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RESEARCH ARTICLE

Effects of early-stage aging on locomotor dynamics and hindlimb muscle force production in the rat

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SUMMARY

Attenuation of locomotor function is common in many species of animals as they age. Dysfunctions may emerge from a constellation of age-related impairments, including increased joint stiffness, reduced ability to repair muscle tissue, and decreasing fine motor control capabilities. Any or all of these factors may contribute to gait abnormalities and substantially limit an animal's speed and mobility. In this study we examined the effects of aging on whole-animal locomotor performance and hindlimb muscle mechanics in young adult rats aged 6–8 months and 'early aged' 24-month-old rats (*Rattus norvegicus*, Fischer 344 × Brown Norway crosses). Analyses of gaits and kinematics demonstrated that aged rats moved significantly more slowly, sustained longer hindlimb support durations, moved with a greater proportion of asymmetrical gaits, were more plantigrade, and moved with a more kyphotic spinal posture than the young rats. Additionally, the external mechanical energy profiles of the aged animals were variable across trials, whereas the younger rats moved predominantly with bouncing mechanics. *In situ* analyses of the ankle extensor/plantar flexor muscle group (soleus, plantaris, and medial and lateral gastrocnemii) revealed reduced maximum force generation with aging, despite minimal changes in muscle mass. The weakened muscles were implicated in the degradation of hindfoot posture, as well as variability in center-of-mass mechanics. These results demonstrate that the early stages of aging have consequences for whole-body performance, even before age-related loss of muscle mass begins.

Key words: aging, locomotion, whole-body mechanics, gait, posture, muscle force production, rat.

INTRODUCTION

Aging is typically accompanied by senescence, the gradual decline in physiological processes that may begin as soon as reproductive maturity (Abrams, 1991). Decreases in reproductive performance and rates of mortality increase with advanced age across a broad phylogenetic range of taxa (Kirkwood and Austad, 2000). In aged vertebrates, including mammals, locomotor performance declines in obvious and familiar ways: older individuals demonstrate gait abnormalities, postural changes, loss of balance control and reduced speed of travel (Bassey et al., 1992; Brown et al., 1999; Brown et al., 2003; Hebert and Gerhardt, 1998). These deficits may be partially attributed to a suite of tissue- and organ-level impairments that include muscle atrophy and weakness ('senile sarcopenia'), changes in the mechanical properties of tendons and ligaments, decreasing fine motor control and decreasing ability to repair damaged tissues (Brown and Hasser, 1996; Devor and Faulkner, 1999; Gosselin, 2000; Hepple et al., 2004). Although many types of neuromuscular research studies assess locomotion in rodent models (e.g. Altun et al., 2007; Brown et al., 2003; Garnier et al., 2008; Hepple, 2003; Hepple et al., 2004), studies of aging traditionally have been conducted to inform the human condition, and often focus on later-stage aging. Thus, despite a tremendous volume of literature on the process of senescence, few aging locomotion studies have addressed early aging deficits, and fewer still have addressed aspects of whole-animal performance.

Measures of whole-animal locomotor performance include traits such as sprint speed, jump distance and locomotor efficiency. Animals may utilize a variety of strategies to minimize the energetic cost of locomotion, including moving their center of mass (CoM) to optimize energy recovery using inverted pendulum and spring-mass mechanics (Cavagna et al., 1977). The inverted pendulum mechanism ('vaulting' mechanics) of conserving external mechanical energy of the CoM is characterized by a pendulum-like exchange of potential and kinetic energies. In general, vaulting mechanics are performed at slower speeds on relatively stiff limbs, although this mode has not been widely documented in small mammals (but see Horner and Biknevicius, 2010). Conversely, spring-mass (or 'bouncing') mechanics are characterized by the in-phase cycling of gravitational potential and kinetic energies and are found in running gaits (i.e. trotting, hopping and galloping). Rather than saving energy *via* passive exchange, elastic strain energy is cyclically stored and released in elastic elements of the body, such as muscles, ligaments and tendons (Alexander, 1988). Bouncing and vaulting mechanics rely on well-functioning muscle–tendon units, the elements of which are known to be compromised by the advanced aging process. Consequently, any age-related changes in CoM mechanics likely represent the net effect of multiple deficits. Although tissue-level contributions to CoM movements are well studied in humans and in other large cursorial mammals, energy-saving mechanisms in small mammals are still poorly understood.

In aging humans, gait abnormalities are often both a response to and a cause of an increased risk of falling (Kang and Dingwell, 2008). For this reason, gait has been extensively studied in aging humans to assess changes in vestibulomotor control and vertical stability (e.g. El Haber et al., 2008; Gabell and Nayak, 1984; Judge et al., 1996; Kang and Dingwell, 2008). However, humans and other bipeds have essentially just two possible choices with regard to gait selection: walk or run. Quadrupeds may vary footfall pattern and timing in many more combinations to alter gait, and thus modify speed, metabolic efficiency and individual limb loading (Hoyt et al., 2006). Furthermore, all gaits require coordinated control of well-functioning musculoskeletal and nervous systems, regardless of the number of limbs involved (Cohen, 1988). As both of these systems exhibit age-related impairments (Kang and Dingwell, 2008), gait transitions are likely to shift concomitantly with age in mammals. Although a substantial number of studies on aging gait in rats have been performed by biomedical researchers (e.g. Dorner et al., 1996; Stoll et al., 1990), some inconsistencies with terms and measurement parameters impede our ability to relate these data with the comparative literature on gait.

Aging is also well known to affect posture (e.g. Onambele et al., 2006; Woollacott, 1993). As organisms approach senescence, joints become more rigid, muscles become smaller and less strong, and tendons become less stiff (Narici et al., 2008). In aging humans, weakness in muscle–tendon units has been shown to be a leading cause of posture imbalance (Onambele et al., 2006). Postural changes in aging quadrupedal animals have not been addressed as frequently, despite the fact that the relationship of posture with gait and CoM mechanics has direct bearing on locomotor economy and minimization of limb load (Reilly et al., 2007).

Sarcopenia, the age-associated loss of skeletal muscle mass (Clark and Manini, 2008; Rosenberg, 1997), is implicated as a primary cause of decreasing locomotor performance in aged individuals (Edström et al., 2007; Sowers et al., 2005). However, some researchers have noted locomotor declines prior to when the onset of significant muscle atrophy is likely to occur (Altun et al., 2007). In our study, we explore the effects of early-stage aging by measuring both *in situ* and *in vivo* metrics of performance in putatively pre-sarcopenic aged animals. We used a well-studied model, the Fischer 344 × Brown Norway rat, a hybrid strain that is known to be hardy and long-lived (Lipman et al., 1996). We compared whole-body measures of locomotor performance in the form of kinetics (forceplate dynamics), kinematics (speed, stride length and frequency) and posture (angles of spinal and ankle flexion) between young adult (6–8 months) and early aged (24 months) rats. Additionally, we measured muscle mass and *in situ* force production of the plantar flexor muscles (soleus, plantaris, and medial and lateral gastrocnemii) to gauge the relative degree of sarcopenia. If muscle loss is a key initiator of locomotory declines in aging mammals, then little difference was expected to occur in performance between young and aged rats, assuming muscle mass was preserved.

MATERIALS AND METHODS

Animals

Data were collected from Fischer 344 × Brown Norway rats, *Rattus norvegicus* (Berkenhout 1769) (Harlan Laboratories, Indianapolis, IN, USA). This strain is recommended by the National Institute on Aging for modeling age-related neuromuscular deficits, as they are known to be longer-lived and more resistant to pathology compared with other inbred strains (Lipman et al., 1996). The rats in our study comprised two age groups (all males): younger adult (6–8 months, N=5, 417±48.4 g) and early-stage aging (24 months, N=5, 578±10.6 g). A 24-month-old rat in this strain is physiologically similar to a 55- to 60-year-old human, and muscle mass and fiber number is reported to be statistically similar to muscles of young adult rats (Russ et al., 2011). Significant signs of sarcopenia in the Fischer 344 × Brown Norway rats do not fully manifest until ~33 months of age (Lushaj et al., 2008). Although these rats reach skeletal maturity at ~6 months, they increase in body mass in the form of adipose and connective tissues until ~30 months of age, similar to the human trajectory (LaMothe et al., 2003; Lushaj et al., 2008; Turturro et al., 1999). Individuals were housed separately in standard laboratory rat cages in a temperature-regulated room (22–23°C) on a 12 h:12 h light:dark cycle and were provided with food and water *ad libitum*. The rats were given several weeks to adjust to their new environment and were closely monitored for health issues. We eliminated two individuals of each age group from the gait and kinetics analyses because of behavioral intractability (for example, freezing rather than moving across the trackway), but *in situ* muscle analyses were performed on all rats. All protocols were in accordance with the Guide for the Care and Use of Laboratory Animals as approved by the Institutional Animal Care and Use Committee of Ohio University.

Gait and kinematics

The rats were encouraged by sound and hand movements to move across a 2-m-long trackway with an integrated force plate (Kistler, type 9281B; Kistler Instrument Corp., Amherst, NY, USA). Attempts were made to capture as many ‘free-ranging’ trials as possible to acquire a range of speeds, and so by design we did not elicit maximal speeds. Video data were recorded with a JVC GR DVL 9800 Mini DV camcorder (JVC Corp., Wayne, NJ, USA) mounted in a lateral view at 60 Hz, a more than adequate frame rate to capture the timing and sequence of footfall events at the observed stride frequencies. Video files were collected and synchronized with force data using Motus 9.0 software (Vicon Peak, Los Angeles, CA, USA). Mean forward speed, individual footfall event times and postural angles were digitized in Motus and exported to Microsoft Excel for further analyses. The timing and order of footfall events were used to define symmetrical gaits following the model of Hildebrand (Hildebrand, 1976). Symmetrical gaits were defined by limb phase, which is the elapsed time between ipsilateral hindlimb and forelimb footfalls divided by total hindlimb stride cycle duration. Duty factor was calculated by dividing hindlimb support duration (the time during which the hindfoot is in contact with the ground) by hindlimb stride duration. Any gait where hindlimbs fell greater or less than 50±5% of the stride cycle apart were treated as asymmetrical and defined following Hildebrand (Hildebrand, 1977).

Plantar flexion (ankle extension) angle was measured at midstance from the left hindlimb (Fig. 1). Plantar flexion angles were obtained by calculating the angle of the foot (distal metatarsal to calcaneus) relative to the ground. In order to grossly approximate differences in trunk flexion between the two age groups, spinal (kyphosis) angles were estimated by digitizing the angle formed by the base of the neck, the highest point of the spine, and the base of the tail. Spinal flexion angle was measured at toe-down, midstance and lift-off.

Mechanics

In order to estimate fluctuations of the external mechanical energies, we recorded whole-body ground reaction forces (GRFs) in vertical, fore–aft and mediolateral directions at 960 Hz for each step. The force output was converted to a digital signal and recorded in Motus 9.0. GRFs were then exported for whole-body mechanics analysis in a

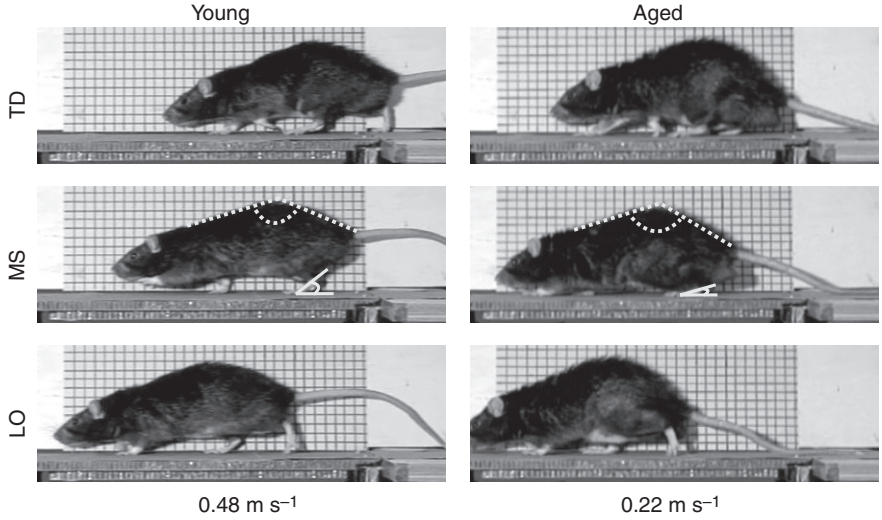

Fig. 1. Digital video stills of young (left column) and aged (right column) rats captured at left hindlimb touchdown (TD), midstance (MS) and lift-off (LO). Angles of spinal flexion and plantigrady (angle of the foot relative to the ground) were digitized from midstance.

custom-made LabVIEW program (National Instruments, Austin, TX, USA). GRFs were used to calculate fluctuations in the external mechanical energies following Cavagna et al. (Cavagna et al., 1977); more detailed methods may be found in other references (Horner and Biknevicius, 2010; Parchman et al., 2003). Briefly, the accelerations of the CoM in all three directions were calculated by dividing GRFs by body mass (M_b; kg), and the velocities (v; m s^{-1}) of the CoM were estimated by taking the first integral of the accelerations after first subtracting body mass from the vertical GRFs. Kinetic energies (E_K) of the CoM in each direction were obtained from the velocities ($E_K=\frac{1}{2}M_b v^2$) and summed to calculate the total kinetic energy (E_{KTot}). Although the integration constant for the craniocaudal direction was set to mean forward velocity (Blickhan and Full, 1992), the constants were estimated as the mean value for the vertical and mediolateral records (Donelan et al., 2002). Only trials that demonstrated nearly steady speeds (i.e. less than 10% net decelerative and accelerative areas, respectively, under the fore–aft GRF curve) during the stride were included for further analyses. Finally, vertical displacement of the CoM (h) was found by integrating vertical velocity with mean vertical displacement set as the integration constant. This value was then used to calculate the change in gravitational potential energy ($E_P=M_b\boldsymbol{g}h$, where $\boldsymbol{g}$ is gravitational acceleration or 9.81 m s^{-2}) across a stride. The sum of E_P and E_{KTot} then yielded the total external mechanical energy (E_{MTot}).

Phase shifts of the fluctuations between E_{KTot} and E_P were calculated in order to describe mechanical gaits. Phase shifts were calculated by dividing the time difference of E_{KTot} and E_P minima by stride duration and then multiplying the resultant value by 360 deg and adding 180 deg (Cavagna et al., 1977). E_{KTot} and E_P cycle out of phase with one another when animals move with inverted pendulum (vaulting) mechanics (phase shift=180–135 deg), whereas in-phase cycling is found during spring-mass (bouncing) mechanics (phase shift=0–45 deg). Phase shifts between 45 deg and 135 deg represent mixed mechanics (Ahn et al., 2004).

Muscle forces

A total of five rats per age group were used in the collection of muscle force properties. These animals included all rats that were used to collect locomotion data plus the two additional rats from each group that did not perform consistently well enough for their locomotion data to be analyzed. Prior to *in situ* muscle testing, each rat was deeply anaesthetized with ketamine/xylazine. The animal was then put in the prone position in a fixed rigid frame that securely immobilized the leg and pelvis. Skin over the posterior leg and popliteal space was reflected to expose the sciatic nerve at the hip joint. A mineral oil pool was formed around the popliteal fossa to maintain muscle temperature with radiant heat. Body temperature (measured rectally) was maintained at ~37°C with a heating pad placed under the trunk of the animal. The tissue overlying the plantar flexor (Achilles) tendon and distal plantar flexor mass was transected and reflected, and then the resting length of the Achilles tendon was measured using digital calipers with the ankle at 90 deg flexion and the hip and knee at 0 deg flexion. The calcaneus was cut distal to the tendon's insertion and the tendon–calcaneus complex was attached in series with a force transducer *via* a custom-made aluminum clamp. We acknowledge that our choice to test the plantar flexors at a fixed ankle angle (90 deg) meant that it was unlikely that any of the muscles was at optimal length (l_o) during the *in situ* testing. However, as locomotion results from the action of the muscle group and not isolated muscle, it is unlikely that all plantar flexor muscles would achieve l_o simultaneously during locomotion. Our observed *in situ* force values were similar to those previously reported for the entire plantar flexor group (Willems and Stauber, 1999). Because specific tension requires the l_o value, we instead divided total plantar flexor force by muscle mass and reported this as muscle quality, as has been performed previously (Dormer et al., 2009; Kan et al., 2005).

Once the calcaneus was securely clamped in place, the length of the muscle–tendon complex was adjusted so that the tendon length was the same as the measured resting length. A Grass S48 stimulator (Astro-Med, West Warwick, RI, USA) was used to activate the plantar flexors *via* sciatic nerve stimulation through a hook electrode (Harvard Apparatus, Holliston, MA, USA). All stimulation pulses were 200 µs in duration and delivered at supra-maximal (125% voltage needed to produce maximum twitch force) intensity. The plantar flexor muscles were then potentiated with a series of ten 100 ms, 100 Hz trains (one train per 10 s), followed by administration of 500 ms trains of 1, 5, 10, 25, 50, 75, 100 and 125 Hz at a rate of one train per 10 s to determine the force–frequency relationship. Two trains of each frequency were delivered, and the mean force response was used to represent the peak force produced at each frequency. The order of administration of the stimulation trains was randomly determined for each animal. Resting tension was recorded during these trials as well. Following contractile testing, the four plantar flexor muscles were dissected, blotted dry and weighed.

Although several indices of sarcopenia have been proposed (e.g. Edström and Ulfhake, 2005), there is considerable debate

Table 1. Kinematic and kinetic stride variables and means (±s.e.m.) for young and aged rats used in the gait and mechanics study and statistical results

	Young		Aged		Age effects across gait	
Variable	Symmetrical	Asymmetrical	Symmetrical	Asymmetrical	*F*	*P*
Strides analyzed	17	21	13	24		
Speed ($m\,s^{-1}$)	0.71±0.12	0.90±0.08	0.44±0.05	0.43±0.09	**20.9**	**0.01**†
Stride frequency (Hz)	3.71±0.17	4.17±0.13	2.75±0.02	3.12±0.16	7.5	0.05‡
Stride length (cm)	19.06±0.68	21.13±0.43	14.37±0.46	13.59±0.45	**8.4**	**0.04**‡
Duty factor (stride cycle)	0.54±0.02	0.51±0.02	0.67±0.01	0.69±0.02	**9.3**	**0.03**‡
Spine angle at midstance (deg)	135.2±0.96	137.0±1.01	126.2±0.82	124.9±0.69	**48.1**	**<0.001**‡
Plantigrady at midstance (deg)	47.8±2.14	34.5±3.60	24.9±4.37	22.6±3.25	**10.8**	**0.03**†
Vertical displacement of the CoM (cm)	0.65±0.10	0.74±0.05	0.56±0.04	0.83±0.10	0.2	0.7‡

All variables are in reference to the left hindlimb. Age effects were measured using a hierarchical mixed-model ANOVA (d.f.=1,4) or ANCOVA, where appropriate. Significant values are in bold.
†Fixed effect=age, random effect=individual nested within age.
‡Fixed effect=age, random effect=Individual nested within age, covariate=velocity.

among researchers as to which parameter is the best measurement. In our study we use muscle wasting as the primary indicator of sarcopenia, but we also measured forces relative to muscle mass and body mass. Force divided by muscle mass is used to measure changes in muscle force isolated from changes in muscle size and is referred to as muscle quality (e.g. Dormer et al., 2009; Kan et al., 2005; Russ et al., 2011). We used muscle mass as our denominator because we tested the plantar flexor muscles as a functional group, and thus we could not determine the optimal length for producing force for individual muscles or estimate physiological cross-sectional area (PCSA). As PCSA is typically calculated using measured muscle mass, length, pennation angle and published values for the fiber length:muscle length ratio at l_o and muscle density in the equation [muscle mass × cos(pennation angle)/(muscle density × fiber length)] (Stone et al., 2007), we feel that our use of mass to calculate muscle quality is appropriate. Muscles tend to lengthen as bones do, and mammals cease bone elongation at sexual maturity. The other terms in the equation are also static: muscle density is an assumed constant, and pennation angle varies little after maturity. Thus, muscle mass would be the main factor in age-related differences in PCSA. We also reported muscle force per unit body mass, as this value may best demonstrate the functional connection between muscles and whole-animal performance, as well as the functional limitations associated with muscle weakness.

Statistical analysis

The analysis of the locomotion data was performed using SYSTAT v. 12.0 (Systat Software, Chicago, IL, USA). Gait and kinematic variables were analyzed using a hierarchical mixed-model ANOVA, with age as a main, fixed factor and individual nested within age as a random factor. Speed was included as a covariate when significant effects were detected (Table 1), but mass did not covary with any of the variables of interest and so was excluded from the analyses. Overall gait choice and CoM phase shift data were analyzed with a chi-square test. Plantar flexor muscle masses and resting tensions were compared using unpaired *t*-tests. Muscle force data were analyzed with general estimating equations (GEEs) using a two-factor (age × frequency) analysis, with frequency as a repeated factor. Separate analyses were performed on absolute muscle force, muscle quality (muscle force/muscle mass) and muscle force as a percentage of both peak force and body mass. In the event of a significant age × frequency interaction, *post hoc* comparisons were evaluated using Mann–Whitney *U*-tests.

RESULTS

Gaits, kinematics and CoM mechanics

We did not elicit maximal speeds from the rats by design, in an effort to capture the range of preferred speeds. The mean gait-wide speed of the younger rats was $0.81\pm0.1\,m\,s^{-1}$ (mean ± s.e.m.), which was nearly double the mean gait-wide speed of the aged rats ($0.44\pm0.07\,m\,s^{-1}$; Table 1). Younger rats showed no preference for symmetrical or asymmetrical gaits (χ^2=0.42, *P*=0.52, d.f.=1), whereas aged rats performed asymmetrical gaits more frequently (χ^2=4.21, *P*=0.05, d.f.=1). Within symmetrical gaits among the young rats, speed had a nearly threefold range of 0.35 to $1.07\,m\,s^{-1}$; among the aged rats, speed spanned a twofold range from 0.24 to $0.51\,m\,s^{-1}$. In the entire range of symmetrical gait speeds, young rats primarily moved in a trot whereas aged rats employed the lateral sequence singlefoot and trot gaits in equal frequencies (Fig. 2). Within asymmetrical gaits, young rat trials exhibited speeds that spanned less than a twofold range (0.60 to $1.03\,m\,s^{-1}$), whereas the speed of aged rats spanned a nearly fourfold range (0.19 to $0.73\,m\,s^{-1}$). Compared with the younger rats, the aged rats used longer duty factors in symmetrical gaits and longer hindlimb body

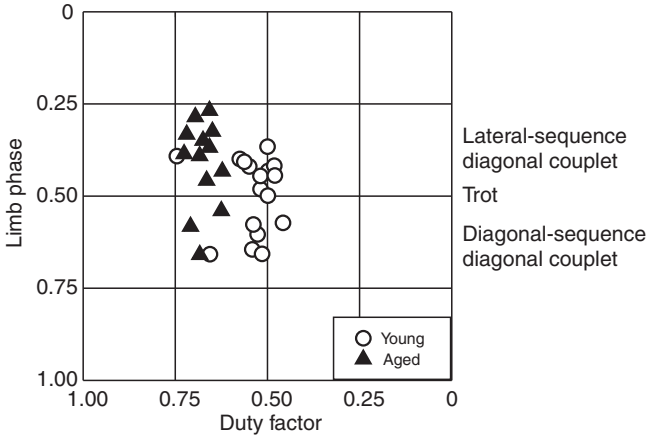

Fig. 2. Symmetrical gait plot [following Hildebrand (Hildebrand, 1976)] showing the relationship of limb phase to duty factor, where limb phase is the portion of a stride that a forelimb follows an ipsilateral hindlimb, and duty factor is the support duration of a hindlimb divided by stride duration. Aged rats (filled triangles) had significantly higher duty factors, but overall symmetrical gait choice between aged and young rats (open circles) was similar and largely remained in the range of diagonal couplets.

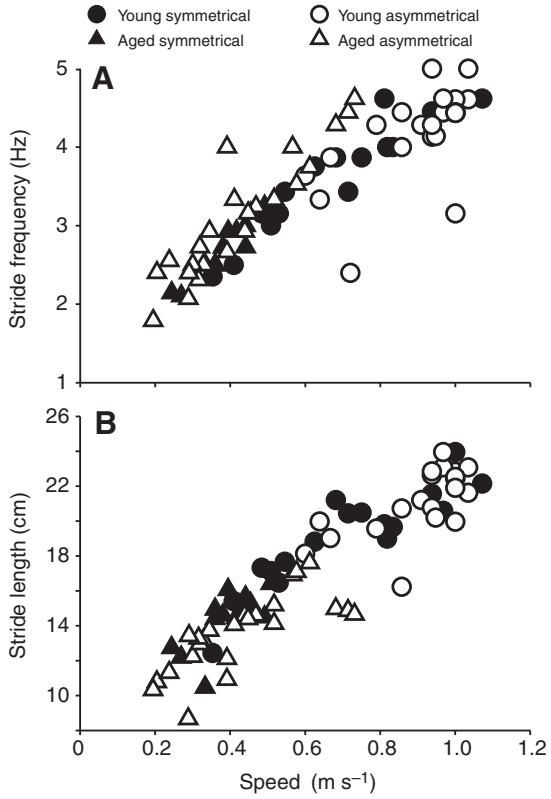

Fig. 3. Kinematic stride variables (A, stride frequency; B, stride length) plotted against speed in young (circles) and aged (triangles) rats. Open symbols correspond to asymmetrical (e.g. gallop, bound) gait trials, and filled symbols correspond to symmetrical (e.g. trot) gait trials. Stride frequency and stride length increased with speed in all gaits and ages, but an ANCOVA demonstrated significantly lower stride frequencies and shorter stride lengths in the aged rats (Table 1).

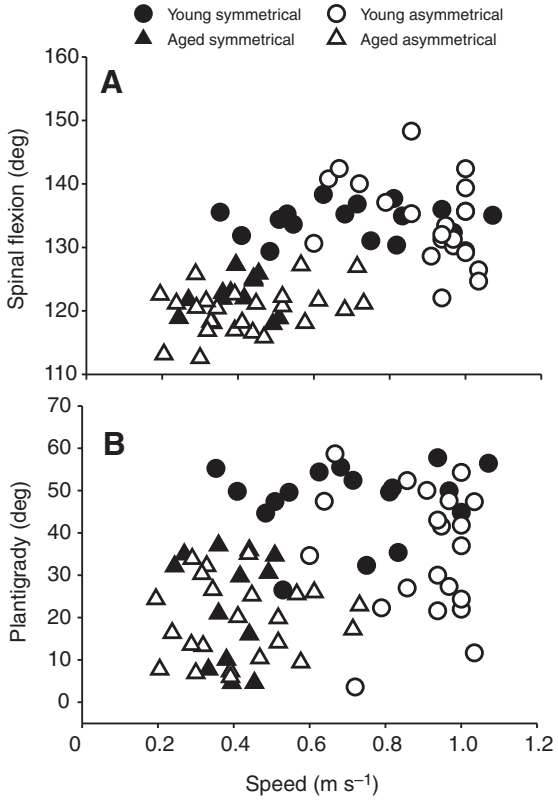

Fig. 4. Posture angles (A, spinal flexion; B, plantigrady) from midstance plotted against speed in young (circles) and aged (triangles) rats. Open symbols correspond to asymmetrical gait trials, and filled symbols correspond to symmetrical gait trials. Aged rats moved with a more kyphotic spine posture and plantigrade foot posture during all gaits (Table 1).

support within asymmetrical gaits (Table 1; Fig. 2). For both age groups, asymmetrical gaits were performed at all speeds, although they were more common at faster speeds. Stride frequency and stride length increased with speed in a similar trajectory in both age groups and across gaits (Fig. 3). A mixed-model ANCOVA demonstrated that, regardless of speed, stride frequency tended to be greater among young rat trials (young 3.94±0.15 Hz, aged 2.94±0.09 Hz; Table 1), and this difference was significant within a gait type ($F_{1,4}$=18.69, P=0.01). Similarly, stride length was significantly longer among younger rat trials among all gaits and speeds (Table 1; Fig. 3B).

Because spine posture remained fairly constant across stance (s.d.=1.76 deg), only the angle of the spine at midstance was used for analyses. Aged rats demonstrated significantly more flexed (kyphotic) spine postures, regardless of gait type. Within an age group, spine angles did not differ significantly between gait type within groups or across stance phase (Table 1; Fig. 4A). Foot posture varied considerably between aged and young rats, with aged rats exhibiting a much more plantigrade foot posture (Table 1; Fig. 4B). Young rats tended to move with a more extended (i.e. less plantigrade) foot posture during symmetrical gaits, but aged rats did not alter foot posture with regard to gait (Table 1).

Differences between age groups in CoM mechanics were also noted (Fig. 5). Whereas young rats converged on bouncing and, less frequently, mixed mechanics (χ^2=27.1, P<0.001, d.f.=2), older rats displayed much more variability and demonstrated no statistical preference for a CoM mechanical gait pattern (χ^2=0.95, P=0.70, d.f.=2). Vertical displacement of the CoM was not significantly different between age groups (Table 1), nor were there differences in the variance of the CoM displacement values.

Muscle mass and force–frequency characteristics

There were no age-related differences in muscle mass of the individual plantar flexor muscles or the overall plantar flexor group mass (Table 2). However, when expressed as a percentage of body mass, relative muscle masses were significantly smaller in the older rats (all P≤0.008; Table 2). In addition, the muscle group resting tensions were not different across ages (young 1.10±0.17 N, aged 1.38±0.36 N; P=0.16). The *in situ* data indicated a reduction in the force-generating capacity of the plantar flexor muscles in aged rats measured at higher frequencies that is consistent with gastrocnemius motor unit discharge rates observed in free-moving rats (Gorassini et al., 2000). As the large gastrocnemii produce the majority of the total plantar flexor force, the loss of higher-frequency force production likely affects optimal locomotor function to some extent. Analysis of the absolute force–frequency relationship revealed significant main effects of age (P=0.04) and frequency (P<0.001), as well as a significant age × frequency interaction (P=0.02) (Fig. 6A). *Post hoc* testing for age differences at specific frequencies was only significant at frequencies ≥75 Hz. When absolute force was expressed relative to body mass (Fig. 6B), the differences between the young and aged rats were markedly increased and significant at all frequencies tested. Muscle quality exhibited a

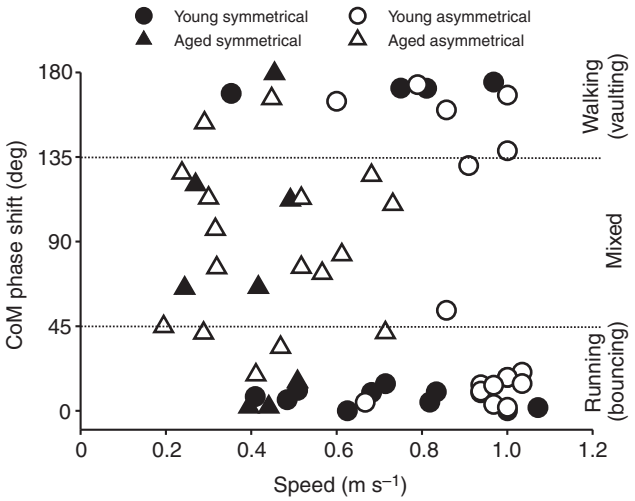

Fig. 5. Center of mass (CoM) mechanics plotted against speed in young (circle) and aged (triangle) rats. Open symbols correspond to asymmetrical gait trials, and filled symbols correspond to symmetrical gait trials. Although young rats tended to move primarily with vaulting or bouncing mechanics, aged rats demonstrated no statistical preference for any mechanism.

significant main effect of frequency ($P<0.001$) but not age ($P=0.15$), although the significant age × frequency interaction ($P=0.002$) persisted (Fig. 6C). Again, significant differences between adult and aged animals were present only at higher frequencies.

DISCUSSION

A range of physiological functions are known to decline progressively with age. From snakes to humans, age-dependent decreases in overall activity, physical strength, coordination and endurance have been reported (Doherty, 2003; Ricklefs, 2008). Consistent with these data, we observed differences in whole-animal and muscle performance between young and aged adult rats that were pervasive and substantial. The aged rats not only moved more slowly, but used fundamentally different gaits and locomotor mechanics than younger rats, and exhibited reduced tetanic plantar flexor forces. Interestingly, the aged rats in this study displayed significant losses in locomotor performance and muscle function despite not exhibiting 'strict' sarcopenia; that is, the plantar flexor muscles were not different in absolute mass from those in the younger rats.

Consistent with their slower locomotor speeds, aged rats moved with longer duty factors. However, speed may not be the only reason why duty factors increase in older animals – stability concerns may also explain gait differences observed between age groups in our study. Moving with higher duty factors could provide longer periods of multiple (>two) limb support and thus augment stability, consistent with observations made in aging humans (El Haber et al., 2008). When moving with symmetrical gaits, aged and younger rats both used diagonal-couplet-dominated gaits at slow to moderate speeds, but the aged rats rarely performed a 'perfect' trot. Instead, they tended to use the imprecise variants of the trot (lateral- and diagonal-sequence diagonal couplets), which, together with high duty factors, ensures long periods of multipodal support. Furthermore, only the aged rats employed the lateral-sequence singlefoot. Other tetrapods that use this gait with high duty factors include turtles and young dogs, both of which need to optimize periods of multipodal support for stability (Hildebrand, 1976; Hildebrand, 1980).

In the younger rats, there were no discrete differences noted between symmetrical gait dynamics and CoM mechanics, and the symmetrical gaits were dominated by bouncing mechanics. This propensity for moving predominantly with bouncing mechanics despite the lack of an aerial phase gait (i.e. 'grounded running') is common among small mammals (Biknevicius and Reilly, 2006; Horner and Biknevicius, 2010). In light of this, it is intriguing that aged rats demonstrated a seemingly random pattern with regard to CoM mechanics. One reason for this lack of mechanical precision may be that well-coordinated mechanisms for adjusting limb stiffness are not viable with aged muscle–tendon units. In vaulting mechanics, the cyclical rising and falling of the CoM with every step is enabled by a fairly (but imperfectly) stiffened limb, and bouncing mechanics are accomplished with the use of well-tuned muscle–tendon and ligament 'springs' (Cavagna et al., 1977). As animals age, there are shifts in the stiffness of muscles, tendons and ligaments. Overall muscle stiffness, or the proportion of passive tension to overall (passive plus active) tension, has been observed to increase with age (Brown et al., 1999; Wolfarth et al., 1997). Although we did not collect formal stiffness data, there were no differences in resting tension recorded during the *in situ* testing (D.W.R., unpublished data), suggesting that muscle stiffness may not have been markedly altered in our aging rats. Furthermore, the preponderance of age-related changes in tendons (e.g. increased elastin content and reduced collagen fibril crimp angle) results in increasing compliance and decreasing strength (Nakagawa et al., 1994; Narici et al., 2008; Vogel, 1980). In addition to impacting CoM mechanics, these changes in tissue properties decrease the capacity for energy recovery during either vaulting or bouncing mechanics, as has been demonstrated in older humans (Cavagna et al., 2008). Although the capacity to recover energy through vaulting or bouncing mechanics in small-bodied mammals is debatable (Pollock and Shadwick, 1994; Reilly et al., 2007), the fact that aging rats appear to be unable to precisely coordinate the movements of their CoM as consistently as younger adult rats reflects a deterioration of locomotor function.

Although asymmetrical gaits such as galloping and bounding are typically associated with faster locomotion, aged rats performed asymmetrical gaits more often than not. Rats of both age groups generally avoided periods of suspension, perhaps because they were not moving at maximal speeds. But, as with symmetrical gaits, the

Table 2. Absolute and relative muscle masses for each plantar flexor muscle

	Absolute muscle mass (g)			Relative muscle mass (% body mass)		
Muscle	Young (N=5)	Aged (N=5)	P	Young (N=5)	Aged (N=5)	P
Soleus	0.172±0.028	0.192±0.009	0.187	0.042±0.003	0.033±0.001	**0.001**
Plantaris	0.429±0.055	0.428±0.031	0.978	0.112±0.014	0.073±0.005	**0.002**
Medial gastrocnemius	1.059±0.176	0.889±0.044	0.096	0.274±0.013	0.152±0.008	**<0.001**
Lateral gastrocnemius	1.017±0.296	1.061±0.056	0.761	0.260±0.038	0.182±0.010	**0.008**
Total plantar flexors	2.677±0.524	2.571±0.114	0.681	0.692±0.043	0.440±0.020	**<0.001**

Values are means ± s.d.
Significant differences between young and aged rats are in bold.

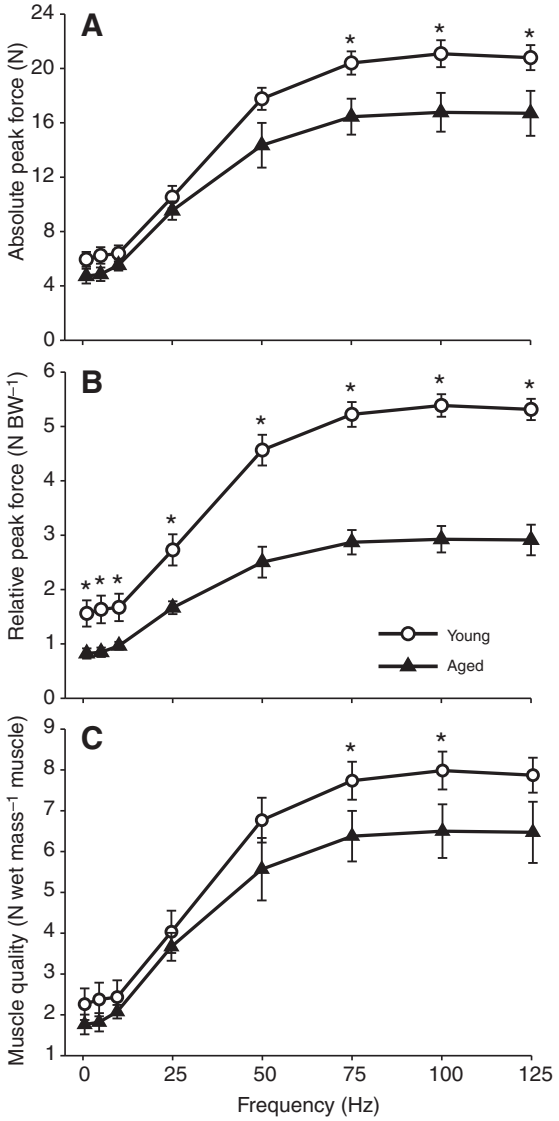

Fig. 6. Muscle mechanics (A, absolute peak force; B, relative peak force; C, muscle quality) plotted against stimulation frequency in the plantar flexor muscles of young (open circles) and aged (filled triangles) rats. Differences were statistically tested using general estimating equations (details in text). Asterisks indicate significant differences between age groups (P<0.05).

substantially higher duty factors in aged rats resulted in a bias towards continuous support of the body with one or both hind limbs during the stride. Younger rats avoided asymmetrical gaits during their slower trials, but aged rats were just as likely to use asymmetrical gaits at the slowest or fastest speeds in their range. These speed and gait choices can be explained partly by structural and functional limitations in the locomotor apparatus of older mammals. Across all gaits, stride lengths are significantly shorter in aged mammals (Bassey et al., 1992; Brown et al., 2003), and these shorter stride lengths are indicative of reduced ranges of joint motion with age (Shaffer and Harrison, 2007). Whether this is caused by age-related increases in muscle stiffness or joint capsule stiffness could not be assessed in this study, but both factors are likely to be influential in reducing the stride lengths (Gosselin et al., 1998). Additionally, the increased kyphosis of the spines in the aged rats would reduce the functional trunk length – and thus stride length – if immobilized by stiffened ligaments and joint capsules.

The adoption of asymmetrical gaits at slower speeds by the older rats might be a strategy to accommodate age-related alterations of the forces affecting the musculoskeletal system and the decreased capacity for muscular force production. The older rats were heavier than young adults, and thus required greater muscular force to counteract the tendency for limb joints to flex during stance. Furthermore, the more plantigrade position of the hindfoot in the aged rats resulted in poor mechanical advantage of the plantar flexor muscles; as the hindfoot approaches a more horizontal position, the moment arm of the GRFs increases, and thus the plantar flexor muscles must produce more force to maintain ankle posture. Initially, these larger limb loads in the aged rats would seem to support the critical force hypothesis for symmetrical–asymmetrical gait transitions, which posits that animals change gait to reduce loading of the musculoskeletal system (Biewener and Taylor, 1986; Farley et al., 1991). However, a previous study found little support for force-motivated gait transitions in another small, crouched mammal – the degu, a caviomorph rodent (Iriarte-Diaz et al., 2006). When adult degus were injected with saline solution to experimentally increase their mass by 20%, they demonstrated no difference in gait transitions when compared with controls. However, the degu study was conducted on young animals that were likely able to compensate for the increased loads by producing greater force within limb muscles. Unfortunately for the aged rats, weakness of the plantar flexor muscles made increasing muscle force to counteract the larger moments experienced by the ankle joint much more difficult (if not impossible) to accomplish.

The only viable solution for an animal that is unable to alter its posture may be to reduce GRFs by shifts in gait and CoM mechanics. This can be accomplished by increasing support duration, thus distributing the force across a longer span of time and thereby reducing the peak vertical forces acting on an individual limb. Indeed, the aging rats did move with higher duty factors in both symmetrical and asymmetrical gaits. Switching to asymmetrical gaits without incorporating an aerial phase also limits the effective GRFs acting on individual limbs (Alexander and Jayes, 1983), although the ability to move faster is compromised. Thus, the asymmetrical gait observed in the aged rats – a shuffle-like imprecise gallop – may not represent a ‘solution’ at all, but rather the net effect of weakened muscles, more compliant tendons and a degraded neuromuscular system.

This study was conducted to investigate the effects of early-stage aging on the locomotion of a commonly studied mammal, and the role of sarcopenia in the declination of locomotor performance in the aged. Our study shows that aging impairs locomotor function in rats by a number of mechanisms acting in concert to produce slower movement, degraded posture and an inability to consistently utilize either CoM energy-saving mechanisms. Moreover, these deficits are present at an age where ‘true’ sarcopenia (loss of absolute muscle mass) has yet to become a major factor, although muscle weakness is apparent. Previous studies have found that in both long- and short-lived wild mammal species, muscular deficits and a reduction in fitness can precede reproductive senescence (e.g. Abrams, 1991; Hindle et al., 2009a; Hindle et al., 2009b). This decrease in fitness may be due in part to early age-related changes, which likely negatively impact individual survivorship in the wild, as the risk of predation increases and foraging capabilities are reduced. Future studies combining whole-body and tissue performance measurements may help to clarify the causal mechanisms of reduced locomotor performance and, ultimately, may lead to a better understanding of the process and timing of senescence.

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