The Journal of Experimental Biology 209, 2395-2408
Published by The Company of Biologists 2006
doi:10.1242/jeb.02310

The cost of running uphill: linking organismal and muscle energy use in guinea fowl (*Numida meleagris*)

Jonas Rubenson, Havalee T. Henry, Peter M. Dimoulas and Richard L. Marsh*
Department of Biology, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA
*Author for correspondence (e-mail: r.marsh@neu.edu)

Accepted 3 May 2006

Summary

Uphill running requires more energy than level running at the same speed, largely due to the additional mechanical work of elevating the body weight. We explored the distribution of energy use among the leg muscles of guinea fowl running on the level and uphill using both organismal energy expenditure (oxygen consumption) and muscle blood flow measurements. We tested each bird under four conditions: (1) rest, (2) a moderate-speed level run at 1.5 m s^{-1}, (3) an incline run at 1.5 m s^{-1} with a 15% gradient and (4) a fast level run at a speed eliciting the same metabolic rate as did running at a 15% gradient at 1.5 m s^{-1} (2.28–2.39 m s^{-1}). The organismal energy expenditure increased by 30% between the moderate-speed level run and both the fast level run and the incline run, and was matched by a proportional increase in total blood flow to the leg muscles. We found that blood flow increased significantly to nearly all the leg muscles between the moderate-speed level run and the incline run. However, the increase in flow was distributed unevenly across the leg muscles, with just three muscles being responsible for over 50% of the total increase in blood flow during uphill running. Three muscles showed significant increases in blood flow with increased incline but not with an increase in speed. Increasing the volume of active muscle may explain why in a previous study a higher maximal rate of oxygen consumption was measured during uphill running. The majority of the increase in energy expenditure between level and incline running was used in stance-phase muscles. Proximal stance-phase extensor muscles with parallel fibers and short tendons, which have been considered particularly well suited for doing positive work on the center of mass, increased their mass-specific energy use during uphill running significantly more than pinnate stance-phase muscles. This finding provides some evidence for a division of labor among muscles used for mechanical work production based on their muscle–tendon architecture. Nevertheless, 33% of the total increase in energy use (40% of the increase in stance-phase energy use) during uphill running was provided by pinnate stance-phase muscles. Swing-phase muscles also increase their energy expenditure during uphill running, although to a lesser extent than that required by running faster on the level. These results suggest that neither muscle–tendon nor musculoskeletal architecture appear to greatly restrict the ability of muscles to do work during locomotor tasks such as uphill running, and that the added energy cost of running uphill is not solely due to lifting the body center of mass.

Key words: muscle, energetics, blood flow, running, uphill, guinea fowl, *Numida meleagris*.

Introduction

For the majority of animals, the metabolic demand of running increases markedly when running uphill as compared with the energy use for level running. For example, human running is nearly twofold more expensive when running on a 15% gradient compared to running at the same speed on the level (Minnetti et al., 1994). The elevated metabolic cost of incline running is commonly explained on the basis of the additional mechanical work done against gravity (Taylor et al., 1972; Kram and Dawson, 1998; Wickler et al., 2005). During steady-speed level running, negative and positive mechanical work of the body are equal, and some fraction of this work may be reciprocally stored and released as elastic strain energy in tendons, reducing the work required of the muscle fibers themselves. By contrast, incline running requires net mechanical energy production and thus necessitates additional net positive muscle fiber work in order to lift the animal's body weight vertically.

While this explanation for the elevated metabolic cost of incline running is intuitively appealing, how mechanical work is modulated and which muscles consume the additional metabolic energy remains unclear. The increase in metabolic rate does not simply reflect the increased mechanical work done, because the overall functions of the muscles have

changed in running uphill. Measures of delta efficiency (increase in gravitational mechanical energy divided by the increase in metabolic energy consumption) in uphill running are often greater than the maximum known efficiency of skeletal muscle (Taylor et al., 1972; Bijker et al., 2001), suggesting that some of the functions requiring energy on the level require less energy when running uphill. Developing hypotheses to explain the metabolic cost of running uphill has been hampered by the lack of information on the energy consumption of individual muscles.

In the present study, we asked whether the additional mechanical and metabolic energy expenditure of incline running is shared across all muscles equally, or, alternatively, are certain muscles preferentially recruited for uphill running? Several authors have argued that a muscle's ability to do useful mechanical work is dependent on its muscle–tendon architecture (for reviews, see Biewener, 1998; Biewener and Roberts, 2000). Although all muscles are capable of producing similar amounts of mass-specific work, short fibered, pinnate, muscles with long external tendons may sacrifice length and position control in favor of high force output and elastic energy storage and release in long tendons (Biewener and Roberts, 2000). As such, pinnate muscles appear better suited for economical isometric force production during level running compared to modulating mechanical work during uphill running. Muscles with long, parallel fibers and little or no external tendon may, on the other hand, be ineffective for elastic energy recovery, but favored for work production. Evidence for this division of labor can be seen from a comparison of *in vivo* work loops and strain trajectories. For example, highly pinnate muscles with tendons (aponeurosis plus free tendon) that are much longer than the fibers, such as the lateral gastrocnemius of running turkeys (Roberts et al., 1997) and the gastrocnemius and plantaris of hopping wallabies (Biewener et al., 1998), shorten little during force production in level running or hopping. In contrast, muscles with a low ratio of tendon length to fiber length, such as the pectoralis of flying pigeons (Biewener et al., 1992) and the vastus lateralis of jumping dogs (Gregersen and Carrier, 2004), shorten substantially while active.

Despite these clear examples of correspondence between architecture and function, current data make the overall importance of pinnate muscles in providing the work during uphill running unclear. Recent studies on the gastrocnemius and plantaris of wallabies (Biewener et al., 2004) and a guinea fowl digital flexor muscle (Daley and Biewener, 2003) indicate that these short-fibered muscles with long external tendons may, in general, contribute little to the additional mechanical work of incline running. However, in turkeys the lateral gastrocnemius and fibularis longus, which have a similar architecture, have been shown to produce substantial work when the birds run uphill (Roberts et al., 1997; Gabaldón et al., 2004). Based on current information, whether pinnate muscles are limited by their architecture in contributing to uphill running is not clear.

In the present study, we explored the distribution of metabolic energy expenditure among muscles during uphill running. We estimated the metabolic energy used by the individual hindlimb muscles of guinea fowl running both on the level and uphill using whole body oxygen consumption and regional blood flow measurements (Marsh et al., 2004; Ellerby et al., 2005; Marsh and Ellerby, 2006). Our goal was, firstly, to determine which muscles are responsible for the elevated metabolic cost of running uphill over that of level running at the same speed and, secondly, to compare these muscles to those responsible for a similar increase in metabolic cost due solely to an increase in level running speed. Thus, this study explores whether the elevated metabolic cost associated with an increased demand for net mechanical work is partitioned differently among hindlimb muscles compared to when no net increase in work is required. Specifically, we tested the hypotheses that the elevated metabolic energy associated with incline running compared to level running at the same speed is: (1) consumed primarily by stance phase muscles because these muscles are responsible for raising the body weight against gravity, and (2) used disproportionately more by parallel fibered muscles with short tendons.

Materials and methods

Animals and training

Eight guinea fowl *Numida meleagris* L. 1.47±0.05 kg body mass (mean ± s.e.m.; 3 female, 5 male), obtained from The Guinea Farm (New Vienna, IA, USA), were cage-reared at the Northeastern University Division of Laboratory Medicine. Birds were maintained on a 12 h:12 h light:dark cycle and provided with unlimited access to food and water. Each bird was trained to walk and run on a motorized treadmill (Trimline 2600, Hebb Industries, Tyler, TX, USA; belt: 1.20 m long, 0.44 m wide) for 30 min per day, 5 days per week, over a period of 2 months prior to testing. Birds were deemed suitable for testing if, after training, they could sustain 30 min of exercise at 2.5 m s^{-1}. All experiments were performed under the approval of the Northeastern University Institutional Animal Care and Use Committee.

Oxygen consumption

The rate of oxygen consumption ($\dot{V}_{O_2}$) was initially measured in birds running at 1.5 m s^{-1} on a level treadmill (moderate-speed run) and at 1.5 m s^{-1} on a 15% gradient (incline run). This speed and incline combination was chosen in order to induce a large increase in metabolic rate that is within the birds' aerobic scope (Ellerby et al., 2003), and at a speed that is above their walk–run transition speed (Gatesy, 1999a). Measurements were subsequently made over a range of faster level running speeds (2.0–3.0 m s^{-1}) in order to determine the level running speed (fast run) that resulted in a $\dot{V}_{O_2}$ similar to the incline run (Fig. 1). A resting $\dot{V}_{O_2}$ was measured in birds sitting quietly within a darkened box on the treadmill belt prior to each running session.

Rates of oxygen consumption were measured using a flow-through respirometry system, the details of which have been

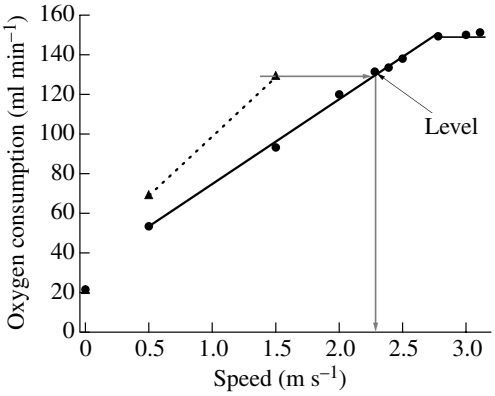

Fig. 1. Representative organismal oxygen consumption of a guinea fowl during rest and during level running (circles) and incline running (triangles) over a range of speeds. The bird was initially tested running at 1.5 m s^{-1} on the level and 1.5 m s^{-1} on a 15% gradient. Subsequent measurements were made over a range of faster running speeds (2.0–3.0 m s^{-1}) in order to determine the speed for which the organismal oxygen consumption matched that at 1.5 m s^{-1} and 15% gradient (as indicated by arrows). Lines were fitted by eye to illustrate the experimental design.

described previously (Ellerby et al., 2003). Briefly, the birds ran with their head and neck inside a loose-fitting transparent mask constructed from the approximately hemispherical tops of two 2 l plastic bottles. A flexible excurrent plastic tube connected the mask to the respiratory system. Room air was drawn through the mask *via* the opening at the bird's neck using a negative pressure pump. The gas exiting the mask was dried and passed through a rotameter-type flowmeter (model IG07-RB, Cole Parmer, Vernon Hills, IL, USA) adjusted to 10.0 l min^{-1} (exercise conditions) or 5.0 l min^{-1} (rest). Excurrent gas was sub-sampled, scrubbed of CO_2 and re-dried before entering a dual-channel oxygen analyzer (Amatek S3A-II, AEI Technologies, Naperville, IL, USA). A continuous stream of CO_2-free, dry room air was pulled through the second cell of the analyzer. The oxygen analyzer was calibrated before and after each testing session using dry, CO_2-free room air assuming a fractional concentration of oxygen of 0.20953. Oxygen consumption was calculated following published procedures (Withers, 1977).

Rates of oxygen consumption were measured continuously during rest and each exercise condition and logged every 5 s on an Apple PowerMac G4 computer *via* a MacLab-2e, 12-bit A/D converter (ADInstruments, Colorado, CO, USA). Steady-state values (after ~2 min at each speed/incline condition) were calculated. After acclimating the birds to the protocol, measurements were repeated a minimum of three times, each on separate days, and an average $\dot{V}_{O_2}$ during rest and for each exercise condition was calculated.

Blood flow measurements

Blood flow to individual muscles and other body tissues was measured using an injectible microsphere technique (see Marsh et al., 2004; Ellerby et al., 2005) in a separate testing session under all three running conditions. Using standard aseptic surgical techniques, the bird's brachial arteries were cannulated under anesthesia (isoflurane, 1.5%) using custom-made polyurethane saline-filled cannulae. The right (injection) and left (withdrawal) brachial artery cannulae were advanced into the left ventricle and the brachiocephalic artery, respectively. A pressure transducer (World Precision Instruments, Sarasota, FL, USA) was used to detect when the ventricular cannula entered the left ventricle. The cannulae were secured in the arteries using 4-0 silk sutures proximal to the cannulae entry sites and further secured to the skin at the elbow. The proximal wings were wrapped with Vetwrapp (3M) hiding the coiled cannulae and the bird was left to recover overnight prior to the blood flow measurements.

During the experimental session, microsphere injections (15 μm diameter polystyrene spheres; Triton Dye-trak VII+, Triton Technologies, San Diego, CA, USA) were made in the following order in all but one bird: (1) after the bird had been resting in a darkened box for approximately 10 min; (2) during a moderate-speed run at 1.5 m s^{-1} and 0% gradient; (3) during a fast run matched for the metabolic cost of the incline run (2.28 or 2.39 m s^{-1} and 0% gradient); and (4) during an incline run at 1.5 m s^{-1} and 15% gradient. In the remaining bird the order was the same except that the uphill and fast runs were reversed. Injections during the running conditions were made after the birds had been running for 2 min and exhibited a steady heart rate as measured by a pressure transducer connected to the injection cannula. The injection and simultaneous blood withdrawal (see below) lasted for approximately 1 min, after which the animal continued to run at the prescribed exercise condition for approximately 30 s. The birds walked at 0.5 m s^{-1} for 2 min before each running condition.

Injection syringes (1 ml) were weighed to the nearest 1 mg before and after filling to determine the volume of microsphere solution in each injection. The injection volumes contained approximately 10^6 spheres (~0.3 ml of solution). The injections were made through a Luer port of three-way stopcock and followed with a flush of 0.7 ml physiological saline. A second Luer port was connected to the pressure transducer from which we monitored pressure to confirm the ventricular location of the cannula and to monitor heart rate except during the injections. 10 s prior to injecting the microspheres the reference arterial blood withdrawal was started at a flow rate of 1.75 ml min^{-1} using a heparinized 3-ml syringe connected to a syringe pump (Genie YA-12, Kent Scientific, CT, USA). The reference withdrawal continued during the injection of microspheres and saline flush, which took approximately 20 s, and continued for approximately 35 s after the flush was completed in order to capture all of the microspheres within the withdrawal cannula. After each injection, the stopcock was removed and rinsed with 100% ethanol together with the injection syringe in order to quantify the number of un-injected spheres.

After completion of microsphere injections, the animals were killed by an overdose of pentobarbital solution and all

but several very small muscles from one leg were dissected out and weighed (Table 1). Muscle nomenclature follows the *Handbook of Avian Anatomy* (Vanden Berge and Zweers, 1993). The muscle samples analyzed were those done previously (Ellerby et al., 2005) with the following differences. (1) The iliofibularis was divided into anterior (antIF) and posterior (postIF) 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 appear to separately innervate the antIF and postIF (T. A. Hoogendyk, personal communication). (2) In the earlier work (Ellerby et al., 2005) all of the digital flexors were analyzed as one group. In the present study, we analyzed four of the digital flexors individually, the superficial flexors of digits II and III (flexor perforans et perforatus digiti II & III, abbreviated as sDF-II and sDF-III), flexor digitorum longus (FDL), and the flexor hallucis longus (FHL). (3) The deep digital flexors to digits II, III and IV are all divided anatomically into medial and lateral heads. The medial heads originate on the posterior surface of the distal femur behind the knee and the lateral heads originate largely on the fibula (Hudson et al., 1959). On the basis of this anatomical arrangement, we combined the lateral and medial heads in two groups designated as deep digital flexors, lateral heads (latDDF) and deep digital flexors, medial heads (medDDF). 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. (4) The femerotibialis muscle group was separated into four heads for analysis, although currently any functional distinctions among these heads are unknown. The nomenclature regarding the divisions of this muscle in birds is subject to some confusion in various sources (Hudson et al., 1959; George and Berger, 1966; Vanden Berge and Zweers, 1993; Gatesy, 1999b), and thus a certain amount of anatomical description is useful here. Current nomenclature (Vanden Berge and Zweers, 1993; Gatesy, 1999b) divides the femerotibialis into three named heads: lateralis, intermedius and medialis, and the lateralis is further subdivided into proximal and distal heads. The femerotialis lateralis pars distalis (FTLD) [the 'externus' (Hudson et al., 1959)] is a small distinct head originating from the distal half of the lateral surface of the femur. The bulk of the muscle, considered as one head by Hudson and colleagues (Hudson et al., 1959), is indistinctly divided into the more lateral, femerotibialis lateralis pars proximalis (FTLP) and the more medial femerotibialis intermedius (FTI). A proximal notch on the anterior surface of the femur forms the only clear division between these heads. We separated them for analysis along a line running from this notch to the patellar tendon. The remaining head, the femerotibialis medialis (FTM), is a distinct spindle shaped head lying along the medial surface of the femur. Selected muscles from the contralateral limb were also analyzed 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 results by tissue are not reported for this study.

Microspheres were recovered from individual muscles and organs from the sacrificed bird using a previously published protocol (Marsh et al., 2004; Ellerby et al., 2005). Prior to processing, a known amount of navy control spheres were added to each tissue sample in order to quantify and correct for the amount of spheres lost in the processing steps. Spheres were subsequently isolated using a series of tissue digestion and rinsing steps [see on-line supplement (Marsh et al., 2004)]. The dye from the isolated microspheres was extracted using cellosolve acetate of known volume and, after centrifugation, the absorbance spectrum of the dye mixture was measured using a scanning spectrophotometer (Ultrospec 3300pro, G.E. Healthcare BioSciences, Uppsala, Sweden). The number of spheres in each experimental color and the navy process control were calculated from the absorbance at their peak-absorbance wavelength and the peak-absorbance wavelength of a low-wavelength contaminant using a matrix inversion calculation implemented in Microsoft Excel. The actual number of spheres used in the final tissue blood flow calculations were corrected for the number of spheres lost in the processing steps using the mean number of navy spheres from four unprocessed tubes containing only navy spheres. The tissue blood flow rate (Q_t) in ml min^{-1} was calculated as:

$$Q_t = \frac{Q_b N_t}{N_b}, \quad (1)$$

where Q_b is the reference blood withdrawal rate (ml min^{-1}), N_t is the number of miscrospheres in the tissue, and N_b is the number of microspheres in the reference blood withdrawal.

In order to further describe the distribution of metabolic energy use amongst muscles during level and incline running, we calculated the fractional increase in blood flow to the muscles between the moderate-speed level run and incline run and between the moderate-speed level run and fast level run. This value has been termed the fractional delta flow (F_{dQ}) (Ellerby et al., 2005) and is equal to the increase in blood flow to a muscle between two exercise conditions divided by the total increase in blood flow to all the muscles between the same exercise conditions. Also, because the size of a muscle will influence the amount of work and force it can produce, and thus its energy use, we calculated the mass-specific increase in blood flow between exercise conditions. Importantly, this latter analysis addresses whether the increase in energy use between exercise conditions in a given muscle (or muscle group) is proportional to its mass, rather than assessing the distribution of total energy use among the muscles.

We also examined the F_{dQ} between exercise conditions amongst specific muscle groupings. We examined the F_{dQ} between the moderate-speed level run and incline run, and between the moderate-speed and fast level runs for: (a) stance muscles divided into parallel fibered 'strap-like' muscles

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

Phase	Muscle	Abbreviation	Mass (g)	Blood flow (ml min^{-1})						
				Rest	Moderate, level	Incline	Fast, level	s.e.m.*	*P*, incline	*P*, fast run
Stance	Ambiens	AMB	1.53±0.06	0.2	0.77	0.99	**1.25**	0.12	0.200	**0.011**
	Caudofemoralis pars caudalis	CFC	2.64±0.09	0.192	0.87	**2.24**	1.49	0.27	**0.003**	0.127
	Caudofemoralis pars pelvica	CFP	3.84±0.21	0.612	2.16	**4.24**	**5.08**	0.66	**0.041**	**0.007**
	Deep digital flexors (combined lateral)†	latDDF	5.55±0.28	0.698	10.92	**15.73**	**13.66**	0.68	**<0.001**	**0.013**
	Deep digital flexors (combined medial)†	medDDF	7.76±0.24	1.064	23.03	**28.23**	**29.25**	1.71	**0.049**	**0.022**
	Flexor perforans et perforatus digiti II	sDF-II	2.21±0.11	0.258	3.76	**4.62**	**4.91**	0.25	**0.026**	**0.005**
	Flexor perforans et perforatus digiti III	sDF-III	6.48±0.30	0.872	22.43	27.21	26.49	1.87	0.091	0.146
	Flexor hallucis longus	FHL	3.73±0.27	0.579	13.00	14.41	**18.98**	1.21	0.426	**0.004**
	Fibularis longus	FL	17.96±0.71	2.048	31.65	**38.68**	36.43	1.97	**0.024**	0.108
	Flexor cruris lateralis pars accessoria	FCLA	6.31±0.22	0.682	3.84	**8.76**	**5.47**	0.48	**<0.001**	**0.030**
	Flexor cruris lateralis pars pelvica	FCLP	29.25±0.81	3.969	37.78	**64.15**	**57.84**	3.30	**<0.001**	**0.001**
	Flexor cruris medialis	FCM	2.88±0.11	0.768	7.50	**11.43**	**11.90**	0.75	**0.002**	**0.001**
	Flexor digitorum longus + fibularis brevis	FDL&FB	9.77±0.39	1.334	26.77	**33.84**	**34.96**	2.07	**0.030**	**0.014**
	Iliotibialis lateralis pars postacetabularis	ILPO	43.25±1.14	7.74	50.91	**98.30**	**84.16**	5.69	**<0.001**	**0.001**
	Ischiofemoralis	ISF	4.36±0.31	1.059	3.70	**6.14**	**5.22**	0.36	**<0.001**	**0.009**
	Iliotrochantericus caudalis	ITC	20.86±0.65	7.023	69.37	**89.74**	77.03	3.80	**0.002**	0.175
	Gastrocnemius intermedia	IG	4.83±0.29	0.554	8.81	**11.07**	**11.32**	0.61	**0.021**	**0.012**
	Gastrocnemius lateralis	LG	18.91±0.38	1.59	26.29	**32.53**	**35.65**	1.72	**0.022**	**0.002**
	Gastrocnemius medialis	MG	13.01±0.45	2.606	27.76	**36.36**	**40.82**	2.09	**0.012**	**0.001**
	Pubo-ischio-femeralis pars lateralis	PIFL	3.56±0.20	3.074	20.02	22.45	**25.61**	1.33	0.216	**0.010**
	Pubo-ischio-femeralis pars medialis	PIFM	9.44±0.45	2.002	35.67	37.44	**42.31**	1.94	0.528	**0.030**
	Iliofibularis (posterior portion)	postIF	12.69±0.56	3.616	10.70	**18.41**	**14.17**	1.04	**<0.001**	**0.036**
Both	Femerotibialis lateralis pars distalis	FTLD	3.56±0.77	0.609	5.62	**7.15**	**7.02**	0.35	**0.005**	**0.033**
	Femerotibialis medialis	FTM	5.15±0.13	0.842	7.22	6.28	**10.97**	0.84	0.440	**0.007**
	Femerotibialis lateralis pars proximalis	FTLP	13.43±0.76	2.752	24.25	**29.39**	**31.06**	1.35	**0.017**	**0.003**
	Femerotibialis intermedius	FTI	14.99±0.62	3.198	31.28	**38.43**	**42.44**	2.28	**0.044**	**0.004**
Swing	Iliofibularis (anterior portion)	antIF	11.34±0.46	4.459	17.75	**22.35**	**26.61**	1.30	**0.025**	**0.001**
	Extensor Digitorum Longus	EDL	4.40±0.30	0.587	3.54	3.94	3.94	0.32	0.395	0.386
	Iliotibialis cranialis	IC	21.15±0.88	5.815	45.67	**54.65**	**60.27**	2.70	**0.034**	**0.002**
	Iliotibialis lateralis pars preacetabularis	ILPR	9.67±0.58	2.512	9.30	**12.53**	**13.78**	0.78	**0.011**	**0.001**
	Iliotrochantericus cranialis	ITCR	5.99±0.30	1.23	7.76	9.40	**11.64**	0.85	0.191	**0.006**
	Obturatorius medialis	OM	7.09±0.59	1.547	14.97	16.58	**19.75**	0.88	0.216	**0.002**
	Tibialis cranialis	TC	16.38±0.45	5.72	54.45	65.24	**79.68**	4.65	0.123	**0.002**

Values given are for the muscles in both legs.

Values in bold indicate significant differences from the moderate-speed run condition (multivariate ANOVA).

*The standard errors reported for the muscle blood flows are the common values for all exercise conditions as calculated from the multivariate ANOVA (excluding rest).

†See Materials and methods for specific muscle names.

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

versus pinnate muscles, (b) stance muscles divided into their primary action (hip, knee or ankle/toe extensors), and (c) muscles divided into those active in stance *versus* swing. [The stance/swing division followed that described earlier (Marsh et al., 2004).

Haemoglobin and plasma lactate concentrations

Directly after completion of the reference blood withdrawal, a 20 μl and a 100 μl blood sample were collected from the withdraw cannula for haemoglobin and lactate analysis, respectively. The sample for haemoglobin analysis was placed in drabkins solution and the sample for lactate analysis was stored in perchloric acid and kept on ice. Haemoglobin and plasma lactate concentrations were measured using standard biochemical assay kits (Sigma Chemical Company, 525A and 826B, respectively). Haemoglobin concentrations remained constant in all birds. One bird was excluded from analysis due to high lactate values. The eight birds analyzed all had blood lactate values below 4 mmol l^{-1}.

Statistics

To test for significant differences in blood flow between running conditions we ran an analysis of variance (ANOVA) using the general linear model within SPSS (version 11) at a significance level of $P<0.05$. An identifier for the individual birds was entered as a factor in the model in addition to the exercise condition. Factoring out the variance among birds is important because the values of blood flow in an individual bird are systematically correlated due to their calculation from a common reference blood flow. The ANOVA model tested for main effects only. We conducted planned contrast analyses between the moderate-speed and fast level running and between the moderate-speed level and incline running, assuming equal variances. A Wilcoxon nonparametric test was used to determine significant differences ($P<0.05$) between the fractional delta flow values due to speed and incline (SPSS version 11).

Lumped values for increases in mass-specific blood flow to pinnate and parallel muscles were compared using paired *t*-tests (using Bonferroni correction) at a significance level of $P<0.05$. We also ran a one-sample *t*-test to test for significant differences between the increase in mass-specific blood flow to muscle groups and the average mass-specific increase in flow to all muscles.

Results

Oxygen consumption

The rate of oxygen consumption during the incline run at 1.5 m s^{-1} and 15% gradient typically increased by 30% over that of level running at 1.5 m s^{-1} (Figs 1 and 2). The level running speed that matched the $\dot{V}_{O_2}$ during the incline run was either 2.28 m s^{-1} or 2.39 m s^{-1}, depending on the bird, and the $\dot{V}_{O_2}$ was generally within 2 ml min^{-1} of the incline run value (Figs 1 and 2). The $\dot{V}_{O_2}$ of the incline run and fast level run were considerably below the maximal $\dot{V}_{O_2}$ of the birds

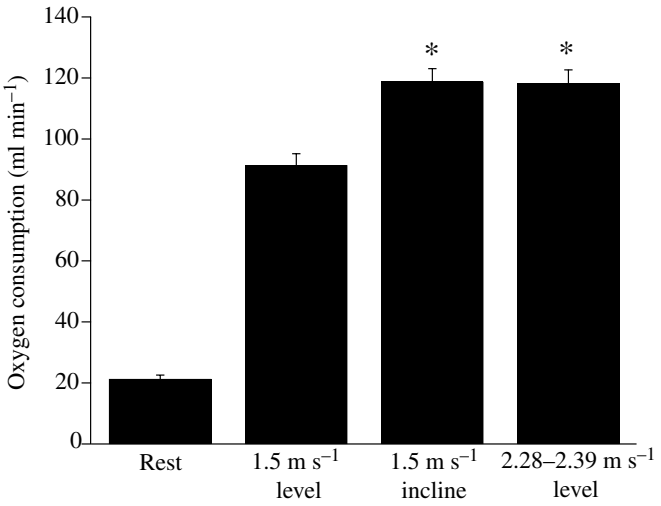

Fig. 2. Organismal oxygen consumption of guinea fowl at rest, running at 1.5 m s^{-1} on the level, running at 1.5 m s^{-1} on a 15% gradient and running at 2.28–2.39 m s^{-1} on the level. Values are means ± s.e.m. (N=8). *Significant difference ($P<0.05$) between the level run at 1.5 m s^{-1} and both the level run at 2.28–2.39 m s^{-1} and incline run at 1.5 m s^{-1} and 15% gradient, as measured by paired *t*-tests. There was no significant difference between the 1.5 m s^{-1} incline run and the 2.28–2.39 m s^{-1} level run.

examined (Fig. 1), indicating that the birds were relying on aerobic metabolism. This was further evident from the low blood lactate concentrations during these runs (<4 mmol l^{-1}).

Total blood flow to the leg muscles and its overall distribution

Total blood flow to the leg muscles increased linearly with total oxygen consumption across exercise conditions (Fig. 3). Commensurate with this finding, the total blood flow to the leg muscles was the same during both the incline run and fast run (Fig. 3), further indicative of the strong correlation between metabolic demand and blood flow.

The mean blood flows (ml min^{-1}) to the limb muscles during rest, the moderate-speed level run, incline run and fast level run are summarized in Table 1. The majority of muscles exhibited a significant increase in blood flow between the moderate-speed level run and both the incline run and fast level run. Several muscles exhibited a significant increase in blood flow only between the moderate-speed level run and the incline run (caudofemoralis pars caudalis, fibularis longus, iliotrochantericus caudalis) or only between the slow level run and the fast level run (ambiens, flexor hallucis longus, puboischio-femeralis pars lateralis and pars medialis, femerotibialis internus, iliotrochantericus cranialis, obturatorius medialis, tibialis cranialis). Only the flexor perforans et perforatus digiti III and the extensor digitorum longus showed no increase in blood flow during either the incline and fast level run.

The fractional increase in blood flow (the increase in blood flow to a muscle between two exercise conditions divided by the total increase in blood flow to all the muscles between the same exercise conditions) to the muscles between the

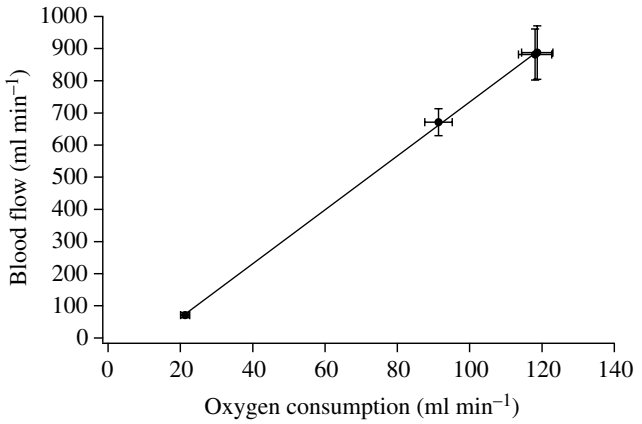

Fig. 3. Organismal oxygen consumption *versus* total leg muscle blood flow of guinea fowl at rest, running at 1.5 m s^{-1} on the level, running at 1.5 m s^{-1} on a 15% gradient and running at 2.28–2.39 m s^{-1} on the level. Values are means ± s.e.m. (N=8). Total blood flow increases linearly with organismal oxygen consumption (y=8.38x–104.3; r^2=0.9997).

moderate-speed level run and incline run and between the moderate-speed level run and fast level run are shown in Fig. 4. Although many muscles had significant increases in blood flow, the muscles that stand out as contributing disproportionately to the total increase during incline running were the flexor cruris lateralis pars pelvica (FCLP), iliotibialis lateralis pars postacetabularis (ILPO), and iliotrochantericus caudalis (ITC), which together contributed 54% of the total increase in blood flow. All of these muscles had higher flows than would be expected if the increased flow were simply distributed according to the mass of the muscles (Fig. 4); together these muscles comprised 27% of the total hindlimb muscle mass.

The largest contributors to the increase in blood flow during fast level running also included the FCLP and ILPO, as well as the femorotibialis (FT) and tibialis cranialis (TC) (~46% of the increase in blood flow combined). Under this running condition, the FCLP, ILPO and FT had mass-specific increases in blood flow that were similar to the average mass-specific increase in flow to all the muscles, but the mass-specific increase in flow to the TC was greater than the average mass-specific increase in flow.

Distribution of blood flow among muscle groups according to architecture and function

Architecturally, the total hindlimb muscle mass of guinea fowl consisted of almost equal proportions of muscles with largely parallel fascicles and short tendons (aponeurosis plus external tendon) (49±0.2% of the mass) and muscles with pinnate fascicles and long tendons (51±0.2% of the mass). When these birds increased speed from 1.5 m s^{-1} to ~2.4 m s^{-1} the increase in blood flow was also almost equally divided between parallel and pinnate fibered muscles acting in both stance and swing (51±5% and 49±5%, respectively).

Because the extra work of running uphill is expected to be

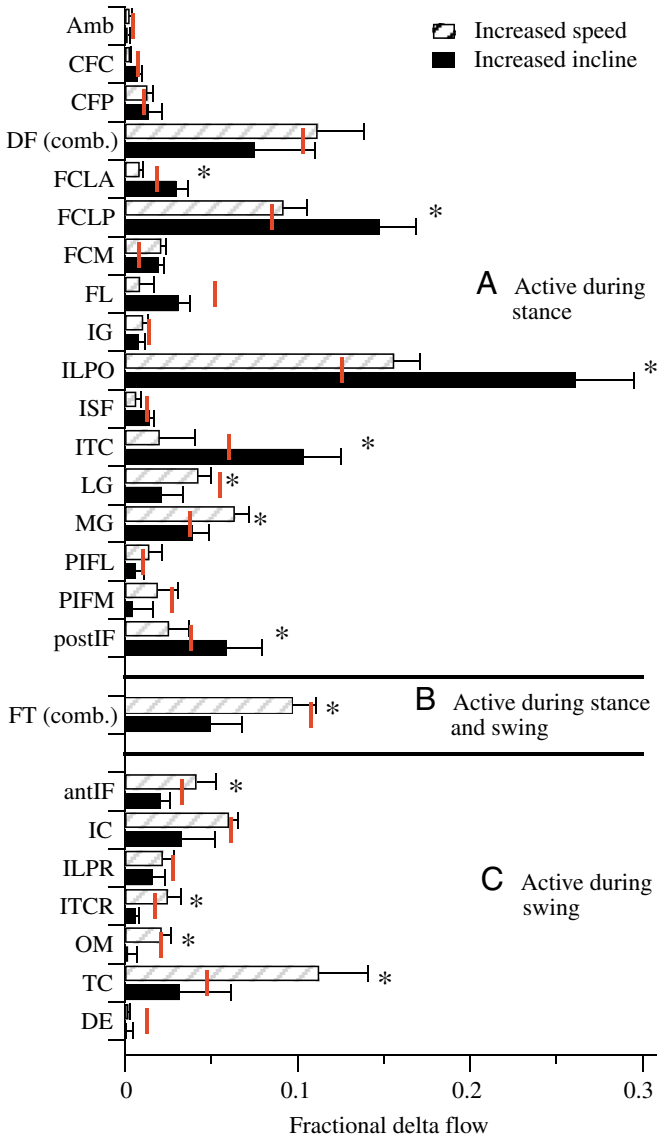

Fig. 4. Fractional increases in blood flow (F_{dQ}) above values for moderate-speed level running due to an increase in speed (hatched bars) or incline (black bars). The digital flexors (sDF-II, sDF-III, latDDF, medDDF) and the femorotibialis muscles (FTLD, FTLP, FTI, and FTM) have been combined into a digital flexor group and femorotibialis group, respectively. Muscles are also grouped into those active during swing and stance (Marsh et al., 2004). The femorotibialis group is assigned both swing and stance phase activity (Marsh et al., 2004). Values are means ± s.e.m. (N=8). *Significant difference (P<0.05) in the F_{dQ} values resulting from an increase in speed or incline (Wilcoxon nonparametric test). The red bars represent the fractional increases in flow predicted if the increased flow was distributed according to muscle mass. Abbreviations are defined in Table 1.

restricted to stance phase, comparing the distribution of blood flow among just those muscles active in stance is useful. Of the stance-phase muscles, parallel and pinnate fibered muscles make up, respectively, 44±0.2 and 56±0.2% of the muscle mass. (This comparison is complicated by the dual function

FT, which is active in both the stance and swing phase. The percentages given include the entire mass of the FT as a pinnate stance-phase muscle.) When the animals increased speed on the level, the increase in blood flow to the stance-phase muscles was approximately equally divided between parallel (51±5%) and pinnate (49±5%) fibered muscles (Fig. 5A). This balance shifted somewhat when the increase in stance-phase flow from level to uphill running was partitioned across these muscle groups. In this case, the parallel fibered muscles received 61±5% of the increase in flow, a value significantly (Wilcoxon signed rank test, P=0.05) greater than the 39±5% going to pinnate stance muscles (Fig. 5A).

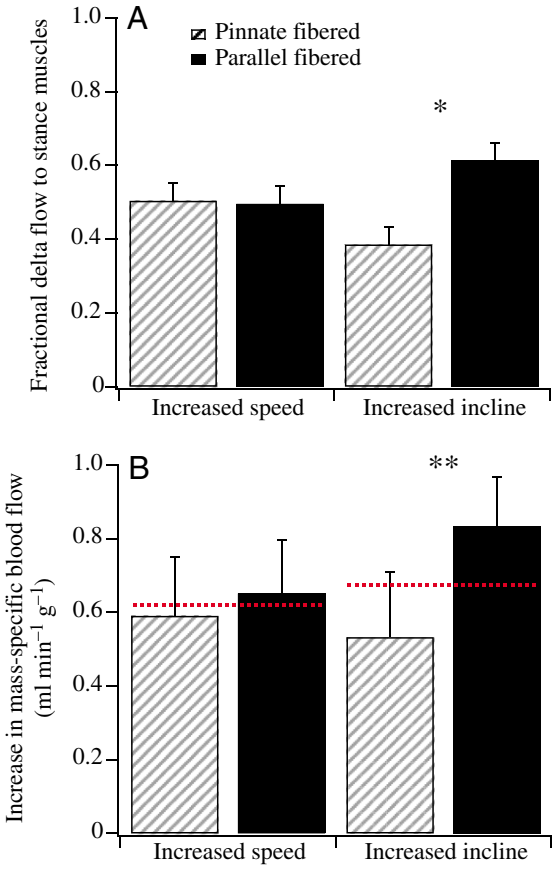

Fig. 5. (A) Fractional increases in blood flow (F_{dQ}) above values for moderate-speed level running due to an increase in speed or incline for parallel-fibered stance muscles (black bars; ILPO, FCLA, FCLP, postIF, FCM, PIFL, PIFM, CFC, CFP, ISF) and pinnate-fibered stance muscles (hatched bars; AMB, ITC, sDF-II, sDF-III, latDDF, medDDF, FHL, FDF&FB, FL, LG, MG, IG, FTLD, FTLP, FTI, FTM). *Significant difference (P<0.05, Wilcoxon test, paired samples) in the values of F_{dQ} between pinnate and parallel groups during incline running. (B) Increases in mass-specific blood flow above values for moderate-speed level running due to an increase in speed or incline for parallel-fibered stance muscles (black bars) and pinnate-fibered stance muscles (hatched bars). The broken red lines represent the average mass-specific increase in blood flow to all stance phase muscles. Values are means ± s.e.m. (N=8). **Significant difference (P<0.005; paired t-test) in the increase in mass-specific blood flow between the pinnate and parallel muscle groups during incline running. The increase in blood flow to the FT muscles was divided in half for the fast running condition because it is active during both stance and swing (Marsh et al., 2004). The increase in blood flow to the FT muscles was assumed to occur completely during the stance phase during uphill running. Abbreviations are defined in Table 1.

We also compared the increase in mass-specific blood flow (ml min^{-1} g^{-1}) between the pinnate- and parallel-fibered stance-phase muscles (Fig. 5B) using paired t-tests corrected for multiple comparisons with the Bonferroni procedure. When comparisons were made within architectural groups, no significant differences were found between the uphill or fast running groups. When pinnate and parallel groups were compared within each running condition a significant difference was found in the mass-specific increase in flow due to incline (P<0.004), but not due to speed.

Another way to ask whether the pinnate and parallel fibered muscles contribute in proportion to their mass is to compare the mass-specific increases in flow to the mean mass-specific increase in flow to all stance-phase muscles using a one-sample t-test (Fig. 5B). With this test, the mean mass-specific increases in blood flow to parallel and pinnate stance-phase muscles were not significantly different from the mean mass-specific increases in flow to all of the stance-phase muscles for either the transition to fast running or uphill running (P>0.05).

With increasing speed in level running, the largest fractional increase in stance-phase muscle blood flow was to muscles with actions at the hip, followed by muscles acting at the ankle and toes, and the lowest fraction going to muscles acting as knee extensors (Fig. 6A). This same rank order was found for the fractional increase in flow between level and uphill running (Fig. 6A), but the F_{dQ} to the hip muscles was significantly larger than that found for increased speed (Wilcoxon signed rank test, P<0.05). The distribution of flow among the stance-phase muscles, according to the joints at which they act, follows the distribution of muscle mass so that the mass-specific flow across joints is approximately constant (Fig. 6B).

The other significant shift in the distribution of the increase in flow was between stance and swing phase muscles (Figs 6A and 7). Approximately 70% of the increase in blood flow due to increasing running speed on the level went to stance-phase muscles (Fig. 7). The distribution of the increase in blood flow between stance- and swing-phase muscles in the transition from level to uphill running was significantly different (Wilcoxon signed rank test, P<0.05), with approximately 90% of the increase in blood flow going to stance-phase muscles (Fig. 7).

Discussion

Running uphill exacts a large metabolic cost compared to running on level ground at the same speed. Yet, which muscles consume the additional metabolic energy of incline running has remained unclear. Using oxygen consumption and blood flow measurements in running guinea fowl, we have demonstrated that the additional metabolic cost of incline running in this species is shared across the majority of hindlimb muscles,

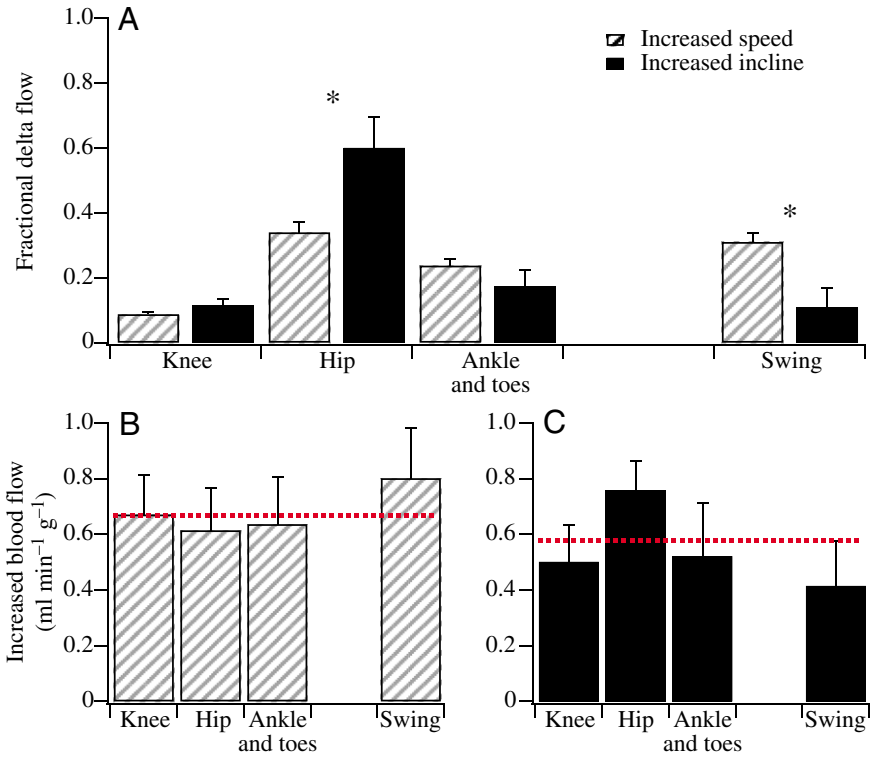

Fig. 6. (A) Fractional increases in blood flow (F_{dQ}) above values for moderate-speed level running due to an increase in speed (hatched bars) or incline (black bars) for muscles grouped by their actions in swing[†] or stance. Within the stance-phase group, muscles were further divided according to the joint at which they have their primary action[‡]. Values are means ± s.e.m. (N=8). *Significant difference (P<0.05, Wilcoxon test) between the values for speed and incline conditions. (B) Increases in mass-specific blood flow due to an increase in speed (grouped as in A). (C) Increases in mass-specific blood flow due to an increase in incline (grouped as in A). Values are means ± s.e.m. (N=8). The broken red lines in B and C represent the average mass-specific increase in blood flow to all hindlimb muscles. [†]Swing and stance phase muscle groups: the increases in flow to all but one muscle complex were assigned to either swing or stance, as indicated in Table 1. The increases in blood flow to the heads of the FT muscle were divided equally between swing and stance during level running because it is active in both phases. During uphill running, the increase in blood flow to this muscle was assumed to result from increased metabolism during stance only. [‡]Grouping of stance-phase muscles by joint action: because the ILPO has extensor moments at both the hip and the knee, the increases in flow to this muscles were divided between the hip (75%) and knee (25%), approximately reflecting the relative moment arms at these two joint. The flow to the other muscles was assigned as follows: Hip: FCLA, FCLP, ITC, postIF, FCM, PIFL, PIFM, CFC, CFP, ISF and ILPO (in part); Knee: FT, and ILPO (in part); Ankle and toes: sDF-II, sDF-III, latDDF, medDDF, FHL, FDL&FB, FL, LG, MG, IG. These assignments are not without ambiguities (see text).

including both stance- and swing-phase muscles. Blood flow measurements indicate that the increase in energy expenditure between level and uphill running is significantly biased toward stance-phase extensor muscles with parallel fibers and short tendons that are considered well suited for performing positive work against gravity. However, our results also show that pinnate stance-phase muscles as well as swing-phase muscles contribute substantially to the increase in metabolic energy expenditure during uphill running and their importance should not be dismissed.

Metabolic energy expenditure and total blood flow

The rates of oxygen consumption ($\dot{V}_{O_2}$) during level and incline running in the present study are similar to those measured in previous studies on guinea fowl energetics (Ellerby et al., 2003; Ellerby et al., 2005). The rate of total blood flow to the leg muscles during level running at 1.5 m s^{-1} and ~2.4 m s^{-1} are, likewise, similar to those obtained previously (Ellerby et al., 2005) on comparably sized guinea fowl. Importantly, the increases in metabolic rate and total blood flow to the leg muscles are proportional (Fig. 3), which is consistent with the view that blood flow is a reliable indicator of skeletal muscle metabolic rate (Ellerby et al., 2005; Marsh and Ellerby, 2006). Examining the contribution of individual muscles with statistically significant increases in flow allowed

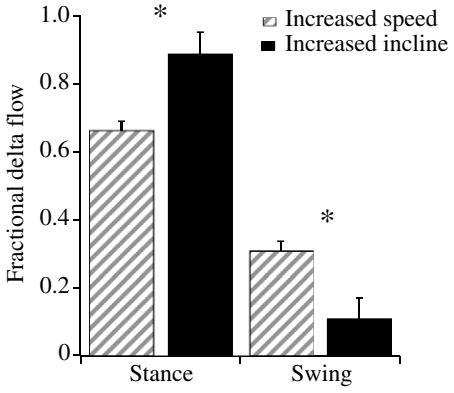

Fig. 7. Fractional increases in blood flow (F_{dQ}) above values for moderate-speed level running due to an increase in speed (hatched bars) or incline (black bars) for stance muscles *versus* swing muscles (for division see Table 1). Stance and swing muscles were assigned following Marsh et al. (Marsh et al., 2004) with two differences. (1) All of the increase in blood flow to the FT during incline running was assumed to occur during stance, and (2) in the present study we measured separately the blood flow to the swing and stance portions of the IF. Values are means ± s.e.m. (N=8). *Significant difference (P<0.05, Wilcoxon test) in the F_{dQ} values for speed and incline conditions.

us to account for 90% of the overall increase in blood flow to the leg muscles. Thus, we are confident that the distribution of energy use among the leg muscles that we describe represents most of the increases in energy use associated with slope and speed.

Although this study did not directly examine maximal aerobic energy expenditure, the results may offer an important clue to the differences in maximal aerobic capacity between level and uphill running. In a previous study of guinea fowl, Ellerby et al. found that the maximal oxygen consumption ($\dot{V}_{O2max}$) in guinea fowl was 6% greater when running uphill compared to the value measured during level running (Ellerby et al., 2003). Studies in humans and horses have also found that $\dot{V}_{O2max}$ is significantly greater during uphill running compared to the value in level running (Hermansen and Saltin, 1969; Paavolainen et al., 2000; McDonough et al., 2002). The present study indicates that the distribution of energy use changes among muscles when running uphill (Fig. 4), supporting the hypothesis that task-specific maximal metabolic rates result from altered muscle recruitment. Likely candidates for the increase in the maximal aerobic capacity during incline running in guinea fowl are the iliotrocantaricus caudalis (ITC) and fibularis longus (FL) muscles. These muscles are in a group of muscles that during level running contribute greatly to increases in energy use at low speeds, but decrease their fractional contribution to increasing energy use at high speeds (Ellerby et al., 2005). In some muscles in this group, e.g. the pubo-ischio-femeralis medialis, the limited increase in energy use at higher speeds likely indicates that the aerobic capacity of the muscle is fully utilized at lower speeds (Ellerby et al., 2005). However, for the ITC and FL our data support the hypothesis that the energy use levels off during high-speed level running because the mechanics of level running do not require large increases in their recruitment at higher speeds, and not because their aerobic capacity is reached. In the present study, the increases in energy use for the ITC and FL with increasing speed on the level were not statistically significant. However, when the mechanical demands of running were altered by uphill running, the increases in energy use by these muscles were substantial, and together accounted for approximately 15% of the total increase in energy use caused by running uphill. This value is large enough that the additional volume of active muscle resulting from the recruitment of these muscles during incline running could explain the increased capacity for aerobic metabolism when running uphill.

Distribution of energy use during level versus incline running

Strap-like muscles with parallel fibers and short tendons have been hypothesized to be primarily suited to function as motors, doing positive work during the locomotor cycle (Biewener and Roberts, 2000). Pinnate muscles, on the other hand, have been viewed to function primarily as struts, doing little mechanical work but instead tensioning tendon springs and allowing the storage and release of elastic strain energy (Biewener and Roberts, 2000). These conclusions have been tempered by recent studies that have found that pinnate muscles in birds are able to increase mechanical work production during incline running and may produce net positive work during level running as well (Daley and Biewener, 2003; Gabaldón et al., 2004). However, these studies of the mechanics of individual muscles are hard to relate quantitatively to the total energy used to perform the extra mechanical work of incline running, and one could still hypothesize that most of the mechanical work is done by the parallel-fibered muscles.

This hypothesis leads to the prediction tested in this study, that the increase in metabolic energy expenditure required to do the positive work against gravity during incline running is consumed primarily by parallel-fibered muscles active during stance. These muscles did increase their energy use to a greater extent in response to an increase in slope than to an increase in speed. However, we also found that a considerable portion of the increase in energy use is due to other muscles, including pinnate stance-phase muscles and muscles active during swing. Indeed, blood flow to the majority of hindlimb muscles increased significantly between running at 1.5 m s^{-1} on the level and on a 15% gradient (Table 1). These findings suggest that the altered demand for mechanical energy production, and thus metabolic energy use, during incline running is likely accommodated by many muscles, including those that are viewed to function as economic force generators during level running.

Although blood flow increased significantly to the majority of leg muscles due to increasing slope or speed, the increase in energy use was distributed differently among the leg muscles between the two methods of altering exercise intensity. One way to highlight how the distribution of energy among muscles was affected by a shift in exercise intensity is to calculate the fraction of the total increase in blood flow between exercise conditions attributed to individual muscles or muscle groups (fractional delta flow, F_{dQ}). The muscle fractional delta flows between the moderate-speed and fast level running conditions were similar to those observed previously (Ellerby et al., 2005). Only minor exceptions exist, possibly because of the slower speeds used for the fast run in the present study. Several novel patterns emerge during uphill running. First, the majority (54%) of the increase in energy during incline running is attributed to only three muscles: the iliotibialis lateralis pars postacetabularis (ILPO), the flexor cruris lateralis pars pelvica (FCLP) and the iliotrocantericus caudalis (ITC). A large contribution to the increase in energy expenditure by the ILPO and FCLP is not unique to incline running, as can be seen from their high F_{dQ} between moderate-speed and fast level running. However, a substantially larger contribution to the elevated energy use is apparent in these muscles during uphill running, and is greater than that predicted on the basis of their mass (Fig. 4). For example, the ILPO, which made up 13% of the hindlimb muscle mass, was responsible for 26% of the increase in energy use with incline, whereas it contributed 16% to the increase in energy use due to speed.

Association between muscle–tendon and musculoskeletal architecture and blood flow

The large contributions of the ILPO and FCLP to the additional metabolic cost of incline running are consistent with the general prediction based on muscle–tendon architecture that muscles with parallel fibers and small external tendons should function to do work. The ILPO is both a hip and knee extensor, and therefore can provide mechanical work against gravity at both of these joints when moving uphill. The mechanical actions of the FCLP are potentially complex. It can act in concert with the FCLA as a pure hip extensor. However, its attachment to the tibia allows it also to function as a knee flexor, and its connection to the intermediate gastrocnemius gives it an ankle extensor action when it is co-active with this muscle (Ellerby et al., 2002). Because, similar to the FCLP, the FCLA shows a much larger increase in fractional energy use due to increasing slope rather than to increasing speed (Fig. 4), we hypothesize that the hip extensor function of the FCLP is of prime importance during uphill running.

An increased energy use resulting from increasing slope was also seen in bi-articular stance-phase muscles that tend to flex the knee, but extend the hip. Particularly prominent in this group is the posterior iliofilbularis (postIF), which was responsible for 6% of the increase in energy use due to increasing slope, an F_{dQ} nearly twice as large as that resulting from an increase in level running speed. Why the bi-articular postIF used more energy during incline running than during fast level running is unclear. One possibility results from the observation that mammalian bi-articular hip and knee flexors (hamstring muscles) may function to transfer energy between the knee and hip joints (Jacobs et al., 1996). If the postIF functions similarly, it would allow knee extensor muscles, such as the femerotibialis, to provide some of the work of lifting the center of mass during uphill running that would otherwise need to be produced by hip extensors.

A surprising finding is the large contribution of the iliotrochantericus caudalis muscle (ITC) to the increase in energy use between level and uphill running. The ITC is a large, highly pinnate muscle, that originates from the illium and inserts on the femoral trochanter *via* an aponeurotic tendon (Gatesy, 1999b). Hutchinson and Gatesy speculated (Hutchinson and Gatesy, 2000) that the primary role of ITC is to produce the internal rotation moment about the long axis of the femur during stance that is required by the horizontal femoral posture in birds (Carrano, 1998). If action about the long axis of the femur is the primary function of the ITC, elevated energy use by this muscle during incline running would most likely result from: (1) an increase in the internal rotation moment at the hip, (2) an increase in the rate of force development that requires recruiting faster, less economical, muscle fibers and/or (3) an increase in the mechanical work due to femoral long-axis rotation. Although we have no direct data dismissing these possibilities, we have no reason to suspect that any occur during uphill running in guinea fowl. During uphill running the average vertical force over one stride is not different from level running, the medio-lateral joint posture appears unchanged (albeit from visual inspection only), and the ground contact times are similar (R.L.M. and J. A. Carr, unpublished data). An alternative possibility is that the ITC is not only involved in providing an internal rotation moment at the hip but also functions to actively extend the hip. Despite its location anterior to the hip, the ITC could contribute to hip extension because its insertion is dorsal to the center of rotation of the hip joint (J.R. and R.L.M., unpublished observations). The increased metabolic energy used by the ITC with uphill running could possibly have resulted from greater force production due to a shift in the load sharing amongst the hip internal rotator and/or hip extensor muscles or altered limb posture, but evidence on these points is lacking. Clarifying the functional reasons for the surprisingly large contribution of the ITC to the increased energy use of incline running will require more detailed analyses of its musculoskeletal architecture and *in vivo* mechanical function.

Despite the uncertainty regarding the determinants of the ITC energetics, the large contribution of this highly pinnate muscle to the increase in energy demand resulting from incline running highlights the fact that muscle–tendon architecture alone has limited power in predicting the effect of an increased demand for mechanical work on the energy use among muscles during locomotion. Depending on the musculoskeletal architecture and the temporal distribution of work required during a movement, pinnate muscles may be equally suited for doing positive mechanical work as are parallel fibered muscles. Although the function of pinnate muscles in providing work has been particularly emphasized during jumping (Roberts and Marsh, 2003), previous studies have also shown that this type of muscle can function to produce work effectively during running, e.g. the lateral gastrocnemius during incline running in turkeys (Roberts et al., 1997; Gabaldón et al., 2004) and the fibularis (peroneus) longus in the same species both in level and uphill running (Gabaldón et al., 2004). For the FL, particularly intriguing similarities exist between data on energy use in running guinea fowl (Ellerby et al., 2005) (this study) and mechanical work production by this muscle in running turkeys (Gabaldón et al., 2004). In guinea fowl, energy use by the FL did not increase significantly as speed was increased above the moderate running speed of 1.5 m s^{-1}, but energy use by this muscle did increase significantly as the birds switched from level running to uphill running at 1.5 m s^{-1} (Table 1). Similarly, in running turkeys mechanical work output by the FL does not increase during level running as speed is increased above 2 m s^{-1}, but increases substantially if the bird runs uphill at this moderate running speed (Gabaldón et al., 2004).

The idea that muscle–tendon architecture does not greatly constrain a muscle's ability to do mechanical work during incline running is also consistent with the overall distribution of energy use by the parallel and pinnate fibered stance-phase muscles considered as groups (Fig. 5A,B). When the birds increased speed in level running these muscle groups supplied equivalent fractions of the increase in energy use. When increase in energy use was caused by switching from level to incline running the balance of energy use by these muscle

groups shifted significantly, and approximately 60% of the increases in energy use occurred in parallel fibered muscles. However, approximately 40% of the increase in metabolic energy use by stance-phase muscles between level and incline running was attributed to pinnate stance-phase muscles.

The large increase in energy use by pinnate muscles during incline running suggests the straightforward hypothesis that these muscles contribute importantly to the increase in mechanical work production required to move uphill. This hypothesis is consistent with the available data on the mechanical function of pinnate ankle extensors in turkeys. However, the possibility exists that some of the increase in energy use in these muscles was due to an increase in force production. Increased force production could have been required if the mean net joint moments increased as a result of altered posture or ground reaction force orientation, or alternatively, if the force sharing among synergist muscles changed. Partial support for this idea comes from the data of Daley and Biewener, who found a significant increase in mean force production in the pinnate gastrocnemius complex between level and incline running at the same speed in guinea fowl (Daley and Biewener, 2003). However, this same study estimated that work production by the lateral gastrocnemius increases more than does force production. Additionally, Gabaldón et al. demonstrated an increase in work output with no increase in force output during uphill running in the pinnate lateral gastrocnemius and fibularis longus of turkeys (Gabaldón et al., 2004). Thus, although increased force production when running uphill could be a reason for the increase in energy use by pinnate muscles, current evidence favors an increase in work output as the major factor.

Blood flow to proximal versus distal limb muscles

The relative contribution of proximal and distal muscles to producing the mechanical work associated with incline running has received considerable attention (Biewener and Gillis, 1999; Gillis and Biewener, 2002; Biewener et al., 2004; Roberts and Belliveau, 2005). Some authors argue that incline running requires a shift in motor recruitment favoring proximal muscles (Biewener and Gillis, 1999; Biewener et al., 2004). This view stems from the observation that distal muscles, in general, posses a highly specialized muscle–tendon architecture (short fibered, pinnate muscles with long compliant tendons) that may limit their role as motors. Some evidence exists for a division of labor between proximal and distal muscles. Increases in muscle strain associated with incline locomotion have been observed in the proximal muscles of rats (Gillis and Biewener, 2002), and large muscle strains have been measured in a proximal muscle of jumping dogs (Gregersen and Carrier, 2004). A recent modeling study (Sasaki and Neptune, 2006) also indicates that the majority of muscle fiber work occurs in proximal muscles during level running in humans, although the gastrocnemius contributes substantially. Moreover, direct measurements of muscle work in the distal limb muscles of wallabies hopping uphill have shown that they produce little of the mechanical work of elevating the center of mass (Biewener et al., 2004). However, in contrast to these findings, distal muscles in turkeys are used to produce considerable amounts of mechanical work during uphill running (Roberts et al., 1997; Gabaldón et al., 2004).

One shortcoming of these previous studies is that they examined only a small fraction of the total hindlimb muscle mass. In an alternative approach, Roberts and Belliveau measured the net joint work at the ankle knee and hip during level and incline running in humans (Roberts and Belliveau, 2005). They found that the majority of the increase in mechanical work with incline running is produced at the hip. However, relating these findings to the distribution of muscle work is difficult due to the limits of inverse dynamic modeling (e.g. co-contraction and energy transfer by two joint muscles).

The present study offers a novel approach in exploring the distribution of energy use among distal and proximal muscles during level and incline locomotion. By grouping muscles that have primary functions at the hip, knee or ankle and toes, we have calculated the relative contribution of each muscle group to the increase in energy associated with running faster or running uphill (Fig. 6A). The complex musculoskeletal architecture of the limb makes some of these assessments of energy use across joints ambiguous. For example, several large hamstring-like muscles in the posterior thigh (FCLP, FCM. postIF) are grouped as hip extensors, and the lateral gastrocnemius and the digital flexors are grouped as ankle extensors. However, these muscles can also produce knee flexor moments and could be expending energy at the knee by co-contracting with knee extensors. This type of energy use is not included in the analyses here, or those by other investigators.

Before considering the uphill data, the substantial contribution of the stance-phase muscles with actions at the hip to the increase in energy expenditure between moderate-speed and fast level running should be noted. Energy use by these muscles represented 34% of the total increase in energy use, or 48% of the increase in stance-phase energy use, resulting from increasing speed. The fact that much of the muscle mass in this group of muscles represents parallel fibered muscles, suggests that increases in work output may play an important role in the increases in energy use due to speed as well as those due to slope. Interestingly, the distribution of the increased energy use due to running faster reflects the distribution of mass among the muscles acting at the different joints during stance and those required for swinging the limb (Fig. 6B). This evidence supports the view that musculoskeletal structure is matched to locomotor demand (Weibel, 2000).

The increase in energy use by stance muscles with actions at the hip that results from increasing slope is even more striking. Approximately 60% of the total increase in blood flow, or 70% of the increase in flow to stance-phase muscles as the birds switched from level to incline running, was due to this group of muscles. This finding provides strong evidence, albeit indirect, corroborating the view that hip muscles produce the majority of the mechanical work of elevating the body during incline running. Future studies examining the

mechanical behavior of proximal muscles are required to fully understand their role during level and incline locomotion.

Blood flow to stance and swing muscles

Our results showed that, as predicted, most (89%) of the increase in muscle energy use between level and incline running occurred in stance muscles (Fig. 7), and thus the fractional contribution of the swing-phase muscles to total energy use was less during uphill as compared to that found during level running. This result contrasts with the relatively constant fraction of energy use by swing-phase muscles resulting from an increase in speed (this study) (Marsh et al., 2004). The large contribution of the stance-phase muscles during uphill running was expected because they are responsible for producing the required increase positive work on the body center of mass.

Because swing times are similar in level and uphill running in guinea fowl (R.L.M. and J. A. Carr, unpublished data) one would expect little change in the mechanical work required to swing the limbs with increasing slope. Contrary to this expectation, several major swing-phase muscles (anterior iliofibularis, iliotibialis cranialis, and iliotibialis lateralis pars preacetabularis) exhibited significant increases in blood flow between level and uphill running. The overall contribution of these muscles to the total increase in energy use was approximately 11% (Fig. 7). One possible explanation of the increased swing-phase energy use is that in guinea fowl all of the joints show greater angular changes over the swing phase (R.L.M., J.R., J. A. Carr and T. A. Hoogendyk, unpublished data). Accomplishing a greater excursion would presumably require a greater amount of mechanical work, and thus energy use. Additionally, during uphill running, the limb segments must be elevated independent of the center of mass during each stride, and therefore small increases in the metabolic cost of swinging the limb may also occur due to work against gravity. Interestingly, increased net joint work at the hip has been observed during the swing-phase of incline running in humans compared to level running at the same speed (Swanson and Caldwell, 2000). Although the increase in energy expenditure between level and uphill running attributed to swing-phase muscles is relatively small, it is an important reminder that swing-phase costs must not be ignored when drawing conclusions on the mechanical determinants of the energy cost of locomotion (Marsh et al., 2004).

Delta efficiency and its biological relevance

Several authors have used delta efficiency (the additional metabolic energy expenditure divided by the additional mechanical energy expenditure between two exercise conditions) to base interpretations on the energetics of locomotion (e.g. Whipp and Wasserman, 1969; Taylor et al., 1972; Donovan and Brooks, 1977). Delta efficiency is often assumed to represent the efficiency of muscles performing work. For instance, in the case of incline running, Taylor and colleagues (Taylor et al., 1972), and later Cohen et al. (Cohen et al., 1978), suggested that delta efficiency is nearly constant, reflecting the narrow range of efficiencies observed for isolated skeletal muscle (Woledge et al., 1985). Superficially, our data could be interpreted as supporting this suggestion. The delta efficiency calculated in this study was 36%, a value similar to that of several other species locomoting uphill (Taylor et al., 1972; Cohen et al., 1978; Kram and Dawson, 1998). Moreover, the metabolic cost of lifting 1 kilogram of body mass 1 meter vertically in guinea fowl (27.4 J kg^{-1} m^{-1}) agrees well with that predicted for animals in general (Cohen et al., 1978).

However, in a detailed comparative analysis of running energetics, the concept of a constant delta efficiency for incline running has been refuted (Full and Tullis, 1990). Indeed, for some species the cost of incline running differs by as much as 150% from that predicted based on a constant efficiency of performing mechanical work against gravity. Furthermore, delta efficiencies calculated for incline running are often much greater (Taylor et al., 1972; Bijker et al., 2001) (this study) than the maximum efficiency of approximately 25% expected for skeletal muscle. These findings suggest that delta efficiency is likely a poor indicator of muscle efficiency during incline running.

The potential errors in estimating muscle efficiency based on delta efficiencies have been summarized well elsewhere (Stainbsy et al., 1980). For delta efficiencies to be valid, the metabolic energy attributed to the baseline measure must not be altered with an increase in workload. This poses a particular problem for incline running. For instance, the metabolic energy attributed to a muscle acting isometrically and facilitating tendon elastic energy storage and release during level running is part of the baseline expenditure. If the action of these muscles is altered during uphill running, along with their metabolic energy expenditure, it follows that the baseline energy use has also been altered.

Conclusion

The metabolic cost of running increases dramatically when animals switch from level running to running uphill, a consequence of doing positive work against gravity. The present results indicate that the additional metabolic cost of incline running is shared across most hindlimb muscles. The increase in energy expenditure is biased toward stance-phase muscles traditionally thought to be ideal for work production, namely proximal, parallel-fibered extensor muscles with short tendons. Nevertheless, considerable energy is expended by pinnate muscles that have often been thought to be specialized for economic force production, as well as by muscles with flexor actions, and also some swing-phase muscles. These findings suggest that neither muscle–tendon nor musculoskeletal architecture greatly restricts the ability of muscles to do work during locomotor tasks such as uphill running, and that the added energy cost of running uphill is not solely related to the work required to lift the body center of mass.

Supported by NIH grant AR47337 to R.L.M. We are grateful to Jennifer Carr and Tom Hoogendyk for assistance in data collection and tissue processing and two anonymous

reviewers for helpful comments and criticisms. We also thank Dr Stephen M. Gatesy for a helpful discussion of the nomenclature of the femerotibialis muscle complex.

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