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The energetic costs of trunk and distal-limb loading during walking and running in guinea fowl *Numida meleagris*

II. Muscle energy use as indicated by blood flow

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Summary

We examined the changes in muscle energy use in guinea fowl running at 1.5 m s^{-1} either unloaded, or carrying trunk loads equal to 23% of body mass, or loads on their distal legs equal to a total of 5% of body mass. We estimated muscle energy use by measuring blood flow to all of the leg muscles using injected microspheres. Total blood flow to the leg muscles increased by approximately 15% under both loading conditions, which matched the percentage increase in net organismal metabolic rate. Significant increases in energy use (inferred from blood flow) above that found in unloaded birds were found in 12 muscles in trunk-loaded birds, with most of the increases restricted to stance-phase muscles, as predicted. Just three of these muscles, the femerotibialis, the iliotibialis lateralis pars postacetabularis and the fibularis longus accounted for 70% of the increased energy use. Noticeably absent from the group of muscles that increased energy use during trunk loading were several large biarticular muscles that have extensor actions at the hip or ankle, but flexor actions at the knee. We concluded that the low energetic cost of carrying trunk loads in guinea fowl may rely on the activation of a group of muscles that together provide support and propulsion across all the major joints, without producing opposing moments at other joints that could potentially waste energy. The specific leg muscles responsible for the increase in metabolism during trunk loading also suggest that the energy cost of producing mechanical work may be an important determinant of the cost of carrying extra mass on the trunk. During distal-limb loading, eleven leg muscles had significant increases in energy use, but unlike during trunk loading, both stance- and swing-phase muscles had large increases in energy use. This distribution of energy use between stance and swing agrees with the prediction that increased mechanical work determines the cost of limb loading, because a substantial fraction of the increased segmental work during distal-limb loading in guinea fowl has been found to occur during stance.

Key words: guinea fowl, *Numida meleagris*, locomotion, backpack loading, blood flow, oxygen consumption, energy use, load carrying.

Introduction

Surprisingly little direct information is available on the mechanical function of the diverse array of muscles active during running, and on the division of energy use among the variety of mechanical tasks they perform. This gap in our knowledge is a consequence of the complexity of the musculoskeletal system, and the difficulty of measuring the energy expenditure of individual muscles. One approach to this problem has been to apply loads to running animals and measure the resulting change in overall energy expenditure by respirometry. For example, additional mass applied to the trunk has been used to influence energy use by stance-phase muscles (Taylor et al., 1980), whereas mass applied to the distal limbs has been used to influence swing-phase cost (Martin, 1985). Other experiments have applied aiding or retarding forces in the horizontal direction to influence the work done in propulsion (Chang and Kram, 1999), or applied forces to aid accelerating the limbs (Modica and Kram, 2005).

Although valuable information can be gleaned from these experiments, deducing muscle function from the results requires indirect inferences, sometimes with numerous assumptions, as explained in the accompanying paper (Marsh et al., 2006). Carrying loads attached to the trunk should influence stance-phase costs without influencing the cost of swing phase, as long as the duty factor does not change very much. The results of these experiments have revealed a diversity of values for load-carrying economy (Marsh et al., 2006). The reasons for the different costs of carrying additional mass on the trunk are not clear. Previous suggestions that the ratio of loaded to unloaded energy cost can reveal the relative

cost of stance and swing (Taylor et al., 1980) are probably not tenable (Marsh et al., 2006). The increase in energy use occurring when the distal limbs are loaded is related presumably to increases in energy use by muscles that must do extra work to move the loaded segment (Martin, 1985; Steudel, 1990; Marsh et al., 2006), but again, direct evidence regarding energy changes at the muscle level is not available. Measuring organismal energy use provides a global picture of the costs of load carrying, but what is needed to fully understand these costs are measures of energy use at the level of individual muscles.

The best available way to simultaneously measure the energy use to all the individual muscles is to measure blood flow to the muscles using microspheres injected into the systemic arterial circulation. This technique is supported by the excellent correlation shown in multiple studies between muscle blood flow and energy use (Marsh and Ellerby, 2006). By using sequential injections of different colored microspheres, this technique is capable of measuring energy use on a muscle-by-muscle basis in the same bird under different exercise conditions. This approach was previously used to determine the energy expenditure of leg muscles in unloaded guinea fowl across a large range of walking and running speeds (Marsh et al., 2004; Ellerby et al., 2005).

In the present paper, we extend the blood-flow technique to examine the alterations in muscle energy use resulting from trunk and distal-limb loading in guinea fowl *Numida meleagris*. Guinea fowl carry trunk loads more economically than do quadrupeds, and more economically than do the large majority of human subjects tested (Marsh et al., 2006). Recent data indicate that the cost of swing phase in human running is probably similar to that found in guinea fowl (Modica and Kram, 2005); thus, the differences in load-carrying economy cannot be due to differences in the relative cost of swing and stance in humans and guinea fowl. Previous inferences about the underlying causes of load-carrying economy were based on assumptions about the distribution of energy use among the stance-phase muscles (Griffin et al., 2003). The present study avoids these assumptions by measuring the distribution of energy use. Alterations in the pattern of energy use among the individual stance-phase muscles may provide some hints as to why the increase in energy use due to trunk loading is smaller than expected from other studies.

Marsh et al. also found a substantial increase in organismal energy cost due to adding mass to the tarsometatarsal segment (Marsh et al., 2006). This increase in energy cost was correlated with an increase in the mechanical work done on the loaded segment, with maximum delta efficiencies of 25%. Despite the goal of the distal-limb loading study (Marsh et al., 2006), which was to alter swing-phase cost specifically, this loading study revealed that approximately 40% of the increase in mechanical energy in the loaded state occurred in late stance during limb extension. Thus, if the increase in metabolic energy use resulting from distal-limb loading results mainly from the requirement for increased mechanical work to move the loaded segment, we predict that energy use should substantially increase in stance-phase muscles as well as in swing-phase muscles.

Materials and methods

Animals and training regime

Guinea fowl *Numida meleagris* L. were obtained from The Guinea Farm (New Vienna, IA, USA) as hatchlings and cage-reared at the Northeastern University Division of Laboratory Medicine. At the time of the measurements the birds were between 10 and 14 months old. Birds had *ad libitum* access to food and water and were maintained on a 12 h:12 h light dark cycle. Body mass was 1.50±0.02 kg (mean ± s.e.m., N=6, range 1.44–1.56 kg, 3 females, 3 males). Three of the birds were also used to collect data reported in the accompanying paper (Marsh et al., 2006). All birds were endurance-trained as described (Marsh et al., 2006) and could sustain 30 min of treadmill exercise at 2.5 m s^{-1}. All procedures involving animals were approved by the Institutional Animal Care and Use Committee.

Loading methods and oxygen consumption measurements

The methods of trunk and limb loading were the same as those used in the accompanying paper (Marsh et al., 2006). Briefly, the trunk loads averaged 23% of body mass and consisted of a canvas backpack and lead weight, which was positioned approximately above the bird's center of mass. Distal-limb loads weighing a total of approximately 5% of body mass consisted of strips of lead positioned distally on the tarsometatarsus.

The rate of oxygen consumption ($\dot{V}_{O_2}$) was measured using an open respirometry system. Respired gases were collected using a lightweight plastic mask. Details of the respirometry setup are given elsewhere (Ellerby et al., 2003). Three of the birds formed part of the accompanying study to determine metabolic rate during load carrying across a range of speeds (Marsh et al., 2006). The aim of the present set of experiments was to determine changes in blood flow with loading at a single running speed (1.5 m s^{-1}). For this reason measurements of $\dot{V}_{O_2}$ for the additional three birds used in the present study focused on this speed, and rest. Resting values were determined with the birds sitting quietly in a darkened box for approximately 10 min. The running protocol involved alternating between 1.5 m s^{-1} and 0.5 m s^{-1} at 2-min intervals under the three loading conditions (unloaded, limb-loaded and trunk-loaded). This alternation of speeds was replicated in the blood-flow experiments. The duration of these intervals had previously been determined to be sufficient to allow heart rate and $\dot{V}_{O_2}$ to stabilize at a given speed (Ellerby et al., 2005). This approach yielded comparable $\dot{V}_{O_2}$ measurements at 1.5 m s^{-1} to those obtained as part of a wider speed range in the earlier set of experiments (Marsh et al., 2006).

Blood-flow measurements

Details of the surgical procedures, cannula construction and microsphere injection procedures were as previously described (Marsh et al., 2004; Ellerby et al., 2005). The blood-flow measurements required a ventricular injection cannula inserted into the left ventricle, and an arterial cannula, which was placed in the brachiocephalic artery, for withdrawal of

reference blood samples. Custom-made polyurethane cannulae were inserted into the left and right brachial arteries, respectively, under isoflurane anesthesia. The birds were allowed to recover overnight post surgery.

Prior to determining resting blood flow, the bird was fitted with the canvas backpack with no weight attached and was left in a darkened box for 10 min. The backpack itself added only 2% to the mass of the bird. In the box, the birds sat quietly with their legs folded under themselves. At the end of this period, injections for measuring resting flow were made. The birds were then removed from the box and performed the following locomotor sequence before the experimental runs were started: walking at 0.5 m s^{-1}, running for 2 min at 1.5 m s^{-1}, and approximately 2 min of walking at 0.5 m s^{-1}. Following this initial exercise the experimental sequence of blood flow measurements was as follows: 1.5 m s^{-1} unloaded, 1.5 m s^{-1} with the trunk load, and 1.5 m s^{-1} with the distal limb loads. The bird maintained each test speed for 2–3 min prior to the injection of microspheres. In between these experimental runs, the birds walked at 0.5 m s^{-1} for approximately 2 min. The trunk load was applied while the bird walked at this speed. Applying the limb loads necessitated removing the bird from the treadmill, removing the trunk load, and applying the weights to the tarsometatarsus.

During the experimental runs the microspheres were injected after 2 min of steady running. Approximately 10 s prior to the injection of microspheres, we began the reference blood withdrawal at a rate of 1.75 ml min^{-1}. The microsphere injections, made *via* the ventricular cannula, contained approximately 1.5×10^6 microspheres (Triton Dye-trak VII+, Triton Technologies, CA, USA). The injections were made through a Luer port of a sterile 3-way stopcock. A pressure transducer was connected to a second Luer port to measure ventricular pressure at all times except during the injections. The microspheres were introduced as a bolus over a 10–20 s period. Immediately following the injection, the injection cannula was flushed with sterile saline to ensure that all the microspheres had been injected into the ventricle. The reference withdrawal was continued for sufficient time after flushing the injection cannula to clear all blood that might contain microspheres from the withdrawal cannula. The injection stopcock was replaced after each injection. Residual spheres in the injection syringes and injection stopcocks were recovered subsequently to determine the actual number of spheres injected. Hemoglobin and lactate values were measured on samples of blood collected at the end of the blood withdrawals and, as expected from previous experiments (Ellerby et al., 2005), these values did not change with the successive exercise bouts (data not reported here).

The tissue flow rate (Q_t) in ml min^{-1} was calculated using the following equation:

$$Q_t = \frac{Q_b N_t}{N_b},$$

where Q_b is the reference blood withdrawal rate in ml min^{-1}, N_t is the number of spheres in the tissue sample and N_b the number of spheres in the reference blood sample. The number of spheres collected in the withdrawal sample was also used to calculate cardiac output (Q_{CO}) according to:

$$Q_{CO} = \frac{Q_b N_i}{N_b},$$

where N_i is the number of spheres injected.

After completion of microsphere injections, the animals were euthanized by overdose of pentobarbital solution and all but several very small muscles from one leg were dissected out and weighed. The muscle samples analyzed were those described previously (Ellerby et al., 2005) with the following differences. (1) The iliofibularis (IF) was divided into anterior and posterior portions, representing the primarily swing and stance-phase compartments of the muscle, respectively. This division started proximally at the point at which the nerve enters the muscle and splits into anterior and posterior branches that innervate the anterior and posterior portions of the muscle (T. A. Hoogendyk, personal communication). (2) In the previous study (Ellerby et al., 2005) all of the digital flexors were analyzed as one group. In the present study, we analyzed three of the digital flexors individually, the superficial flexors of digits II and III (flexor perforans et perforatus digiti II and III (sDF-II and sDF-III respectively), and the flexor digitorum longus (FDL). The remaining digital flexors were processed as a group and designated mixed digital flexors (mixDFs); this group consisted of the deep flexors of digits II and III (perforatus digiti II and III), the flexor of digit IV, the plantaris and the flexor hallucis longus). (3) The only digital extensor removed was the extensor digitorum longus (EDL), which resides in the shank. The other digital extensors are in the tarsometatarsal segment and are extremely small. Selected muscles from the contralateral limb were also taken as a check that the microspheres were adequately mixed in the ventricle and distributed evenly throughout the circulatory system. The heart and samples of the flight muscles were also removed for analysis. The brain and most of the abdominal organs were also removed as detailed previously (Ellerby et al., 2005), but the detailed results by tissue are not reported for this study.

Tissue samples were placed in centrifuge tubes for processing along with a known amount of a color (navy) of microspheres not injected into the animal. The navy spheres acted as a control to correct for the loss of any microspheres during processing. Final sphere amounts were referenced to an unprocessed control tube containing an identical amount of navy spheres and scaled accordingly. Typically, 80% or more of the microspheres were successfully recovered. Following extraction of spheres from the tissues, the mixture of dyes recovered was quantified using a Ultrospec 3300pro (Amersham, Piscataway, NJ, USA) scanning spectrophotometer. The numbers of spheres of each color in the sample was calculated from the absorbance profiles of pure colors using a matrix inversion procedure and corrected for percent recovery. Details of the digestion, sphere recovery, and

calculations are given in the online supplement previously published (Marsh et al., 2004) (http:// www.sciencemag.org/ cgi/content/full/303/5654/80/DC1).

Statistical analyses

Statistical comparisons were done using ANOVA as implemented in the General Linear Model in SPSS (Macintosh version 11). When measuring blood flow with the microsphere technique there is significant variation among the animals tested (Marsh et al., 2004; Ellerby et al., 2005). Measurement errors in all of the values for a given exercise condition in an individual animal are correlated because these values are calculated using a single reference blood withdrawal sample, which is subject to random errors. Therefore, a code for the individual animal was entered as a factor into the model in addition to exercise condition. This procedure allowed us to remove the inter-individual variation in flow and test for the effects of loading.

Our experiment was designed to test for significant differences between unloaded and loaded values of blood flow when the birds ran at 1.5 m s^{-1}. Therefore, the majority of comparisons were done using a multivariate ANOVA model including animal and exercise condition as factors, and not including the resting values of flow. The variances among the loaded and unloaded conditions were similar and parametric statistics were utilized. The unloaded condition was treated as the control, and blood flows during both loading conditions were compared to the control value using two different procedures. (The analyses presented here used total blood flow, but none of the results were altered if net blood flow above rest was used in the model.) First, the experimental design called for *a priori* linear contrasts that tested for significant differences between each loading condition and the unloaded control. Second, we ran the *post-hoc* Dunnett's *t*-test, which also compares each experimental group to the control. The Dunnett *t*-test has a lower probability of Type II errors, i.e. finding a significant difference where none exists. We also ran an ANOVA model including the resting values to compare the total flow to the non-exercise related organs among all groups, using the *post-hoc* Scheffé procedure.

Mean values for the exercise conditions are presented ± s.e.m., as calculated from the ANOVA model with both loading condition and animal as factors.

Results

Effects of loading on metabolic rate, cardiac output, and summed blood flows

Rate of oxygen consumption ($\dot{V}_{O_2}$) was 17.7±1.1 ml min^{-1} at rest and increased to 83.8 ml min^{-1} when the birds ran unloaded at 1.5 m s^{-1}. When compared with this unloaded control value, $\dot{V}_{O_2}$ was significantly increased by both trunk and limb loading (ANOVA contrasts, $P<0.001$ and $P=0.002$, respectively) (Fig. 1A). The net metabolic rate (gross metabolic rate – resting rate) during trunk and distal-limb loading increased by 16% and 15%, respectively, above the unloaded control. Loading condition also had a significant effect on cardiac output for both trunk and limb loading (ANOVA, $P<0.001$ and $P=0.006$, respectively) (Fig. 1B).

Blood flow to tissues not involved in exercise metabolism decreased by a small but significant amount when the comparison was done between either loaded condition and the unloaded control. The summed flow to the brain and abdominal organs decreased by approximately 20 ml min^{-1} during both trunk and limb loading (ANOVA, $P=0.024$ and 0.007, respectively) (Fig. 1C). If the comparisons are done including the resting condition in the ANOVA

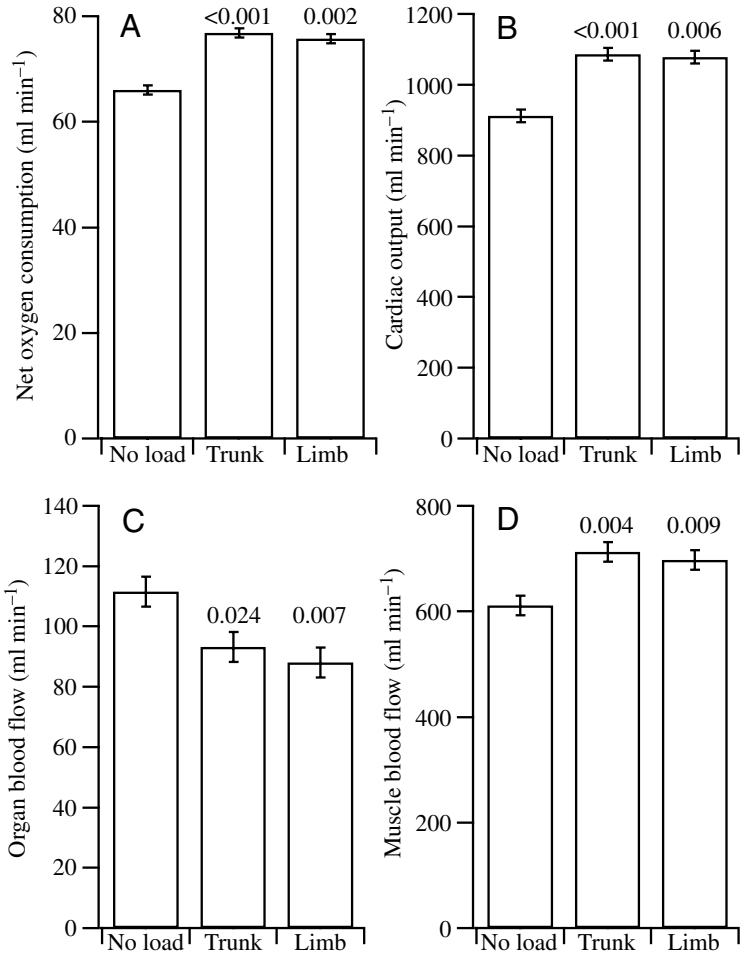

Fig. 1. Organismal oxygen consumption and blood flows in guinea fowl during unloaded, trunk-loaded and distal-limb-loaded running at 1.5 m s^{-1}. (A) Net oxygen consumption, calculated as the mean values during running minus the mean resting value. (B) Cardiac output measured by dilution of the injected microspheres. (C) Summed flow to the brain and abdominal organs. (D) Summed blood flow to all of the leg muscles. Values are means ± s.e.m. ($N=6$). *P* values are indicated above each bar.

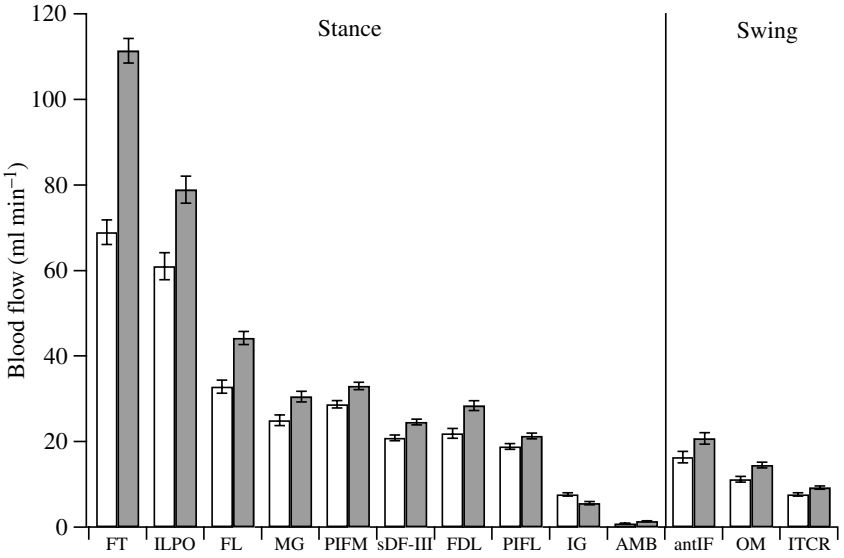

Fig. 2. Blood flow (mean ± s.e.m., *N*=6) to the muscles that showed significant changes in flow when guinea fowl carried a load on their backs equal to 23% of body mass. Open bars, control values for unloaded running at 1.5 m s^{-1}; shaded bars, the loaded values. Muscles are grouped into those active during swing and stance as indicated by EMG activity, previously measured during unloaded running. The FT is grouped with the stance muscles under the assumption that all of the increase in flow due to a trunk load is due to stance-phase metabolic activity (see text). Abbreviations are defined in Table 1.

model, the organs flows did not differ among the experimental groups when compared using Scheffé's *post-hoc* tests. The inability to detect significant changes in organ blood flow with the resting values in the ANOVA model related in part to the variability in the resting values of blood flow to the organs. Resting organ flow ranged from 55–270 ml min^{-1} among the various birds. The sum of the flows to the flight muscles (pectoralis and supracoracoideus) declined by approximately 10 ml min^{-1} (ANOVA, $P<0.004$) from the unloaded control condition to either loaded conditions.

As expected, blood flow increased significantly to the heart (difference, ~18 ml min^{-1}, $P<0.03$) and the leg muscles. When the flow to all the leg muscles was summed, the total leg muscle flow increased by 17% and 14% above the control values during trunk and limb loading respectively. The increase was significant for both trunk and limb loading (ANOVA, $P=0.004$ and 0.009, respectively) (Fig. 1D).

Effects of loading on blood flow to individual leg muscles

When the birds carried loads on their backs, 12 muscles showed statistically significant increases in blood flow compared to the unloaded control values based on ANOVA using linear contrasts (Table 1, Fig. 2). One muscle, the gastrocnemius intermedia (IG), had a significant decrease in flow (Fig. 2). The more conservative Dunnett *t*-test confirmed the statistical significance of the change in flow to all of these muscles except for the anterior portion of the iliofibularis (antIF), a swing-phase muscle, and the puboischiofemoralis lateralis (PIFL). Summing the significant differences in the muscles responding to trunk loading accounted for all of the overall difference in leg muscle blood flow, and 90% of the

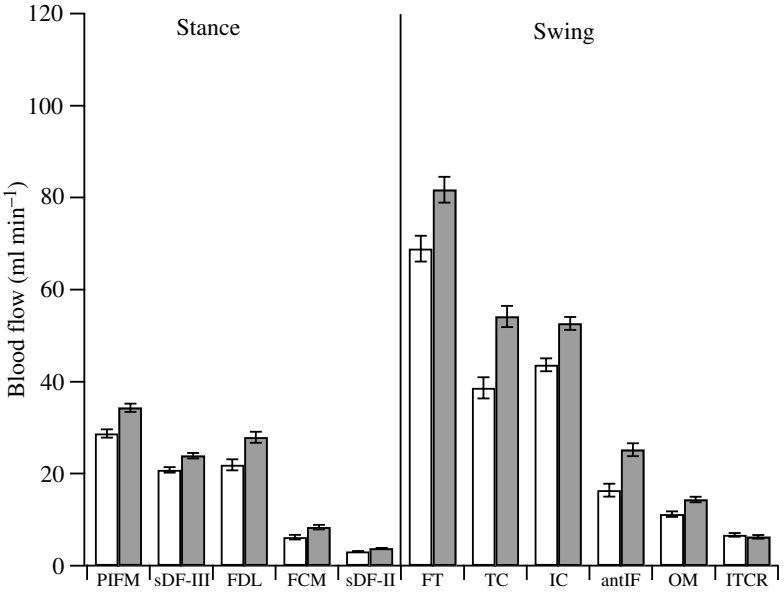

Fig. 3. Blood flow (mean ± s.e.m., *N*=6) to the muscles that showed significant changes in flow when guinea fowl ran with distal limb loads totaling approximately 5% of body mass. Open bars, control values for unloaded running at 1.5 m s^{-1}; shaded bars, the loaded values. Muscles are grouped into those active during swing and stance as indicated by EMG activity, previously measured during unloaded running. The FT is grouped with the swing-phase muscles under the assumption that all of the increase in flow due to a load on the distal limb is due to swing-phase metabolic activity (see text). Abbreviations are defined in Table 1.

Table 1. *Muscle masses and blood flows for the leg muscles of guinea fowl*

				Blood flow (ml min^{-1})						
					Run				Load *vs* No load	
Phase	Muscle	Abbreviation	Mass (g)	Rest	No load	Trunk load	Limb load	s.e.*	*P*, Trunk	*P*, Limb
Stance	Ambiens	AMB	1.47±0.08	0.25	0.89	**1.43**	0.81	0.07	**<0.001**	0.407
	Caudofemoralis pars caudalis	CFC	2.90±0.26	0.28	1.40	1.55	1.24	0.13	0.412	0.382
	Caudofemoralis pars pelvica	CFP	4.27±0.79	0.90	4.73	5.07	4.21	0.32	0.462	0.271
	M. flexor perforans et perforatus digiti II†	sDF-II	1.97±0.06	0.34	3.12	3.34	**3.77**	0.10	0.143	**0.001**
	M. flexor perforans et perforatus digiti III†	sDF-III	6.59±0.29	1.43	20.85	**24.63**	**23.91**	0.65	**0.002**	**0.007**
	Flexor digitorum longus‡	FDL	8.12±0.19	1.62	21.91	**28.43**	**27.94**	1.15	**0.003**	**0.004**
	Mixed digital flexors§	mixDFs	17.41±0.59	4.40	46.44	46.87	52.71	2.62	0.909	0.122
	Flexor cruris lateralis pars accessoria	FCLA	5.44±0.3	0.70	4.14	3.58	3.15	0.48	0.429	0.179
	Flexor cruris lateralis pars pelvica	FCLP	28.57±1.26	4.88	35.59	30.39	35.96	2.14	0.117	0.904
	Flexor cruris medialis	FCM	2.74±0.11	0.99	6.18	4.86	**8.46**	0.46	0.070	**0.006**
	Fibularis longus	FL	15.8±0.57	3.96	32.83	**44.23**	37.45	1.55	**<0.001**	0.061
	Gastrocnemius intermedia	IG	4.28±0.51	1.54	7.64	**5.68**	7.38	0.42	**0.008**	0.673
	Iliotibialis lateralis pars postacetabularis	ILPO	41.20±1.62	8.98	60.98	**78.91**	65.09	3.16	**0.002**	0.379
	Ischiofemoralis	ISF	2.98±0.26	0.64	1.97	2.72	2.76	0.36	0.172	0.152
	Iliotrochantericus caudalis	ITC	18.07±0.7	9.22	58.08	61.07	56.37	2.59	0.434	0.652
	Gastrocnemius lateralis	LG	18.01±0.58	3.49	22.52	19.75	23.04	0.92	0.058	0.698
	Gastrocnemius medialis	MG	22.37±0.86	4.32	25.02	**30.59**	24.90	1.26	**0.011**	0.947
	Puboischiofemeralis pars lateralis	PIFL	3.26±0.27	5.03	18.85	**21.32**	20.82	0.71	**0.034**	0.078
	Puboischiofemeralis pars medialis	PIFM	8.57±0.49	3.63	28.70	**33.08**	**34.35**	0.87	**0.005**	**0.001**
	Iliofibularis (posterior portion)	postIF	13.46±0.87	3.15	9.33	6.10	11.55	1.04	0.053	0.164
Both	Femerotibialis	FT	34.32±1.15	12.73	68.92	**111.38**	**81.72**	2.85	**<0.001**	**0.01**
Swing	Iliofibularis (anterior portion)	antIF	10.54±0.53	3.51	16.42	**20.74**	**25.29**	1.37	**0.050**	**0.001**
	Extensor digitorum longus	EDL	4.95±1.08	1.18	6.28	6.43	6.81	0.22	0.656	0.122
	Iliotibialis cranialis	IC	20.99±1.50	7.80	43.64	45.02	**52.66**	1.45	0.514	**0.001**
	Iliotibialis lateralis pars preacetabularis	ILPR	8.60±0.28	2.84	6.74	6.30	6.36	0.44	0.489	0.555
	Iliotrochantericus cranialis	ITCR	4.98±0.18	1.00	7.64	**9.30**	**9.62**	0.42	**0.021**	**0.009**
	Obturatorius medialis	OM	6.03±0.62	1.98	11.22	**14.57**	**14.45**	0.65	**0.005**	**0.006**
	Tibialis cranialis	TC	15.37±0.96	5.27	38.67	45.61	**54.19**	2.33	0.061	**0.001**

Values given are for the muscles in both legs.

Values in bold indicate significant differences from the unloaded condition, as assessed by multivariate ANOVA.

Values for mass are means ± s.e.m. (N=6).

*The standard errors (s.e.) reported for the muscle blood flows are the common values for all conditions, as calculated from the multivariate ANOVA.

†Avian anterior pointing toes (digits) are numbered II, III, IV from the medial to the lateral side of the foot. Digits II and III receive insertions from two digital flexors. The most superficial flexors of these digits are designated as perforans et perforatus based on the anatomy of their tendons.

‡The flexor digitorum longus sends branches of its tendon to all of the anterior pointing toes.

§The mixed digital flexors included the deep flexors of digits II and III, the flexor of digit IV, the plantaris, and the flexor hallucis longus.

Mean resting values are included for completeness, although they were not included in the ANOVA model.

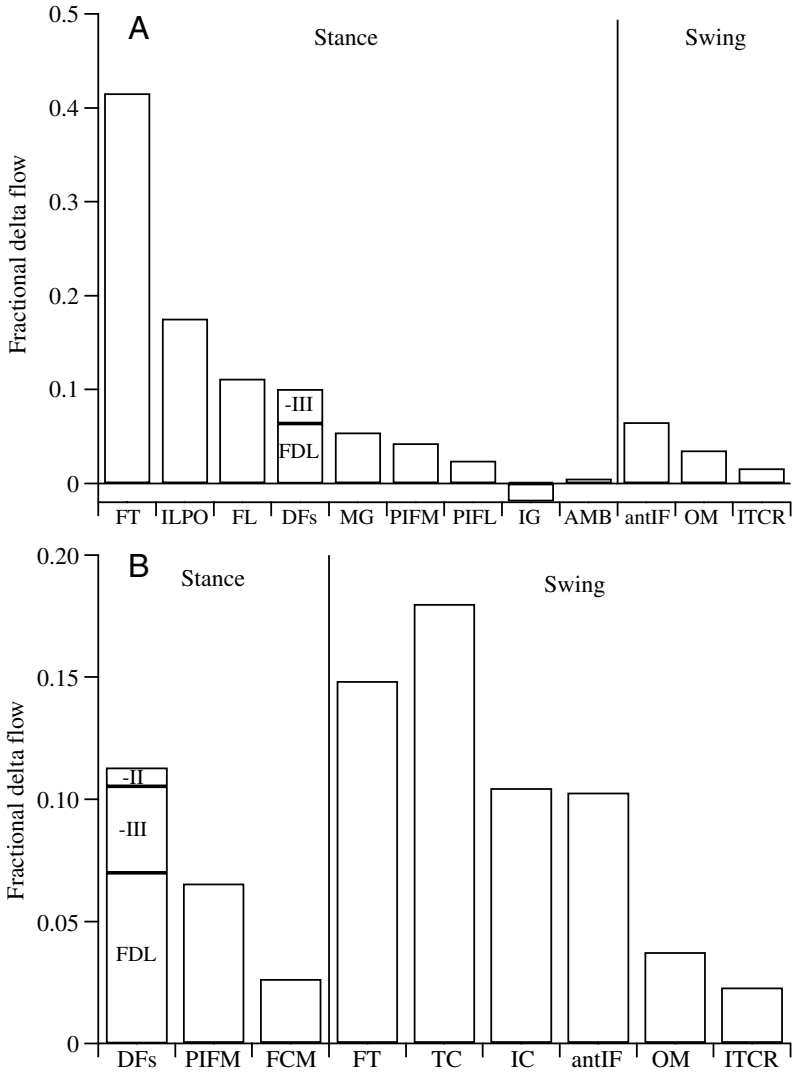

Fig. 4. Fractional delta flow in the leg muscles that have significant changes in blood flow in response to (A) trunk or (B) limb loading. Fractional delta flow is the ratio of the change in flow to an individual muscle divided by the total increase in flow to all of the leg muscles combined. The FT is grouped with the stance muscles for trunk loading and swing-phase muscles for limb loading (see text). Abbreviations for muscle names are given in Table 1.

increase in flow occurred in stance-phase muscles, assuming that all of the increase in flow to the dual function femerotibialis (FT) occurred during stance. (This assumption seems reasonable given the fact that the swing-phase activity in the FT occurs in mid-swing during knee extension and is unlikely to be influenced by trunk loading.)

When the distal limbs were loaded, 11 muscles had statistically significant increases in blood flow during limb loading compared to the unloaded control values (Table 1, Fig. 3). (All of the comparisons were significantly different with both the linear contrasts procedure and the Dunnett *t*-test.) Of these muscles, six were swing-phase muscles and five were stance-phase muscles. The sum of the significant changes in flow in response to limb loading accounted for 80% of the overall difference in flow during limb loading. Considering only the significant changes in flow in response to distal-limb loading, the increase in flow to the swing-phase muscles accounted for 74% of the total, if in this case we attribute all of the change in the FT to swing phase. (This assumption again seems reasonable because the major stance-phase activity of the FT occurs in mid-stance when limb loading would be unlikely to influence the load on the muscle.) If this calculation is done by summing the changes in flow to all of the muscles, not just the ones with significant differences, then the swing-phase muscles account for 60% of the increase.

Discussion

The division of muscle energy expenditure among different mechanical functions during walking and running has sometimes been inferred indirectly through changes in organismal energy use brought about by loading the trunk (Taylor et al., 1980) or the distal limbs (Martin, 1985; Steudel, 1990). Trunk loading has been assumed to alter energy expenditure of stance-phase muscles only (Taylor et al., 1980), whereas distal-limb loading has been assumed to increase energy consumption by mainly swing-phase muscles (Martin, 1985). However, precise inferences from these types of studies can be problematical (Marsh et al., 2006). One of the biggest limitations of these loading studies, as well as other types of investigations seeking to reveal the links between mechanical function and metabolic cost, has been the inability to track the energy use of individual muscles in the limbs.

We overcame this limitation by using muscle blood flow to estimate the changes in muscle energy use brought about by loading. One benefit of the microsphere technique is that it allows a number of sequential measurements of blood flow to all body tissues to be made under different levels of exercise. Muscle blood flow to active muscle is known to be controlled locally and the flow rate is proportional to metabolic rate in active skeletal muscles (Marsh et al., 2004; Ellerby et al., 2005; Marsh and Ellerby, 2006). The proportionality between metabolic rate and blood flow to active muscle was shown again in the present study. The approximately 15% increase in net metabolic rate of the whole animal resulting from back or distal-limb loading was accompanied by a proportional increase in leg blood flow in both cases (Fig. 1). The alteration in muscle energy use is not general across the limb, but instead reveals the specific muscles that respond to trunk or limb loading.

Using the blood flow technique in the context of trunk and limb loading is challenging because the changes in metabolic

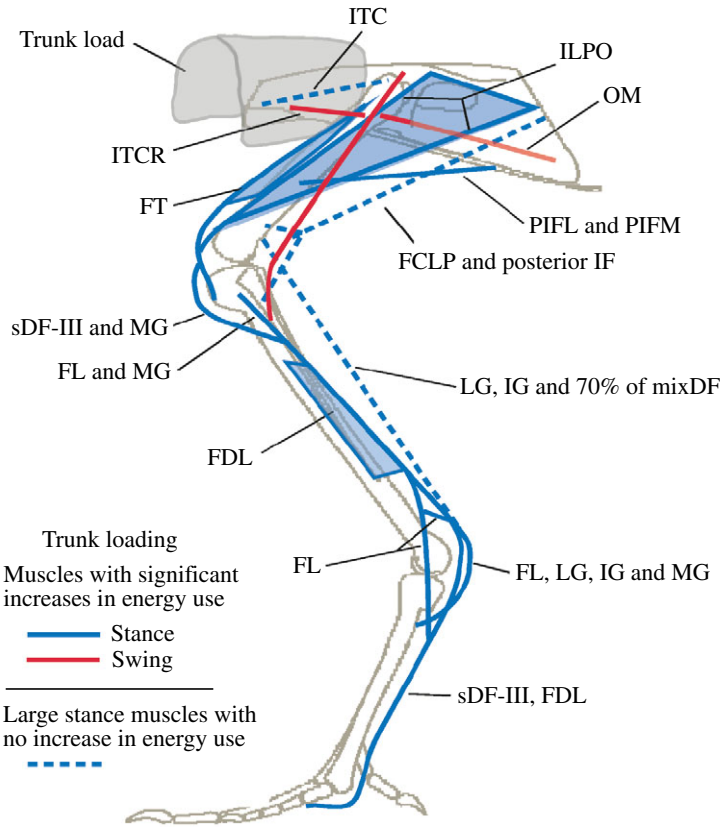

Fig. 5. Approximate line of action for selected hind limb muscles of the guinea fowl. Blue solid lines and solid red lines indicate the lines of action for stance and swing-phase muscles, respectively, that significantly increase energy use in response to a load on the trunk. broken blue lines indicate biarticular stance-phase muscles that had unchanged (FCLP, postIF and LG) or decreased (IG) energy use in response to trunk loading. The small ambiens muscle, which has a significant increase in flow (Table 1), is not shown. The lines of action are drawn to show the major actions of the muscles at the joints, and do not necessarily indicate precisely the muscle origins and insertions or to quantitatively indicate the moment arms. For muscles sharing a similar line of action, only one line is shown. For the ankle extensors, a common line of action is shown along the tibiotarsus, but separate lines indicate origins and insertions where different.

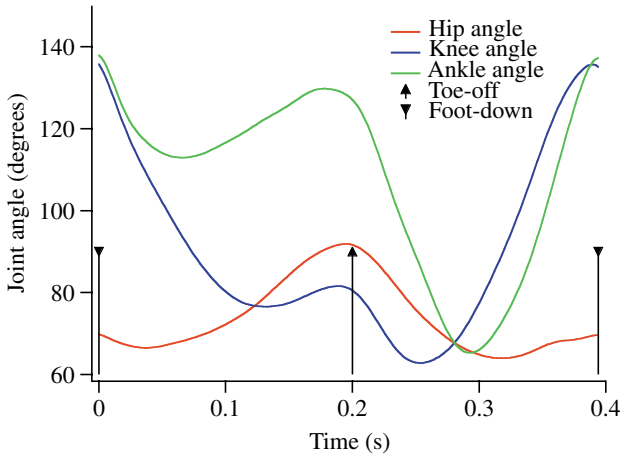

Fig. 6. Joint angles recorded from a guinea fowl running at 1.5 m s^{-1} (J. A. Carr and R.L.M., unpublished data).

rate are considerably smaller then those found across a large range of running speeds (Ellerby et al., 2005). For this reason, we may have failed to statistically detect some biologically relevant alterations in energy use (Type II statistical errors). For trunk loading, the data suggest that this type of error was not very important because the increases in blood flow to the muscles with statistically significant changes in flow accounted for all of the overall increase in flow to the leg muscles. For limb loading, somewhat more uncertainty exists, but we still identified statistically significant increases in flow to individual muscles accounting for 80% of the total increase in flow to the leg muscles. One source of uncertainty stems from combining some of the digital flexors for analysis of microsphere content. The deep flexors of digits II and III and the flexor of digit IV all have two heads, one of which originates on the distal posterior femur and thus has a knee flexor moment and the other originates largely on the proximal fibula and thus has no action at the knee. With excellent hindsight, we can suggest that these heads with differing anatomical actions should have been analyzed separately. Nevertheless, we conclude that we are likely to have captured the major patterns of shifting energy use when guinea fowl carry loads on their backs, or attached to their distal limbs.

Redistribution of blood flow

In a previous study of blood flow during unloaded level running (Ellerby et al., 2005), no significant redistribution of flow from the non-exercise related tissues was detected. The present results indicate that guinea fowl are capable of some redistribution of blood flow during changes in exercise intensity, but only if we restrict the comparisons to the control and loaded running groups, excluding the values from resting birds. Similar to the earlier study (Ellerby et al., 2005), we found no significant differences if the exercise values of organ flow were compared to the resting values, in part because of the variability in the resting flow values to the non-exercise related organs. However, we did note a small, but statistically significant, decrease in mean organ flow between the values for control birds running unloaded at 1.5 m s^{-1} and those measured when the birds ran with either limb or trunk loads. We also measured a significant decrease in flow to the flight muscles between unloaded and loaded conditions. Decreases in blood flow occurred in some leg muscles, but only in the case of the IG during trunk loading was this decrease significant. The decreases in blood flow to the internal organs or resting muscles such as the flight muscles should not be taken to represent a decrease in energy use in these tissues equivalent to the same amount of blood delivered to the active muscles. Unlike the situation in active skeletal muscle, blood flow to digestive organs and the kidneys is not primarily

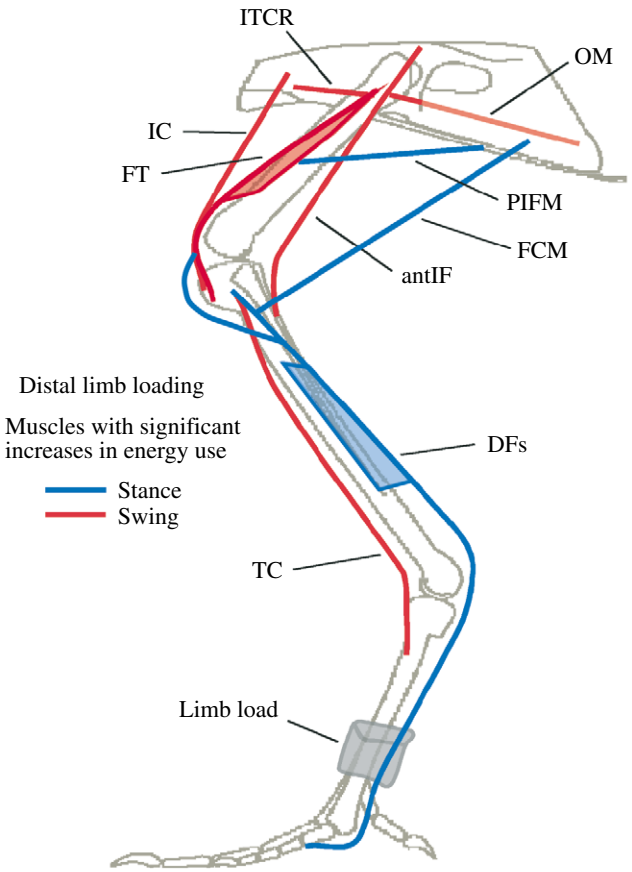

Fig. 7. Approximate lines of action for selected hindlimb muscles of the guinea fowl. Blue solid lines and solid red lines indicate the lines of action for stance and swing-phase muscles, respectively, that significantly increase energy use in response to a load on the distal limb. The lines of action are drawn to show the major actions of the muscles at the joints, and do not necessarily indicate precisely the muscle origins and insertions or to quantitatively indicate the moment arms.

controlled by metabolic rate (Gallavan, Jr and Chou, 1985; Regan et al., 1995). The very low extraction that occurs in the non-exercising condition gives these organs a substantial reserve to decrease flow without altering metabolic rate (Rowell, 1974).

Alteration in muscle energy use by trunk loading

Guinea fowl have been found to carry trunk loads more economically than most humans and all quadrupedal mammals previously studied (Marsh et al., 2006). One possible explanation for these data is that the economical load carrying is related to guinea fowl having a substantial part of the cost of running attributable to energy use by swing-phase muscle. Marsh et al. estimated that swing-phase energy use represents approximately one fourth of total energy use during walking and running in guinea fowl (Marsh et al., 2004). This result was confirmed in the present study. When running unloaded at 1.5 m s^{-1} the swing-phase muscles received 27% of the total flow to the leg muscles (Table 1), assuming that one half of the flow to the dual function FT during unloaded level running is related to swing-phase metabolic activity (Marsh et al., 2004). Previous studies have suggested that the relative costs of stance and swing could be inferred by comparing the ratio of loaded to unloaded energy use (cost ratio) with the ratio of loaded to unloaded mass (mass ratio) (Taylor et al., 1980). The assumptions inherent in this line of reasoning are that trunk loading affects only the cost of stance phase and that the mass-specific cost of transporting the added load is the same as the mass-specific cost of transporting the normal body mass. With these assumptions, if swing-phase costs are substantial, the cost ratio will be smaller than the mass ratio because loading the trunk should only influence the stance-phase cost. Given the swing-phase costs as estimated from the blood flow studies, the economy of load carrying in guinea fowl is consistent with this line of reasoning (Marsh et al., 2006). However, taking into consideration data from load-carrying experiments in a broad range of species as well as estimates of the cost of swing phase in humans, Marsh et al. concluded that comparing the cost ratio to the mass ratio does not appear to be a reliable indicator of the relative cost of stance and swing (Marsh et al., 2006). They suggested that factors other than the relative cost of stance and swing play a major role in determining the cost of carrying extra load on the trunk.

Because the increase in energy use due to trunk loading is largely due to increases in energy use by the active locomotor muscles, the economy of load carrying ought to be related to which muscles alter their functions in response to the load. Without data on energy use by individual muscles, previous investigators have had to make numerous assumptions to connect organismal energy use to muscle function. For example, to relate the increase in energy use due to trunk loading in walking humans to altered muscle energy use, it was assumed (Griffin et al., 2003) that (1) trunk loading increases energy use only in stance-phase muscles; (2) muscle energy use was proportional to the active muscle volume required for force production; (3) all of the stance-phase extensors participated in supporting the increased load; and (4) the summed muscle force at a joint was distributed such that equal stress was maintained in these extensors (Griffin et al., 2003). The data presented here on energy use by individual muscles allow us to ask whether the distribution of energy use among the leg muscles changes with trunk loading and if so, whether these changes provide any clues to the economical load carrying found in guinea fowl.

One way to highlight which muscles respond to an increase in exercise intensity is to calculate the ratio of the change in flow to an individual muscle (dQ) to the increase in total blood flow to the legs; this ratio has been termed ‘fractional delta flow’ or F_{dQ} (Ellerby et al., 2005). All of the stance-phase muscles have extensor actions at one or more joints and could potentially support and accelerate the increased load. However, significant increases in flow occurred in just 8 of the 18 stance-phase muscles measured, and within this group just three muscles, the FT, posterior iliotibialis lateralis (ILPO), and fibularis longus (FL) accounted for 70% of the increase in flow

(Fig. 4). These data confirm the assumption that trunk loading influences mostly stance-phase energy use. However, they also clearly indicate that energy use is not distributed across all of the stance-phase extensors.

Does the specific distribution of increases in muscle energy use suggest any hypotheses that might explain the economy of load carrying found in guinea fowl? The problem we face in answering this question is that as a result of this study we have detailed information for all the leg muscles on the changes in energy use caused by trunk loading, but this detailed information is not matched by an equally detailed knowledge of the mechanical functions of all the individual muscles. Thus, any hypotheses must necessarily be based on indirect inference. We also assume that the overall timing of EMG activity remains similar to that found in the unloaded condition, i.e. the division between stance- and swing-phase muscles.

One such hypothesis is based on the anatomical actions of the stance-phase muscles (Hudson et al., 1959; Gatesy, 1999). The muscles that significantly increase energy use in response to trunk loading all have actions such that they could contribute to supporting body weight, and/or accelerating body mass, without generating opposing moments at other joints (Fig. 5). To accomplish these tasks the muscles should have extensor actions at the hip, knee and ankle joints, and a flexor action at the toe joint (tarsometatarsal–phalangeal joint). The FT, which has the largest F_{dQ} (Fig. 4) is a monoarticular knee extensor. The ILPO, the second largest contributor to the response to trunk loading, is a bi-articular muscle with extensor actions at both the knee and the hip. The FL, accounting for 11% of the increased flow, originates mostly on the tibiotarsus and extends the ankle *via* its attachment to the tibial cartilage (Fig. 5). This muscle can also contribute to digital flexion *via* an accessory tendon that attaches to the tendon to digit III. The major portion of the gastrocnemius medialis (MG) originates on the tibiotarsus below the knee and is a monoarticular ankle extensor. A smaller portion of this muscle originates from the patellar tendon and provides a knee extensor moment (Fig. 5). The PIFL and puboischiofemoralis medialis (PIFM) are monoarticular hip extensors (Fig. 5). The digital flexors are a complex set of seven muscles in the shank, some of which are divided into two heads. All of the digital flexors have tendons crossing the ankle and toe joints and thus tend to extend the ankle and flex the toes. However, these muscles have diverse origins, with some heads originating on the shank, and others crossing the knee. Some of the heads that originate above the knee have knee flexor actions and others have opposing knee extensor actions. In this study, we examined the individual contributions of three of these muscles and combined the rest for analysis. Of the individual muscles analyzed the FDL and sDF-III responded to trunk loading with significant changes in flow. The FDL originates on the shank. The origin of sDF-III is similar to the MG, with a portion originating below the knee and a portion originating from the patellar tendon, and thus this portion of the muscle has a knee extensor action in addition to its actions at the ankle and toe joints. Clearly, the increases in energy use in response to trunk loading are found in a selected set of stance-phase muscles.

The lack of a significant increase in blood flow in some large bi-articular stance-phase muscles that consume considerable amounts of energy during unloaded running also supports this anatomically derived hypothesis. These muscles include the posterior portion of the iliofibularis (postIF), flexor cruris lateralis pars pelvica (FCLP), flexor cruris medialis (FCM), gastrocnemius lateralis (LG), and gastrocnemius intermedia (IG), which exert extensor moments at either the hip or the ankle, but flexor moments at the knee (Fig. 5). The only muscle to show a significant decrease in flow, the IG, is in this group. The deep digital flexors that we combined for analysis (mixDFs) also showed no significant change in energy use. Of the total mass in this mixed muscle group, 70% was from heads that have flexor moments at the knee. Based on blood flow, the combined energy use from these biarticular muscles accounts for 26% of the stance-phase energy use in unloaded birds running at 1.5 m s^{-1}. The mechanical roles of these muscles during unloaded running that result in this substantial energy use cannot be specified with certainty at this time. However, the lack of increase in energy use when the birds carry trunk loads suggests that these bi-articular stance muscles have no significant role in supporting the increased weight or accelerating the increased mass associated with this loading regime.

The specific stance-phase muscles responsive to trunk loading may also indicate that an important component of the added energy cost is the increased mechanical work, rather than just the cost of supporting the added body weight, as was assumed in some earlier studies (e.g. Taylor et al., 1980). In late stance the ankle, knee and hip all extend (Fig. 6), and the center of mass is lifted and accelerated (Heglund et al., 1982). The FT, ILPO, FL, MG and PIFM share a similar pattern of electromyogram (EMG) activity (Gatesy, 1999; Marsh et al., 2004), with activity occurring later in stance when they could contribute to the positive work being done on the center of mass. Direct evidence from sonomicrometry and force recordings indicates that the FL performs positive work to extend the ankle during unloaded level running in turkeys (Gabaldon et al., 2004). During running, the ILPO in guinea fowl first lengthens in early stance and then shortens while active in the last half of stance (Buchanan, 1999; Marsh, 1999). By inference, this muscle is also performing positive work in late stance, to extend the hip and knee. The mechanical function of the FT in stance is not known, but the major stance-phase EMG burst occurs with appropriate timing to contribute to active knee extension. The length of the PIFM or PIFL has not been recorded directly using sonomicrometry, but these muscles have parallel fascicles and no significant tendon (Gatesy, 1999). Thus, the length of the fascicles in the monoarticular hip extensors when active in late stance is expected to track hip extension and thus perform positive work. The conclusion that the cost of accelerating the extra mass during trunk loading is an important part of total energy cost in guinea fowl is supported by data on the energetics and

mechanics human running. The energy required to produce the horizontal force that accelerates the body mass forward in unloaded running is an important contributor to the total cost (Chang and Kram, 1999), and loading the trunk increases the horizontal ground reaction forces substantially (Chang et al., 2000).

Although the majority of the increase in energy use with trunk loading was found in stance, three swing-phase muscles did show significant increases in flow. Why energy use by swing-phase muscles would be changed by trunk loading is not clear. The accompanying study (Marsh et al., 2006) found that the duration of swing is unaltered by trunk loads and stance duration increases by only 4%. However, the possibility exists that more subtle changes in the kinematics of the swinging limb occurred without substantial changes in duty factor. The changes in energy use in these muscles could also be due to enhanced stance activity, because for the antIF and iliotrochantericus cranialis (ITCR) some EMG activity is seen during stance (Gatesy, 1999) (T. A. Hoogendyk and R.L.M., unpublished).

Our conclusion is that the data presented here, support the hypothesis that the very selective pattern of increased energy use among stance-phase muscles in response to trunk loading in guinea fowl contributes to the economical load-carrying found in this species. Specifically the hypothesis is that the low energetic cost of carrying loads results from the activation of a group of muscles that together provide support and propulsion across all the major joints in the leg, without producing opposing flexor moments that could potentially increase energy use.

Alteration in muscle energy use by distal-limb loading

The goal of distal-limb loading studies has been to selectively influence the costs of swing phase (Martin, 1985; Steudel, 1990). In the case of loads on the human foot (Martin, 1985), this goal is likely met because the foot is short and undergoes little change in segmental energy before toe-off (Williams and Cavanagh, 1983). In guinea fowl the most convenient place to attach a distal-limb load is on the elongated tarsometarsus, as was done in the present study. However, because of the length of this segment and the digitigrade running style that characterizes all birds, this segment begins to accelerate forward during the latter part of stance, due to ankle extension and digital flexion. As a result, approximately 40% of the increase in mechanical work due to loading the tarsometarsus occurred during stance (Marsh et al., 2006).

In the accompanying study (Marsh et al., 2006), we concluded that the increase in energy use during distal-limb loading in guinea fowl was likely due to the increase in mechanical work done on the tarsometatarsal segment, so we hypothesized that the metabolic burden of supplying this work would be shared by both stance- and swing-phase muscles. This hypothesis is supported by our data on blood flow (Table 1, Figs 3, 4). The exact proportions of the increased energy use attributed to swing and stance depend on the distribution of energy use in the FT, and whether the proportions are calculated based on summing the flows to all of the leg muscles, or only those with statistically significant changes. In unloaded level running, the FT is active during both swing and stance (Marsh et al., 2004). Unlike in the IF, the EMG activity in this muscle is not conveniently regionalized to allow separation into stance and swing compartments. For distal-limb loading, we have assumed that the increased energy use by this muscle was due to swing activity. If only the statistically significant changes in flow are summed, the distribution of energy use between swing and stance is predicted to be 74% and 26%. Considering the flow to all of the muscles, and assigning all of the energy use by the FT to swing phase, results in 58% of the increased energy use being attributed to swing and 42% to stance, a remarkably close match to the distribution of increased mechanical work found in the accompanying study (Marsh et al., 2006). If some of the increase in energy use of the FT occurs during stance, the proportion of stance-phase energy use would be predicted to be higher. Regardless of these uncertainties, the data indicate that a substantial part of the increase in energy use due to limb loading occurs in muscles active during stance. This finding supports the conclusion in that the increase in energy use during distal-limb loading is linked to the increase in mechanical work required to move the loaded segment, because a considerable part of the increase in segmental work occurs during stance (Marsh et al., 2006).

During limb loading, the increases in energy use by swing-phase muscles are distributed across most of the muscles classified previously as being active during this phase of the stride (Marsh et al., 2004), not just the tibialis cranialis (TC), which acts directly on the loaded segment (Fig. 7). This broad distribution makes sense even though the increase in mechanical energy is confined to the tarsometatarsal segment (Marsh et al., 2006) because the changes in segmental energy are expected to be due to both the muscles acting directly on this segment, and to muscles that transfer work to this segment through joint reaction forces and the action of two-joint muscles (Martin and Cavanagh, 1990). A more complete inverse dynamic analysis, and optimization modeling incorporating the data presented here on muscle energetics, might allow a better prediction of which muscles are involved in providing the extra work (Marsh et al., 2006).

The likely role during limb loading of increased energy use by the stance-phase digital flexors (DFs) is clear, although the functional importance of the significant increases in energy use by the flexor cruris medialis (FCM) and PIFM, also classified as stance-phase muscles, is less certain. The segmental energy of the tarsometatarsus increases in late stance during ankle extension and flexion of the tarsometatarsal–phalangeal joint (Marsh et al., 2006). These joint movements are precisely the expected functions of the DFs (Fig. 7). The FCM is a biarticular muscle capable of producing hip extensor and knee flexor moments, and the PIFM is a monarticular muscle that will produce a hip extensor moment when active (Fig. 7). The role of these moments in doing work on the loaded tarsometatarsal segment is not intuitively obvious. Instead of

doing positive work, the FCM and PIFM could participate in absorbing work in late swing when the segmental energy of the limb decreases. We have not recorded EMG activity from these muscles, but data published elsewhere (Gatesy, 1999) indicate that they may be active in late swing. A similar swing-phase role has been attributed to the human hamstrings during running (Nilsson et al., 1985). Recording EMG activity in selected muscles during loading experiments may help to clarify the function of these and other muscles, such as the FT, whose role in coping with the increased loads is not entirely clear.

We conclude that our hypothesis that the energy cost of distal-limb loading in guinea fowl is directly related to the increase in mechanical work required to move the loaded segment is supported by the distribution of energy use among both stance- and swing-phase muscles. The increase in energy use resulting from limb loading was distributed broadly across many swing-phase muscles. Additionally, similar to the increase in stance-phase segmental work, a substantial amount of the increased energy use occurred in stance-phase muscles.

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References

Buchanan, C. I. (1999). Muscle function and tendon adaptation in guinea fowl (*Numida meleagris*) trained to run on different slopes. PhD thesis, Northeastern University, Boston, MA, USA.

Chang, Y. H. and Kram, R. (1999). Metabolic cost of generating horizontal forces during human running. *J. Appl. Physiol.* **86**, 1657-1662.

Chang, Y.-H., Huang, H.-W. C., Hamerski, C. M. and Kram, R. (2000). The independent effects of gravity and inertia on running mechanics. *J. Exp. Biol.* **203**, 229-238.

Ellerby, D. J., Cleary, M., Marsh, R. L. and Buchanan, C. I. (2003). Measurement of maximum oxygen consumption in guinea fowl *Numida meleagris* indicates that birds and mammals display a similar diversity of aerobic scopes during running. *Physiol. Biochem. Zool.* **76**, 695-703.

Ellerby, D. J., Henry, H. T., Carr, J. A., Buchanan, C. I. and Marsh, R. L. (2005). Blood flow in guinea fowl *Numida meleagris* as an indicator of energy expenditure by individual muscles during walking and running. *J. Physiol.* **564**, 631-648.

Gabaldon, A. M., Nelson, F. E. and Roberts, T. J. (2004). Mechanical function of two ankle extensors in wild turkeys: shifts from energy production to energy absorption during incline *versus* decline running. *J. Exp. Biol.* **207**, 2277-2288.

Gallavan, R. H., Jr and Chou, C. C. (1985). Possible mechanisms for the initiation and maintenance of postprandial intestinal hyperemia. *Am. J. Physiol.* **249**, G301-G308.

Gatesy, S. M. (1999). Guineafowl hind limb function. II: Electromyographic analysis and motor pattern evolution. *J. Morphol.* **240**, 127-142.

Griffin, T. M., Roberts, T. J. and Kram, R. (2003). Metabolic cost of generating muscular force in human walking: insights from load-carrying and speed experiments. *J. Appl. Physiol.* **95**, 172-183.

Heglund, N. C., Cavagna, G. A. and Taylor, C. R. (1982). Energetics and mechanics of terrestrial locomotion: iii. Energy changes of the centre of mass as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **79**, 41-56.

Hudson, G. E., Lanzillotti, P. J. and Edwards, G. D. (1959). Muscles of the pelvic limb in galliform birds. *Am. Midl. Nat.* **61**, 1-66.

Marsh, R. L. (1999). How muscles deal with real-world loads: the influence of length trajectory on muscle performance. *J. Exp. Biol.* **202**, 3377-3385.

Marsh, R. L. and Ellerby, D. J. (2006). Partitioning locomotor energy use among and within muscles: muscle blood flow as a measure of muscle oxygen consumption. *J. Exp. Biol.* **209**, in press.

Marsh, R. L., Ellerby, D. J., Carr, J. A., Henry, H. T. and Buchanan, C. I. (2004). Partitioning the energetics of walking and running: swinging the limbs is expensive. *Science* **303**, 80-83.

Marsh, R. L., Ellerby, D. J., Henry, H. T. and Rubenson, J. (2006). The energetic costs of trunk and distal limb loading during walking and running in guinea fowl *Numida meleagris*. I. Organismal metabolism and biomechanics. *J. Exp. Biol.* **209**, 2050-2063.

Martin, P. E. (1985). Mechanical and physiological responses to lower extremity loading during running. *Med. Sci. Sports. Exerc.* **17**, 427-433.

Martin, P. E. and Cavanagh, P. R. (1990). Segment interactions within the swing leg during unloaded and loaded running. *J. Biomech.* **23**, 529-536.

Modica, J. R. and Kram, R. (2005). Metabolic energy and muscular activity required for leg swing in running. *J. Appl. Physiol.* **98**, 2126-2131.

Nilsson, J., Thorstensson, A. and Halbertsma, J. (1985). Changes in leg movements and muscle activity with speed of locomotion and mode of progression in humans. *Acta Physiol. Scand.* **123**, 457-475.

Regan, M. C., Young, L. S., Geraghty, J. and Fitzpatrick, J. M. (1995). Regional renal blood flow in normal and disease states. *Urol. Res.* **23**, 1-10.

Rowell, L. B. (1974). Human cardiovascular adjustments to exercise and thermal stress. *Physiol. Rev.* **54**, 75-159.

Steudel, K. (1990). The work and energetic cost of locomotion. I. The effects of limb mass distribution in quadrupeds. *J. Exp. Biol.* **154**, 273-285.

Taylor, C. R., Heglund, N. C., McMahon, T. A. and Looney, T. R. (1980). Energetic cost of generating muscular force during running: comparison of small and large animals. *J. Exp. Biol.* **86**, 9-18.

Williams, K. R. and Cavanagh, P. R. (1983). A model for the calculation of mechanical power during distance running. *J. Biomech.* **16**, 115-128.