followed by incremental 1 m/s increases every 60 s until the horses completed the 8 m/s step of the test. Packed cell volumes, both resting (rHCT) and exercise (eHCT) were measured in duplicate using the microhaematocrit technique. Resting circulating blood volume (rBV) was calculated using PV and rHCT and standard calculations (Persson 1967; McKeever et al. 1987, 1988; Rose and Hodgson 1994) where:

\[
\text{Blood Volume} = \frac{\text{Plasma Volume}}{100} \times \frac{\text{PCV}}{100}
\]

Resting red cell (rRCV) volume was calculated by subtracting PV from rBV. Note, this measurement does not include the volume of the splenic reserve. Estimated total blood volume (tBV) was calculated using the same formula for BV but with the PCV obtained at the 8 m/s step of an incremental exercise test (Persson 1967; Rose and Hodgson 1994). This volume is only an estimate of total blood volume as exercise induced fluid shifts influence ePCV. However, this volume does include an estimate of the splenic reserve with the estimated splenic volume (SpV) calculated by subtracting rBV from tBV.

*Statistical analysis:* Results are expressed as mean ± s.e. Data were analysed using ANOVA and t tests (Sigmastat Version 2.03). Data were found to be normally distributed and the null hypothesis was rejected when P<0.05.

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**Results**

*Experiment 1*

Exercise caused significant (P<0.05) increases in heart rate, rectal temperature and skin temperature of both old and young mares (Figs 1b,c). Exercise also caused increases (P<0.05) in the haematocrit, total protein and blood lactate concentration of both groups (Figs 2a–c).

As far as comparisons between age groups, there were no differences (P>0.05) between old and young mares when the core and rectal temperatures were compared at rest or when measured at 10 min post exercise (Figs 1a,b). Similarly, there were no...
statistically significant differences (P ≤ 0.05) between young and old horses for skin temperatures measured at rest, at the 40°C end point of exercise, or at 10 min post exercise (Fig 1c). There were no significant differences (P ≤ 0.05) between young and old horses for haematocrit, lactate concentration or total protein concentration measured at rest, at 40°C or at 10 min post exercise (Figs 2a–c). Similarly, there were no differences (P ≤ 0.05) between the groups (P ≤ 0.05) for haematocrit, plasma lactate concentration and total protein concentration measured in samples collected at any of the other 5 min sample points (data not shown) during the exercise test.

The average heart rates of older mares were approximately 43 beats/min higher (P ≤ 0.05) than the younger mares when they reached a core temperature of 40°C and remained about 13 beats/min higher (P ≤ 0.05) through the end of the recovery period at 10 min post exercise (Fig 3a).

Younger mares did, however, run for almost twice as long (P ≤ 0.05) as older mares before reaching a core temperature of 40°C (Fig 3b). Finally, there were no differences (P = 0.11) in changes in bodyweight (corrected for faecal losses) when measured post exercise in the young (-9.9 1.1 kg) compared to the old horses (-6.9 1.3 kg). Likewise, there were significant differences between young and old horses (P ≤ 0.05) when the weight loss was expressed as a per cent change in bodyweight (young = 4.4 0.5% vs. old = 3.1 0.5%).

**Experiment 2**

Young horses had a larger (P ≤ 0.05) PV (Fig 4a) compared to older horses (28.5 1.4 l vs. 24.1 1.6 l), rBV (Fig 4b and 41.3 2.0 l vs. 35.3 2.3 l) and rRCV (Fig 4c and 13.3 0.6 l vs. 11.1 0.8 l) compared to old horses. There were no significant differences in PCV (54 1% vs. 51 1%) at rest (Fig 5a) during exercise (Fig 5a) or estimated SpV (Fig 6) (14.2 1.0 l vs. 9.8 0.8 l). One should view the latter information with caution as there were relatively low numbers of old horses. There appears to be a large difference in the means for SpV. The statistical power to detect
a difference in SpV was low which should be taken into account so as not to make a type II error when interpreting this parameter.

Discussion

The significant finding of Experiment 1 was that older horses exercising at an absolute work intensity of 1625 watts reached a

Fig 4: Mean s.e. (a) plasma volume (PV), (b) resting blood volume (rBV), and (c) resting red cell volume in young and old mares. Asterisk (*) indicates a difference (P<0.05) between the means for young and old horses.

Fig 5: Mean s.e. (a) packed cell volume at rest (PCV) and during exercise (ePCV) for young and old horses. Mean s.e. (b) blood volume calculated using packed cell volume at rest (rBV) and total blood volume (tBV) calculated using the ePCV. Asterisk (*) indicates a difference (P<0.05) between young and old horses. A symbol (D) indicates a difference due to exercise (P<0.05).

Fig 6: Mean s.e. for estimated splenic volume (SpV = tBV - rBV). There was no difference between young and old mares (P = 0.54).

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core temperature of 40°C in almost half the time required by the younger mares. Exercise is an energetically costly endeavour and the transduction of potential energy to kinetic energy results in the generation of ATP to fuel work; however, it also generates a great deal of heat that must be dissipated (McConaghy 1994). The 2 groups of horses used in the present study were similar in weight and both groups had similar mechanical efficiencies. Therefore, the absolute energetic cost of the activity for any given submaximal activity should have been similar and in theory, both groups should have generated a similar amount of metabolic heat (McConaghy 1994). The primary difference between the 2 groups during exercise was age. Therefore, it appears that the older horses could not eliminate the excess heat as well and thus, they had a faster rate of heat gain and reached the core temperature cut-off point of 40°C in a much shorter time compared to the young animals.

Another major observation in the first experiment was that HR, measured when core temperature reached 40°C, was substantially greater in the older mares than the younger mares. The greater heart rate seen in the older mares at this endpoint suggests that their hearts had to work harder to muster sufficient cardiac output to accommodate the combined demand of increased blood flow to the organs and muscles as well as to the skin for thermoregulation (McKeever and Hinchcliff 1995; McKeever 1998, 2005). Interestingly, the more rapid heart rate in the older horses was not enough to compensate and they were still unable to dissipate the heat generated from exercise as quickly as younger mares. However, it should be noted that the young and old horses had almost identical core temperatures and heart rates 10 min after exercise. This normalisation in the old horses supports the suggestion that the old mares could handle the demands of thermoregulation alone but not the combined demand of exercise and thermoregulation.

One possible reason for the decrease in thermoregulatory capacity and higher heart rate seen in the old mares may be related to the lower resting absolute plasma volume which could have reduced preload. While we did not make more sophisticated measurements of cardiovascular function, a reduction in preload would have the potential to cause a reduction in stroke volume with a potential impact on cardiac output. This is the case in man (Haskell and Phillips 1995; Kenney 1995, 1997; Lakatta 1995) and Betros et al. (2002) have also reported an age-related decline in maximal oxygen pulse (\( \text{OP}_{\text{max}} \)) is an indicator of stroke volume). The work intensity used in the present study was around 40–50% of maximal aerobic capacity and it is well recognised that stroke volume plateaus around that point (McKeever and Hinchcliff 1995). Cardiac output is the product of stroke volume and HR. Therefore, if stroke volume was compromised by age, then it is highly likely that the greater HR observed in the old horses was required to achieve the increase in cardiac output necessary to meet the dual demand during exercise for increased blood flow to working muscle and to the skin for thermoregulation, sweating and evaporative cooling.

The above speculation regarding a lower stroke volume with age is consistent with what is observed in man (Haskell and Phillips 1995; Kenney 1995; Lakatta 1995) and has been suggested in older horses as well (Betros et al. 2002). Mechanistically, studies in man suggest that an ageing-induced reduction of stroke volume could include any of the 4 major determinants of stroke volume, namely preload, contractility, ventricular chamber size and afterload (Haskell and Phillips 1995; Kenney 1995; Lakatta 1995; McKeever 1998, 2005). The present study does not present data that can address the effect of ageing on contractility, ventricular chamber size or afterload. However, we can speculate on factors related to preload because in Experiment 2 we measured absolute plasma volume and calculated blood volume. The data demonstrates that older horses started off with lower vascular volumes than the younger horses. Since vascular volume affects venous return and cardiac filling, then one could speculate that there was the potential for a preload related effect on cardiovascular function that may be one part of the explanation for the observed differences in heart rate at the endpoint of exercise. More mechanistic studies are needed to address the effect of ageing on all the determinants of stroke volume including preload, contractility, ventricular chamber size and afterload.

Interestingly, the data from Experiment 2 demonstrates a paradox in the interpretation of some of the information from Experiment 1 that suggests the fluid shifts were the same in old and young horses. Haematocrit and PP concentrations can be used as markers of hydration status and can also be used to estimate percentage (relative) changes in plasma volume during exercise (Van Beaumont et al. 1972; Convertino, et al. 1983; McKeever et al. 1993a,b). The fact that there were no differences in HCT or PP at rest, or at any point during exercise, could be interpreted to mean that pre-exercise hydration status was the same.

This is first study to document that old horses have a lower plasma and blood volume compared to their young counterparts. While the data from Experiment 1 would support the conclusion that relative changes in plasma volume were the same, it does not give insight into absolute values for vascular volume at rest. Nevertheless, this observation is consistent with information reported for man (Kenney 1995).

The smaller absolute vascular volume seen in old horses in Experiment 2 may partially explain why their cardiovascular systems could not meet the combined demand of exercise and thermoregulation as observed in the first experiment. Older humans commonly have lower total body water, plasma volume and reserves of fluid for sweating (Armstrong and Kenney 1993; Kenney 1995, 1997). Therefore, while the relative reduction in plasma volume during exercise was similar, the older horses started off with a significantly lower absolute plasma volume. This would lead to lower venous return, stroke volume and cardiac output and a compromise of thermoregulatory stability (McKeever and Hinchcliff 1995). The lower absolute BV and PV observed in the present study would result in lower venous return and cardiac output during exercise, thus decreasing cardiovascular efficiency. The lower PV seen in the older horses would also mean a smaller fluid reserve for the formation of sweat and, therefore, a greater propensity towards thermoregulatory instability (Harrison 1985; McKeever et al. 1987; Convertino 1991; McKeever 1998).

Finally, it was observed that there was a similar bodyweight change in both groups of horses. While only an estimate, one would presume that since the weight loss was corrected for faecal losses, then the change in weight would reflect primarily sweat and respiratory water losses. Therefore, the older horses had an inability to keep cool during exercise despite possibly similar sweating rates. While pure speculation, this may have been due, in part, to the lower plasma volume and reserve of fluid for sweat production as well as an impairment of skin blood flow and thus a mismatch between the ability to transfer heat from the core to the surface to be eliminated via evaporative heat loss mechanisms (Kenney 1995, 1997; Ho et al. 1997). The mechanism in man for an age-related decline in skin blood flow during exercise involves an
impairment of the sensitivity of mechanisms affecting vascular tone (Kenney 1995, 1997; Ho et al. 1997). Those mechanisms involve neural influences and vasoactive hormones like angiotensin (Wade and Freund 1990; Zambraski 1990; McKeever 1998). Work reported by Kenney (1995) has added more insight into the effect of age by demonstrating that ageing alters plasma renin activity during exercise as well as changes in the sensitivity of skin blood vessels to circulating levels of angiotensin. An ageing disruption of the renin angiotensin cascade has also been documented in the horse where it has been shown that the PRA response to exercise is substantially lower in old horses compared to young ones (McKeever and Malinowski 1999).

Conclusions

Data from the present study confirm that older horses had decreased ability to thermoregulate during exercise and that those differences may be due different absolute PV at the start of exercise. The increased susceptibility of older horses to overheating exemplified by this study should enable veterinarians, owners and riders of horses to identify certain horses as more likely than others to develop hyperthermia during exercise. Training regimens and athletic events should be designed accordingly to prevent heat stress in the older equine athlete. Recognising that older horses have a decreased ability to thermoregulate during exercise should lead to improved monitoring practices for heat stress and, therefore, a decreased occurrence of exercise-induced hyperthermia during equine athletic activities.

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Conflicts of interest

The authors declare no potential conflicts.

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2Becton-Dickenson, Parsippany, New Jersey, USA.
3Physiostemp Instruments, Clifton, New Jersey, USA.
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