Intraspecific scaling of the minimum metabolic cost of transport in leghorn chickens (Gallus gallus domesticus): links with limb kinematics, morphometrics and posture

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ABSTRACT
The minimum metabolic cost of transport (CoT\text{min}; J kg\textsuperscript{-1} m\textsuperscript{-1}) scales negatively with increasing body mass (\(\propto M\text{b}^{0.32}\)) across species from a wide range of taxa associated with marked differences in body plan. At the intraspecific level, or between closely related species, however, CoT\text{min} does not always scale with \(M\text{b}\). Similarity in physiology, dynamics of movement, skeletal geometry and posture between closely related individuals is thought to be responsible for this phenomenon, despite the fact that energetic, kinematic and morphometric data are rarely collected together. We examined the relationship between these integrated components of locomotion in leghorn chickens (Gallus gallus domesticus) selectively bred for large and bantam (miniature) varieties. Interspecific allometry predicts a CoT\text{min} \(\sim 16\%\) greater in bantams compared with the larger variety. However, despite 38\% and 23\% differences in \(M\text{b}\) and leg length, respectively, the two varieties shared an identical walking CoT\text{min}, independent of speed and equal to the allometric prediction derived from interspecific data for the larger variety. Furthermore, the two varieties moved with dynamic similarity and shared geometrically similar appendicular and axial skeletons. Hip height, however, did not scale geometrically and the smaller variety had more erect limbs, contrary to interspecific scaling trends. The lower than predicted CoT\text{min} in bantams for their \(M\text{b}\) was associated with both the more erect posture and a lower cost per stride (J kg\textsuperscript{-1} stride\textsuperscript{-1}). Therefore, our findings are consistent with the notion that a more erect limb is associated with a lower CoT\text{min} and with the previous assumption that similarity in skeletal shape, inherently linked to walking dynamics, is associated with similarity in CoT\text{min}.

KEY WORDS: Terrestrial locomotion, Size, Body mass, Geometric similarity, Energetics

INTRODUCTION
Body size has a significant influence on the morphology and metabolism of animals (Schmidt-Nielsen, 1975, 1984; Biewener, 1989). In animals that locomote terrestrially, the absolute amount of metabolic energy required to move a given distance increases with increasing body size, but not in direct proportion (slope <1) (Bruinzeel et al., 1999; Halsey and White, 2012). In relative terms, the mass-specific energy per unit distance (the cost of transport, CoT; J kg\textsuperscript{-1} m\textsuperscript{-1}) is lower in larger species than in smaller ones. Often, at optimal self-selected speeds within a gait, animals incur a minimum cost of transport (CoT\text{min}) and it seems reasonable to expect natural selection to favour strategies that minimise the CoT\text{min}. For example, if the movement requirements of animals were similar, they would be expected to share optimum limb dynamics, and similar morphological proportions to allow it (Alexander and Jayes, 1983). The evolutionary allometry of CoT\text{min} with body mass (\(M\text{b}\)) is widely reported. For example, across more than 90 species of mammals and birds (7 g to 260 kg), CoT\text{min}=10.7\(M\text{b}^{0.32}\) (Taylor et al., 1982). Adding amphibians, reptiles and invertebrates (<1 g) to this data set yielded a similar result (CoT\text{min}=10.8\(M\text{b}^{0.32}\); Full and Tu, 1991) and African elephants (Loxodonta africana, \(M\text{b}=1542\) kg) fall within the 95\% confidence intervals (CIs) of this equation (Langman et al., 1995). The scaling exponent, however, is known to differ between walking and running (Margaria et al., 1963; Minetti et al., 1999; Rubenson et al., 2004, 2007; Maloiy et al., 2009; Nudds et al., 2011; Watson et al., 2011), and also between small crouched- and large upright-postured vertebrates (Reilly et al., 2007; Nudds et al., 2009). Furthermore, there is overlooked variation in CoT\text{min} at a given \(M\text{b}\), associated with variation in body form (Full et al., 1990). The general trend of decreasing CoT\text{min} with \(M\text{b}\), however, holds for over three orders of magnitude. Where outliers exist, their relatively more or less economical CoT\text{min} compared with other species of the same \(M\text{b}\) is attributed to adaptations associated with activity patterns (Watson et al., 2011), dominant locomotor mode (Dawson and Taylor, 1973; Fish et al., 2000, 2001; Griffin and Kram, 2000; Nudds et al., 2010), ecological niche (Bruinzeel et al., 1999), climate (Yousef et al., 1989; Maloiy et al., 2009) or having a protective shell (Baudinette et al., 2000; Zani and Kram, 2008). Ultimately, the reasons underlying the allometry of CoT\text{min} with \(M\text{b}\) and the factors that determine the CoT are not yet fully understood (Cavagna et al., 1977; Fedak et al., 1982; Heglund et al., 1982a,b; Heglund and Taylor, 1988; Kram and Taylor, 1990; Roberts et al., 1998; Pontzer, 2005, 2007a,b). Between disparate species, musculoskeletal morphology and shape vary with size (Schmidt-Nielsen, 1975, 1984; Biewener, 1989; Reilly et al., 2007), speed requirements (Garland, 1983), climate (Janis and Wilhelm, 1993), ecological niche (Bruinzeel et al., 1999) and locomotor mode (Griffin and Kram, 2000; Abourachid, 2001; Nudds et al., 2010). Within species or between closely related species, however, variation in shape is reduced, meaning insight can be gained into the factors that dictate the CoT and how it scales with \(M\text{b}\), independent of shape (Griffin et al., 2004; Day and Jayne, 2007; Langman et al., 2012). For example, miniature, Arabian and draft horses (Equus ferus caballus) showed no difference in CoT\text{min} when trotting, despite spanning 8- and 2-fold differences in \(M\text{b}\) and leg length, respectively (Griffin et al., 2004). Similarly, there was little
difference in walking $\text{CoT}_{\text{min}}$ within camels (Camelus dromedaries, $M_b=240–580$ kg) (Yousef et al., 1989; Maloiy et al., 2009) or donkeys (Equus asinus, $M_b=170–583$ kg) (Yousef et al., 1972; Maloiy et al., 2009), or between adult Asian elephants (Elephas maximus) and sub-adult African elephants ($M_b=1435–3545$ kg) (Langman et al., 1995, 2012). It is assumed that similarity in $\text{CoT}_{\text{min}}$ across individuals of differing body masses is due to their being geometrically, posturally and physiologically similar and locomoting with dynamically similar gaits (Griffin et al., 2004; Langman et al., 2012). Surprisingly, despite this explanation being widespread in the literature, there is no empirical evidence linking $\text{CoT}_{\text{min}}$ across a size range with similar limb kinematics and skeletal proportions for a walking gait (the only gait over which dynamic similarity can be investigated; Alexander and Jaynes, 1983). In humans, the only bipedal species to have been examined across a size range (children–adults), walking $\text{CoT}_{\text{min}}$ scaled in a similar manner to that found across species (i.e. $\alpha M_b^{1/3}$) (Weyand et al., 2010), which is contrary to findings from within quadruped investigations where $\text{CoT}_{\text{min}}$ varied across sizes. To fully understand these results, it is necessary to expand the available data for bipeds and to investigate the relationships between the $\text{CoT}_{\text{min}}, M_b$, limb kinematics and skeletal proportions.

Domestic leghorn chickens, Gallus gallus domesticus (Linnaeus 1758), are selectively bred for large and bantam (miniature) varieties, providing an opportunity to investigate how size influences $\text{CoT}_{\text{min}}$ independent of shape in an avian species. Rubenson et al. (2007) derived an interspecific scaling equation of walking $\text{CoT}_{\text{min}}$ against $M_b$ ($\text{CoT}_{\text{min}}=17.80\pm2.98 M_b^{0.471\pm0.032}$) using minimum measured values of the net cost of transport ($\text{CoT}_{\text{net}}$, the amount of energy required to move 1 kg over 1 m minus maintenance and postural costs) for a range of birds and mammals (0.29–1.542 kg). The aim of this study was to investigate whether large ($N=5$; mean$\pm$s.e.m. $M_b=1.92\pm0.13$ kg, range$=1.62–2.19$ kg) and bantam ($N=9$; $M_b=1.39\pm0.03$ kg, range$=1.29–1.54$ kg) leghorns would show a 16% difference in $\text{CoT}_{\text{min}}$ as predicted by the Rubenson et al. (2007) equation, and to compare their $\text{CoT}_{\text{net}}$ with that of animals of a similar $M_b$. Importantly, we simultaneously determined whether the two varieties of leghorn walked in a dynamically similar way and were geometrically and posturally similar to gain insight into the links between these integrated components of terrestrial locomotion.

RESULTS

Morphological measurements

Mean linear dimensions measured from large and bantam leghorns are presented in Table 1. The skeletal measurements of the bantams were, on average, ~83% of those of the larger variety. Predicted hindlimb dimensions (Table 1) for the bantams, based on the percentage difference in sternum length between the two varieties, all fell within the range predicted from the large variety data (mean$\pm$s.e.m.), indicating that the axial and appendicular skeletons of the two varieties were geometrically similar. Independent samples $t$-tests (equal variances assumed unless otherwise stated) showed that, represented as a proportion of total skeletal leg length ($l_{\text{skeletal}}=femur+tibiotarsus+tarsometatarsus$ lengths), the femur (0.28 in both varieties) was not significantly different in both varieties, but both tarsometatarsus lengths ($r=1.00$, d.f.$=4$, $P=0.374$). Similarly, the tibiotarsus ($r=0.07$, d.f.$=12$, $P=0.948$) and tarsometatarsus lengths ($r=–1.26$, d.f.$=12$, $P=0.233$) were the same proportion of total leg length in the two varieties (0.42 and 0.30, respectively). Femur width, as a proportion of femur length was also similar ($r=0.63$, d.f.$=12$, $P=0.128$) between the two varieties (0.11 and 0.10 in bantam and large leghorns, respectively). Similarly, the tibiotarsus width:length ratio (0.07 in both varieties) did not differ (equal variances not assumed: Levene’s test, $F=5.25$, $P=0.041$) between varieties ($r=0.07$, d.f.$=5.70$, $P=0.326$) and nor did the tarsometatarsus width:length ratio, which was 0.10 in both (d.f.$=12$, $P=1.00$). The two varieties therefore share similar hindlimb skeletal proportions.

The ratio of hip height to skeletal leg length, $h_{\text{hip}}/l_{\text{skeletal}}$ a measure of posture (Gatesy and Biewener, 1991), was on average ~5% greater in the bantam compared with the large variety (0.79$\pm$0.02 and 0.74$\pm$0.01, respectively), but was not statistically different between varieties ($r=1.96$, d.f.$=12$, $P=0.074$). The predicted $h_{\text{hip}}$ for the bantams (Table 1), however, fell outside of the range predicted from the large variety’s $h_{\text{hip}}$ data, being approximately 1 cm shorter than measured. Bantam $h_{\text{hip}}$ was 0.87 times that of the larger birds, which was a greater fraction than found for the skeletal element measurements. Therefore, the bantams adopted a more erect posture compared with the large variety.

Walking kinematics

Duty factor decreased linearly with speed ($U$, m s$^{-1}$) and neither the slope nor the intercept of this relationship differed between varieties (Fig. 1A, Table 2). Stride frequency ($f_{\text{stride}}$, Hz) increased at the

Table 1. Hindlimb segment measurements and sternal keel lengths from the birds used in experiments and geometric predictions for appendicular measurements of the bantams

| Length/width | Bantam (mm) | Large (mm) | Bantam prediction (mm)* |
|--------------|-------------|------------|------------------------|
| $l_{\text{kel}}$ | 90.00±1.30†  | 107.40±5.29 | 70.71–73.64‡  |
| $l_{\text{fem}}$ | 71.04±0.83  | 85.92±1.74  | 106.77–110.44  |
| $l_{\text{tib}}$ | 107.2±1.23  | 129.29±2.19 | 67.28–80.23   |
| $l_{\text{tars}}$ | 75.48±1.19  | 93.15±2.36  | 253.94–264.12  |
| $l_{\text{skeletal}}$ | 253.70±3.11 | 308.37±6.06 | 73.3–7.73     |
| $w_{\text{fem}}$ | 7.78±0.11  | 8.96±0.24  | 7.03–7.45     |
| $w_{\text{tib}}$ | 7.18±0.11  | 8.62±0.25  | 7.60–7.97     |
| $w_{\text{tars}}$ | 7.57±0.15  | 9.27±0.22  | 187.55–197.54 |
| $h_{\text{hip}}$ | 200.00±3   | 229.00±6   | 135.1–175.5   |

*Predicted value ranges for the bantams were calculated as (large linear dimension$\pm$s.e.m.$\times0.84$ based on the percentage difference in keel length between the varieties. Bold values represent geometric predictions that were not significantly different from observed bantam measurements.†$N=6$ for bantam sternum measurements.‡$h_{\text{hip}}$ measurements are given to the nearest millimetre because the measurements were made in metres to the nearest millimetre.
same rate with \( U \) in the two varieties, but was 0.37 Hz greater in the bantam variety across all \( U \) (Fig. 1B, Table 2). Similarly, the incremental increase in stride length (\( U_{\text{stride}} \)) with \( U \) was the same in the two size groups, whilst \( U_{\text{stance}} \) was longer by 0.09 m across all \( U \) in the large variety (Fig. 1C, Table 2). The duration of the swing phase of the limb (\( U_{\text{swing}} \)) decreased curvilinearly with \( U \) at the same rate in the two groups, but was 0.05 s longer in the large variety across all \( U \) (Fig. 1D, Table 2). Stance phase duration (\( U_{\text{stance}} \)) also decreased curvilinearly with \( U \) and at the same rate in the two size groups. \( U_{\text{stance}} \) was, however, 0.08 s longer in the large variety across all \( U \) (Fig. 1D, Table 2). Therefore, each parameter responded to increasing \( U \) in the same way in the two varieties and differences in their absolute values (related to size) were fixed across all speeds.

### Metabolic power and CoT

The positive relationship between mass-specific metabolic power (\( P_{\text{met}} \), W kg\(^{-1} \)) and walking \( U \) (Fig. 2A) was similar (both the slopes and intercepts) for the two varieties (Table 2). Calculating CoT\(_{\text{min}} \) as the slope of this relationship (slope method) therefore gives 16.20 J kg\(^{-1} \) m\(^{-1} \) in each variety. During quiet standing, resting metabolic rate (RMR, W kg\(^{-1} \)) did not differ (Fig. 2A, Table 2) between bantam and large leghorns (7.24±0.42 and 7.21±0.48 W kg\(^{-1} \), respectively), indicating that they shared the same mass-specific energetic cost of general maintenance and maintaining their posture combined. Therefore, the relationship between net mass-specific metabolic power (net-\( P_{\text{met}} \), W kg\(^{-1} \); the metabolic rate required for locomotion exceeding that required for standing quietly) and \( U \) (Fig. 2A) was also similar for the two size groups (Table 2).

Total cost of transport (CoT\(_{\text{tot}} \), J kg\(^{-1} \) m\(^{-1} \)) decreased curvilinearly with \( U \), indicating that the highest walking speeds of the birds were most metabolically optimal. CoT\(_{\text{tot}} \) (J kg\(^{-1} \) m\(^{-1} \); net-\( P_{\text{met}} / U \)), however, was not correlated with \( U \) and fell within a similar range for the two size groups (bantam: 9.44–16.10 J kg\(^{-1} \) m\(^{-1} \); large: 9.72–15.33 J kg\(^{-1} \) m\(^{-1} \)) (Fig. 2B, Table 2). Calculating CoT\(_{\text{min}} \) as the minimum measured CoT\(_{\text{net}} \) (subtraction method), taken as the mean of all CoT\(_{\text{net}} \) values across all speeds and both varieties, gives 13.04 J kg\(^{-1} \) m\(^{-1} \). Predicted walking CoT\(_{\text{min}} \) values for large and bantam leghorns based on Rubenson et al. (2007) were 13.09 and 15.24 J kg\(^{-1} \) m\(^{-1} \), respectively. Both varieties therefore shared a CoT\(_{\text{min}} \) closer to that predicted for the larger variety, contrary to the 16% difference predicted. This corresponds to the bantams having a CoT\(_{\text{min}} \) ~14% lower than predicted for their \( M_b \), which fell within the 95% CIs of Rubenson et al.’s (2007) equation. The net cost per stride (J kg\(^{-1} \) stride\(^{-1} \)) was lower in bantams than in the larger variety by 1.17 J kg\(^{-1} \) stride\(^{-1} \) across all speeds (Fig. 2C, Table 2).

## DISCUSSION

Across species, CoT\(_{\text{min}} \) is reported to scale hypoallometrically with \( M_b \) (Taylor et al., 1970, 1982; Fedak et al., 1974; Kram and Taylor, 1990; Full and Tu, 1991; Langman et al., 1995; Roberts et al., 1998). However, we found that bantam and large varieties of leghorn chickens have identical CoT\(_{\text{min}} \) despite the smallest and largest individuals differing 1.7-fold in \( M_b \) and 1.35-fold in leg length. An independence of CoT\(_{\text{min}} \) from body size was previously reported within large quadrupedal species (>90 kg) spanning 1.5- to 8-fold ranges in \( M_b \) and up to 2-fold ranges in leg length (Griffin et al., 2004; Maloiy et al., 2009; Langman et al., 2012). The present data represent the first evidence of a lack of correlation between \( M_b \) and CoT\(_{\text{min}} \) within an avian species. No effect of \( M_b \) or leg length suggests that size itself does not influence the CoT but, rather, some other factor, perhaps correlated with body size, may be responsible.

The simultaneous collection of kinematics and morphological data here allow us to investigate further previous hypotheses on what is driving the interspecific CoT\(_{\text{min}} \) versus \( M_b \) relationship. Larger species perform the same amount of mass-specific mechanical work as smaller species, whilst using less mass-specific metabolic energy during terrestrial locomotion (Fedak et al., 1982; Heglund et al., 1982a,b; Alexander, 2005). How this is possible is not fully understood. It is generally accepted that \( M_b \) has no independent influence over CoT (Pontzer, 2005, 2007a,b). Leg length, however, is often discussed as the morphological factor explaining the allometry of CoT\(_{\text{min}} \) (Kram and Taylor, 1990; Schmidt, 1984; Biewener, 2003; Alexander, 2003) as longer legs allow longer \( U_{\text{stance}} \) for the muscles to apply force through recruiting slower, less metabolically expensive muscle fibres (metabolic rate is inversely proportional to \( U_{\text{stance}} \) during which the muscles apply force) (Kram and Taylor, 1990). In addition, longer limbs allow lower \( f_{\text{stride}} \) requiring fewer muscle contractions. In the present study, however, the different sized birds shared the same mass-specific CoT\(_{\text{min}} \) despite the bantams having shorter limbs, shorter \( U_{\text{stance}} \) and higher \( f_{\text{stride}} \) compared with the larger variety. Using the maximum height of the limb as a strut (effective limb length, \( h_{\text{hip}} \)) as the indicator of size
has been shown to better predict CoTmin across species (h_{hip}, r^2=0.98) than using the sum of the skeletal element lengths (s_{skel}, r^2=0.78) (Steudel and Beattie, 1995; Pontzer, 2007a). Over a small size scale of analysis, however, it has been demonstrated that between-individual differences in limb arrangement (e.g. limb excursion angle), the cost of swinging the limb and the coefficient of converting metabolic energy into muscle force ‘k’ (which were not measured in this study) prevent a clear relationship between h_{hip} and CoTmin (Pontzer, 2005, 2007b). In agreement with Pontzer’s (2005, 2007b) findings, despite the greater absolute h_{hip} of the larger variety, compared with the bantams, they did not have a lower CoTmin. It may be that variation in limb excursion angle (i.e. the difference in posture), rather than h_{hip}, dominated variation in CoTmin. Indeed, by using a model to predict the rate of force production associated with both supporting body weight and swinging the limb as a function of all of these parameters, Pontzer (2007a) found this was a better predictor of metabolic rate than contact time, limb length or M_{b} at both interspecific and intraspecific levels. Equally, the shared CoTmin of the two varieties may be due to their identical appendicular and axial skeletal geometry, consistent with previous assumptions in intraspecific analyses (Langman et al., 2012).

Another potential explanatory factor is limb posture (linked to effective limb length). Across vertebrates, the limb bone lengths scale positively and almost geometrically with M_{b} but become increasingly more aligned with one another and less crouched (Biewener, 1989). A prominent step-change exists in the scaling of both CoTmin and the mechanical cost of transport (E_{mech}, J kg^{-1} m^{-1}) across species associated with crouched postures in those <1 kg and upright postures in those >1 kg, making their efficiency of transport (CoTmin/E_{mech}) approximately 7% and 26%, respectively (Reilly et al., 2007; Nudds et al., 2009). Unlike larger species with a more upright posture, small crouched-postured (non-cursorial) species do not benefit from elastic energy savings or pendular mechanisms (Reilly et al., 2007). Furthermore, a more vertical limb decreases the muscular force required to support a unit of body weight and improves the mechanical advantage of the muscles (Biewener, 1989). The change in posture with increasing size means that muscle stress is nearly independent of M_{b} (rather than M_{b}^{1/3}). Griffin et al. (2004) suggested that between closely related individuals, consistent limb posture might account for consistent CoTmin across a range of body sizes as muscle stress would in this case scale geometrically (αM_{b}^{1/3}). The volume of active muscle would therefore increase with size and counter any metabolic savings associated with having longer legs (Griffin et al., 2004). However, in the present study the shared CoTmin of the chicken groups did not correspond to a similar posture. When comparing the posture of the two size groups as h_{hip}/s_{skel}, the limbs...
Previous research suggests a relationship between the mass-specific energetic parameters and walking speed. The data points and best-fit lines are as shown in Fig. 1. The lines of best fit are (A) metabolic power, $P_{\text{met}} = 16.20U + 6.93$ (bantam) and $16.20U + 5.86$ (large); and net metabolic power, $net-P_{\text{met}} = 16.00U - 0.88$ (bantam) and $16.00U - 1.26$ (large); (B) total cost of transport, $CoT_{\text{tot}} = 22.39U^{-0.50}$ (bantam) and $19.95U^{-0.50}$ (large); and net cost of transport, $CoT_{\text{net}} = 4.77U + 11.89$ (bantam) and $4.77U + 10.53$ (large); and (C) net cost per stride = $7.10U + 2.42$ (bantam) and $21.21U + 0.24$ (large). Mass-specific resting (standing) metabolic rates are also included in A at 0 m s$^{-1}$. Data points are means ± s.e.m.

The lower than predicted $CoT_{\text{min}}$ in the bantams was also associated with lower mass-specific energy requirements per stride, compared with the larger variety. All birds shared a $CoT_{\text{min}}$ closer to that predicted for the larger variety and the $CoT_{\text{min}}$ of the bantams was approximately 14% lower than predicted from their $M_b$. Our findings indicate that with $U$, the two varieties shared identical rates of change in all parameters, which would be expected to imply geometric, postural and dynamic similarity. Each kinematic parameter differed between the two varieties only by a fixed value across all speeds. The larger variety took longer strides by 9 cm, took less frequent strides by 0.37 Hz and had longer durations of both swing and stance phases of the limb by 0.05 and 0.08 s, respectively. At a given absolute $U$, duty factor is generally higher in larger species than in smaller ones (Gatesy and Biewener, 1991); however, the duty factors of the chickens were not significantly different between size groups. Similarly, a selection of felid species spanning a 46-fold range in $M_b$ were found to use similar duty factors at a similar walking speed (Day and Jayne, 2007). For what was previously an expectation (Griffin et al., 2004; Maloiy et al., 2009; Langman et al., 2012), the present data offer the first empirical evidence of a link between identical walking $CoT_{\text{min}}$ in individuals of differing size and similar limb dynamics and skeletal geometry. We can speculate that for a given skeletal shape, regardless of $M_b$, walking $CoT_{\text{min}}$ may be consistent. Some additional studies in which shape was controlled for also support this idea. For example, adding back loads up to 50% of $M_b$ has a negligible effect on the $CoT$ in quadrupedal rats, dogs and horses as well as bipedal humans, guinea fowl and other birds (Taylor et al., 1980; Ellerby and Marsh, 2006; Tickle et al., 2010, 2013). Furthermore, obese and thin humans of the same height (likely to be similar in skeletal proportions) show no difference in $CoT_{\text{min}}$ (Browning et al., 2006).

In contrast to our findings, a comprehensive study of 48 humans spanning a 6-fold range in $M_b$ and 1.5-fold range in height concluded that $CoT_{\text{min}}$ was $\alpha M_b^{-1/3}$ (Weyand et al., 2010). This result, however, may be associated with ontogenetic differences in shape, because the human subjects ranged from 5 to 32 years of age and the data were intentionally separated into four size groups to reduce individual variability (Weyand et al., 2010). Indeed, dividing the $CoT$ by body height accounted for the observed differences between the human size groups. Therefore, at any given speed, all subjects incurred the same $CoT$ to cover the same horizontal distance relative to their own body height (Weyand et al., 2010). Small (2 g) ghost crabs ($Ocypode quadrata$), one of the few invertebrate species examined, were found to have a higher $CoT$ than larger ones (47 g), despite their similar appearance in shape (Tullis and Andrus, 2011). In the absence of detailed kinematic and morphometric measurements, however, it is not possible to conclude much from this result. It is, of course, possible that the link we found here between energetics, kinematics and skeletal morphometrics may not be characteristic of species with more than two legs.

**Conclusions**

Leghorn chickens selectively bred for large and bantam varieties shared the same walking $CoT_{\text{min}}$ despite a 1.70-fold difference in $M_b$ and 1.35-fold difference in total leg length between the smallest and largest individuals. These data represent the first evidence of $CoT_{\text{min}}$ being independent of $M_b$ within a small crouched-postured bipedal species. Our findings also provide the first evidence (for what was previously only assumed) of a link between this and similar walking dynamics and skeletal geometry. In contrast to interspecific trends, however, $h_{hip}$ did not scale geometrically between varieties and represented a greater proportion of total leg length in the bantam variety compared with the large variety. All birds shared a $CoT_{\text{min}}$ closer to that predicted for the larger variety and the $CoT_{\text{min}}$ of the bantams was approximately 14% lower than predicted from their $M_b$. Our findings are therefore in agreement with the general consensus that for a given body size, $CoT_{\text{min}}$ decreases with limb erectness. The lower than predicted $CoT_{\text{min}}$ in the bantams was also associated with lower mass-specific energy requirements per stride, compared with the larger variety, which may be linked to differences in their posture and/or their derived muscle morphology/physiology. We emphasise the importance of intraspecific in addition to interspecific investigations as well as the combination of kinematics, morphometric and posture measurements towards gaining insight into the factors that dictate $CoT$.

**MATERIALS AND METHODS**

**Study species**

Adult (>16 week) male bantam ($N=9$; mean ± s.e.m. $M_b=1.39\pm0.03$ kg) and large ($N=5$; $M_b=1.92\pm0.13$ kg) leghorn chickens were purchased from a local.
breeder and housed in the University of Manchester’s animal unit. All housing was provided ad libitum, and the birds were not fasted prior to experiments. Birds were trained for 1 week to locomote on a motorised treadmill (T60 Tunturi³, Finland) prior to data collection. All experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986, were approved by the University of Manchester Ethics Committee and performed under a UK Home Office Project Licence held by J.R.C. (40/3549).

Respirometry

An open flow respirometry system (all equipment Sable Systems International⁴, Las Vegas, NV, USA) was used to measure the birds’ rates of oxygen consumption (\(\dot{V}_{\text{O}_2}\) ml min⁻¹) and carbon dioxide production (\(\dot{V}_{\text{CO}_2}\) ml min⁻¹). Perspex® respirometry chambers were built (bantam: 66×46.5×48 cm, large: 97.5×53.5×48 cm) and mounted upon the treadmill. Air was pulled through the chambers using a FlowKit 500 at flow rates (FR) of 150 l min⁻¹ (bantam) and 250 l min⁻¹ (large). Excurrent airflow was sub-sampled (0.11 l min⁻¹) for gas analysis. Water vapour pressure (WVP) was measured using an RH-300 water vapour analyser (Oxilla II). Ambient air (scrubbed of granular, Sigma-Aldrich, Steinheim, Germany) and passed through a CO₂ analyser (Oxilla II) prior to data collection. All experiments were carried out in accordance with the Animals (Scientific Procedures) Act 1986, were approved by the University of Manchester Ethics Committee and performed under a UK Home Office Project Licence held by J.R.C. (40/3549).

Statistical analyses

The slopes and the intercepts of the relationships between the dependent variables (metabolic or kinematics measures) and \(U\) were investigated for differences between chicken varieties using general linear models (GLMs). Models included variety as a fixed factor, \(U\) as a covariate and the interaction term variety × \(U\). If the interaction term was non-significant (indicating similar slopes between varieties), it was removed from the model and the updated model was re-run (assuming parallel lines) in order to test for differences in intercepts. Where the relationship between a dependent variable and \(U\) was curvilinear, the data were log₁₀ transformed. All best-fit lines were taken from coefficients tables produced by the GLMs. Between-variety differences in hindlimb skeletal element proportions (% total leg length) were investigated using independent samples t-tests. Hindlimb proportion data were tested for equality of variance using a Levene’s test for equality of variance.

Acknowledgements

We would like to thank John Lees and Karlina Ozolina for their assistance with respirometry data collection.

Competing interests

The authors declare no competing or financial interests.

Author contributions

The study was conceived and designed by J.R.C. and R.L.N. K.A.R. executed the study. Data were interpreted and analysed by K.A.R. with assistance from R.L.N. and J.R.C. K.A.R., R.L.N. and J.R.C. drafted and revised the manuscript.

Funding

This research was supported through funding provided by the Biotechnology and Biological Sciences Research Council (BBSRC: G011138/1 and 00221116/1 to J.R.C.), K.A.R. was supported by a Natural Environment Research Council (NERC) doctoral training account (DTA) PhD stipend and Collaborative Awards in Science and Engineering (CASE) partnership with The Manchester Museum. Deposited in PMC for immediate release.

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