

RESEARCH ARTICLE

The metabolic cost of walking on gradients with a waddling gait

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SUMMARY

Using open-flow respirometry and video footage (25 frames⁻¹), the energy expenditure and hindlimb kinematics of barnacle geese, *Branta leucopsis*, were measured whilst they were exercising on a treadmill at gradients of +7 and –7 deg, and on a level surface. In agreement with previous studies, ascending a gradient incurred metabolic costs higher than those experienced on level ground at comparable speeds. The geese, however, are the first species to show an increased duty factor when ascending a gradient. This increased duty factor was accompanied by a longer stance time, which was probably to enable the additional force required for ascending to be generated. Contrary to previous findings, the geese did not experience decreased metabolic costs when descending a gradient. For a given speed, the geese took relatively shorter and quicker strides when walking downhill. This ‘choppy’ stride and perhaps a lack of postural plasticity (an inability to adopt a more crouched posture) may negate any energy savings gained from gravity’s assistance in moving the centre of mass downhill. Also contrary to previous studies, the incremental increase in metabolic cost with increasing speed was similar for each gradient, indicating that the efficiency of locomotion (mechanical work done/chemical energy consumed) is not constant across all walking speeds. The data here suggest that there are species-specific metabolic responses to locomotion on slopes, as well as the established kinematics differences. It is likely that a suite of factors, such as ecology, posture, gait, leggedness and foot morphology, will subtly affect an organism’s ability to negotiate gradients.

Key words: barnacle geese, energetics, kinematics, locomotion, respirometry.

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INTRODUCTION

Most terrestrial animals are required, at some point, to locomote up or down gradients. Ascending an incline should be more mechanically costly than locomotion on level ground, because of the requirement for additional work to increase the potential energy of the centre of mass (CoM) as it ascends the slope (Parsons et al., 2008). To produce this extra mechanical work, a greater volume of limb muscle may be recruited (Carlson-Kuhta et al., 1998; de Leon et al., 1994; Gardiner et al., 1982; Leroux et al., 1999; Pierotti et al., 1989; Robert et al., 2000; Roy et al., 1991) or muscle-shortening strains increased (Gillis and Biewener, 2002). Additional muscle activity means that whole-animal metabolic costs (rate of O₂ consumption, \dot{V}_{O_2}) should also increase (Ellerby et al., 2003), assuming that additional costs are met aerobically. Accordingly, increased metabolic costs were found in birds (Bamford and Maloij, 1980; Ellerby et al., 2003; Rubenson et al., 2006; Snyder and Carello, 2008; Warncke et al., 1988), humans (Dill, 1965; Margaria et al., 1963; Minetti et al., 2002; Yousef et al., 1972), a range of quadrupeds (Armstrong et al., 1983; Bedford et al., 1979; Brooks and White, 1978; Chassin et al., 1976; Clapperton, 1964; Cohen et al., 1978; Eaton et al., 1995; Farley and Emshwiller, 1996; McDonough et al., 2002; Raab et al., 1976; Robbins et al., 1979; Snyder and Carello, 2008; Taylor et al., 1972; White and Yousef, 1978; Wickler et al., 2000; Wickler et al., 2003; Wunder and Morrison, 1974; Yousef et al., 1972) and invertebrates (Full and Tullis, 1990; Tullis and Andrus, 2011) when ascending gradients. Although there are notable exceptions – the marabou stork *Leptoptilos crumeniferus* (Bamford and Maloij, 1980), cockroach

Gromphadorhina portentosa (Herreid et al., 1981) and ant *Camponotus* sp. (Lipp et al., 2005) – generally, for most species, as gradient increases so does \dot{V}_{O_2} at all locomotory speeds (Bedford et al., 1979; Brooks and White, 1978; Chassin et al., 1976; Clapperton, 1964; Cohen et al., 1978; Eaton et al., 1995; Ellerby et al., 2003; Full and Tullis, 1990; Raab et al., 1976; Robbins et al., 1979; Wunder and Morrison, 1974; Yousef et al., 1972). The relative cost of ascending or descending a gradient may also be size dependent, with a lower percentage increase or decrease (compared with locomotion on the level) of locomoting uphill or downhill, respectively, for smaller animals (Taylor et al., 1972).

Based upon similar reasoning, locomoting down a decline is predicted to be less metabolically costly than locomotion on the level, because here the CoM is moving ‘with’ and not ‘against’ gravity. Descending a gradient is not metabolically free, however, because the muscles must absorb mechanical energy to decrease the potential energy of the body. The absorption of potential energy is facilitated by leg muscles, which appear to shift function in response to gradient. In both bipeds (Roberts et al., 2007) and quadrupeds (Lammers et al., 2006) the leg muscles appear to generate additional energy (an increase in positive work) to propel the CoM uphill (against gravity), whereas on a decline they shift function to energy absorption (negative work) and operate as braking struts (Lammers et al., 2006; Roberts et al., 2007; Smith et al., 1998). Opposite to the situation during incline locomotion, less limb muscle is recruited relative to that during level locomotion when travelling downhill (Kai et al., 1997; Smith and Carlson-Kuhta, 1995).

Most empirical studies have recorded reduced metabolic costs for animals descending declines (Armstrong et al., 1983; Byrnes et al., 1985; Cohen et al., 1978; Margaria et al., 1963; Minetti et al., 2002; Raab et al., 1976; Robbins et al., 1979; Taylor et al., 1972; White and Yousef, 1978; Yousef et al., 1972). Ants are an exception, but this may be due to the resolution of the respirometry equipment not allowing detection of the small differences expected in very small animals (Lipp et al., 2005). Although there are exceptions (Yousef et al., 1972), \dot{V}_{O_2} also generally decreases with a steepening decline (Cohen et al., 1978). The saving in metabolic costs when descending relative to locomotion on a horizontal surface tends not to be as great in absolute terms as the increased costs associated with ascending a slope of a similar angle (Armstrong et al., 1983; Cohen et al., 1978; Raab et al., 1976; Robbins et al., 1979; Taylor et al., 1972; White and Yousef, 1978). This implies that the whole-animal mechanical efficiency (mechanical work done/chemical energy consumed) is lower on declines.

Limb and body posture, and kinematics may also change in response to gradients (Carlson-Kuhta et al., 1998; Jayne and Irschick, 1999; Leroux et al., 1999; Smith et al., 1998). Although there are some broad trends in the responses of animals to locomoting on gradients, the findings of previous studies suggest that there are subtle species-specific differences in the kinematic responses (Gillis and Biewener, 2002). Hence, more comparative data are required before the constraints acting upon the locomotor system when negotiating slopes can be fully understood. Most research examining terrestrial locomotion with respect to gradients, including bird studies, has focused on species whose movements are primarily facilitated through terrestrial locomotion. These species are presumably highly adapted for efficient walking or running. Although capable of terrestrial locomotion, the barnacle goose has conflicting demands for flight and swimming, which compromise its morphology in terms of terrestrial locomotion. One obvious consequence of these competing demands is the barnacle goose's waddling walking gait. During their annual moult, barnacle geese are grounded and during the rearing period they spend much of their time walking with their goslings in search of food. Hence, effective terrestrial locomotion is still fundamental to a barnacle goose's survival, and as a generalist species with competing locomotor demands they may give different insight into the constraints operating on the vertebrate locomotor system than species more focused on cursoriality (Nudds et al., 2010).

In this study, barnacle geese had their metabolic rates measured whilst exercising at a range of speeds upon a treadmill on 7 deg positive and negative gradients, and on the level. It was predicted that, because barnacle geese are not specifically adapted to a terrestrial locomotory lifestyle, the metabolic cost of their uphill and downhill locomoting would be higher and not as low, respectively, relative to their level walking when compared with specialist terrestrial species.

MATERIALS AND METHODS

Study species

Five barnacle geese *Branta leucopsis* (Bechstein 1803) as used in a previous study (Nudds et al., 2010) were raised from eggs within the BSF Animal Unit at the University of Manchester. Geese were housed within a 12 m² room and provided with an area of turf and a 30 cm deep raised pond. They were maintained on a 13h:11h light:dark cycle and were given food *ad libitum* (Poultry Grower Pellets, Small Holder Range, Norfolk, UK), and were not fasted prior to the experiments. The geese were 6 months old when used in the experiments described here, at which point their body masses

(mean 1.79 kg, range 1.5–2.1 kg) had stabilised and were comparable to those of an older 17 month old cohort (mean 1.75 kg, range 1.5–2.1 kg), which were previously reared under the same conditions. The geese were imprinted from hatching and from the age of 3 months were trained regularly to walk inside a respirometry chamber upon a Tunturi T60 (Turku, Finland) treadmill. All experimental procedures were conducted in accordance with the Animals (Scientific Procedures) Act 1986 under a UK Home Office Licence held by Dr Codd (40/3001) and were approved by the University of Manchester Ethics Committee.

Indirect calorimetry

A standard open-flow respirometry system was used to measure rates (ml min⁻¹) of O₂ consumption (\dot{V}_{O_2}) and CO₂ production (\dot{V}_{CO_2}) (Lighton, 2008; Withers, 2001). The geese walked within a transparent walled chamber constructed of Perspex with internal dimensions of 66×48.5×46.5 cm and a volume of 148 l. The chamber was sealed upon the treadmill with rubber and brush seals. Air was pushed through the chamber by two vacuum pumps (models 2750CGH160 and 2688CHI44, Thomas, Sheboygan, WI, USA) at 255 l min⁻¹. A high flow rate relative to the chamber size was used to ensure quick stabilisation of the measured gas volumes, reducing the amount of time the geese had to walk. Excurrent air was sub-sampled at 0.110 l min⁻¹ downstream of the chamber and passed through an RH300 (Sable Systems International, Las Vegas, NV, USA) for measurement of water vapour pressure and relative humidity. The air was then scrubbed of H₂O (using magnesium perchlorate) and passed through a CA-10A CO₂ analyser (Sable Systems International), then scrubbed of CO₂ (using Ascarite II) prior to passing through a dual absolute and differential O₂ analyser (Oxzilla II, Sable Systems International). Therefore, the air entering the O₂ analyser was both H₂O and CO₂ free. A second O₂ analyser channel continuously measured O₂ levels in ambient air. Before analysis by the second O₂ channel, Ascarite II and magnesium perchlorate were used to scrub CO₂ and H₂O from the ambient air. The sub-sampling pumps (TR-SS3 mass flow meters, Sable Systems International) were positioned downstream of the gas analysers. Voltage outputs were recorded with a UI2 interface and ExpeData version 1.25 (both Sable Systems International). Scrubbing chemicals were supplied by Acros organics (Thermo Fisher Scientific, Fair Lawn, NJ, USA).

Because water was scrubbed from the sub-sampled air stream prior to O₂ and CO₂ analysis, the primary flow rate (FR) was adjusted to a dry-corrected flow rate (FR_c) using equation 8.6 in Lighton (Lighton, 2008):

$$FR_c = FR (P_b - P_{H_2O}) / P_b, \quad (1)$$

where P_b is barometric pressure and P_{H_2O} is water vapour pressure. \dot{V}_{O_2} was calculated from equation 10.1 of Lighton (Lighton, 2008) as:

$$\dot{V}_{O_2} = \frac{F_{I_{O_2}} - F_{E'_{O_2}}}{(1 - F_{E'_{O_2}})}, \quad (2)$$

where $F_{I_{O_2}}$ is the concentration of O₂ flowing into the respirometry chamber and $F_{E'_{O_2}}$ is that measured in CO₂-free air by the O₂ analyser after leaving the chamber. The accuracy of the respirometry system was tested by leaching N₂ into the respirometry chamber at 2 l min⁻¹ (Fedak et al., 1981). The error in \dot{V}_{O_2} measured was <1.96% with the treadmill belt running at both 1.00 and 2.00 m s⁻¹ and the time to reach a stable reading was 163 s.

The metabolic rates of the geese were measured at five walking velocities ($U=0.25, 0.50, 0.75, 1.00$ and 1.25 m s⁻¹) and at three

gradients: 7 deg decline, 7 deg incline and on the level (i.e. 0 deg gradient). The data for walking on the level were obtained from a previous publication (Nudds et al., 2010). During the walking trials a companion bird was housed in a mesh-fronted holding crate and was visible to the walking goose. A mirror was also attached to the front of the respirometry chamber facing the walking goose. The companion goose and mirror reduced anxiety for the walking goose and ensured a steady walking performance. Each goose was walked at all of the five velocities on one of the gradients during a single experiment and the order of velocities and gradients used was randomised. The geese were made to walk until measured gas concentrations had stabilised (typically between 5 and 10 min). The maximum U sustainable by all the geese for a period long enough to obtain stable gas concentration readings on each gradient was 1.25 m s^{-1} . At this speed the geese were still using a walking gait (Nudds et al., 2010). Between each walking U , before the first U measured and after the final U measured, the geese were allowed to rest until the gas concentrations recorded were stable (again 5–10 min). A stable reading when resting was taken to indicate recovery from the previous bout of walking. For each speed the O_2 and CO_2 concentrations were derived as the mean of the last 60 s (60 samples) of the period of stable readings.

Kinematics

A Sony HDR-SR8E video camera (25 frames s^{-1}) was used to film the geese at each of the five walking speeds. The video footage was analysed using Tracker 2.51 (Open Source Physics). The position of the right foot (nearest to the camera) was tracked over five strides, to calculate the gait kinematics parameters: stride length (l_{stride}), stride frequency (f_{stride}), stance phase (t_{stance}), swing phase (t_{swing}) and duty factor.

Data analyses

Differences in the relationship between the dependent variables \dot{V}_{O_2} , respiratory exchange ratio (RER), kinematics variables and U for different gradients were investigated using general linear models (GLMs). Gradient was included as a factor, goose as a random factor and U as a covariate. Goose was included in the GLM, because of the repeated measures taken for each individual, i.e. each goose was measured once at each U and for each gradient (5 speeds \times 3 gradients = 15 measurements per goose). Initially, both the slopes and intercepts of the relationship between the dependent variable and U were tested for differences between gradients. If the interaction term (gradient \times U) was non-significant, indicating similar slopes (i.e. the incremental change in the dependent variable with U was similar for each gradient), it was removed from the GLM and the GLM was rerun assuming parallel lines (similar slopes) and hence testing for a difference in the intercepts only. Where differences were found, *post hoc* Fisher's least significant difference (LSD) tests ($\alpha=0.05$) were used to indicate specific differences between the gradients.

All statistical analyses were performed using IBM SPSS Statistics v.19. Results are displayed as means \pm s.e.m. for \dot{V}_{O_2} and RER, and as means \pm s.d. for kinematics variables.

RESULTS

Rate of oxygen consumption

A GLM showed the slope of the relationship between \dot{V}_{O_2} and U was similar for each gradient (Table 1). Simplifying the GLM by specifying a common slope (Fig. 1) indicated a difference in the intercepts of the relationship between \dot{V}_{O_2} and U for the three gradients (Table 1). During incline walking, the geese had a higher

\dot{V}_{O_2} than when both decline and level walking across all speeds (Table 1, Fig. 1). Previous studies have not conducted GLM analyses. Instead, separate linear regression lines were used to determine the relationship between \dot{V}_{O_2} and U for each gradient independently. Differences in the slopes of the \dot{V}_{O_2} against U regressions were then taken as such without statistical validation. Similar separate regression analyses here resulted in lines of best fit for the three gradients tested of $y=36.85+65.51x$, $r^2=0.98$, $P<0.001$ (level), $y=34.40+70.68x$, $r^2=0.98$, $P<0.001$ (decline) and $y=40.17+80.17x$, $r^2=0.99$, $P<0.001$ (incline).

The RERs were similar on each gradient (mean 0.69 ± 0.01 , 0.72 ± 0.01 and 0.73 ± 0.02 for decline, level and incline, respectively). RER also increased predictably with U and the incremental increase in RER with U was similar for each gradient (Table 1). The mean RER for each U in ascending order (0.25, 0.50, 0.75, 1.00 and 1.25 m s^{-1}) was 0.67 ± 0.03 , 0.70 ± 0.01 , 0.71 ± 0.02 , 0.72 ± 0.02 and 0.77 ± 0.02 .

Kinematics

With the exception of duty factor, the incremental change in each kinematics variable with U was similar among gradients (Table 1, Fig. 2). On all gradients, duty factor decreased with increasing U , but was higher at all speeds during incline walking than during decline and level walking, which were similar at all speeds (Table 1, Fig. 2A).

t_{stance} and t_{swing} also decreased with U at all gradients. t_{swing} was of shorter duration during incline walking than when both decline and level walking (Table 1, Fig. 2B). Although a Fisher's LSD *post hoc* test did not detect a difference, t_{swing} also appeared to be of shorter duration on a decline than when walking on the level. In contrast, t_{stance} was of longer duration at all speeds during incline walking than when walking on level ground, which in turn showed a longer t_{stance} than when walking on a decline (Table 1, Fig. 2B).

l_{stride} was shorter and f_{stride} quicker across all speeds during decline than during both incline and level walking, which were similar at all speeds (Table 1, Fig. 2C,D).

For each GLM performed there was a significant effect of goose upon the dependent variable (Table 1). The absolute values of kinematics variables and \dot{V}_{O_2} differed between individuals. The overall (direction of) between-gradient effects and changes with U , however, were similar for each goose.

DISCUSSION

Across all speeds the metabolic cost (\dot{V}_{O_2}) of walking uphill in the barnacle geese was higher than during downhill and level walking, which had similar costs. The increase in metabolic cost when moving up an incline agrees with previous studies (Armstrong et al., 1983; Bamford and Maloij, 1980; Bedford et al., 1979; Brooks and White, 1978; Chassin et al., 1976; Clapperton, 1964; Cohen et al., 1978; Dill, 1965; Eaton et al., 1995; Ellerby et al., 2003; Farley and Emshwiller, 1996; Margaria et al., 1963; McDonough et al., 2002; Minetti et al., 2002; Raab et al., 1976; Robbins et al., 1979; Rubenson et al., 2006; Snyder and Carello, 2008; Taylor et al., 1972; Warncke et al., 1988; White and Yousef, 1978; Wickler et al., 2000; Wickler et al., 2003; Wunder and Morrison, 1974; Yousef et al., 1972). The finding that descending a decline is not metabolically cheaper than locomoting on the level, however, is contrary to previous findings (Armstrong et al., 1983; Byrnes et al., 1985; Cohen et al., 1978; Margaria et al., 1963; Minetti et al., 2002; Raab et al., 1976; Robbins et al., 1979; Taylor et al., 1972; White and Yousef, 1978; Yousef et al., 1972). Likewise, the incremental cost of increasing speed was similar at each gradient, which is inconsistent with most previous

Table 1. The results of the GLMs and associated Fisher's LSD statistical tests used to determine whether gradient (grad) differentially affected \dot{V}_{O_2} , RER and each of the five kinematics variables

Dependent variable	Interaction term (grad × U) ^a	Final ANCOVA	LSD <i>post hoc</i> between gradient comparisons (mean difference)			
			Decline – level	Decline – incline	Level – incline	±95% CI
\dot{V}_{O_2}	$F_{2,65}=1.14$ $P=0.325$	Grad, $F_{2,67}=10.25$, $\eta_p^2=0.23$, $P<0.001$ Goose, $F_{4,67}=12.41$, $\eta_p^2=0.43$, $P<0.001$ U, $F_{1,67}=321.54$, $\eta_p^2=0.83$, $P<0.001$	1.42	-12.89 ^b	-14.31 ^b	6.95
RER	$F_{2,65}=2.61$ $P=0.081$	Grad, $F_{2,67}=2.50$, $\eta_p^2=0.07$, $P=0.090$ Goose, $F_{4,67}=4.80$, $\eta_p^2=0.223$, $P=0.002$ U, $F_{1,67}=15.74$, $\eta_p^2=0.19$, $P<0.001$				
Duty factor		Grad, $F_{2,65}=7.83$, $\eta_p^2=0.19$, $P=0.001$ Goose, $F_{4,65}=2.74$, $\eta_p^2=0.14$, $P=0.036$ U, $F_{1,65}=1002.84$, $\eta_p^2=0.94$, $P<0.001$ grad*U, $F_{1,65}=3.17$, $\eta_p^2=0.09$, $P=0.049$	-0.006	-0.043 ^b	-0.036 ^b	0.01
$\log_{10} t_{\text{stance}}^c$	$F_{2,65}=7.54$ $P=0.474$	Grad, $F_{2,67}=20.87$, $\eta_p^2=0.38$, $P<0.001$ Goose, $F_{4,67}=7.64$, $\eta_p^2=0.313$, $P<0.001$ U, $F_{1,67}=4526.09$, $\eta_p^2=0.99$, $P<0.001$	-0.027 ^b	-0.051 ^b	-0.024 ^b	0.016
$\log_{10} t_{\text{swing}}^c$	$F_{2,65}=1.36$ $P=0.264$	Grad, $F_{2,67}=15.38$, $\eta_p^2=0.32$, $P<0.001$ Goose, $F_{4,67}=3.26$, $\eta_p^2=0.16$, $P=0.017$ U, $F_{1,67}=405.30$, $\eta_p^2=0.86$, $P<0.001$	-0.018	0.032 ^b	0.049 ^b	0.018
$\log_{10} l_{\text{stride}}^c$	$F_{2,65}=0.31$ $P=0.733$	Grad, $F_{2,67}=9.39$, $\eta_p^2=0.22$, $P<0.001$ Goose, $F_{4,67}=10.52$, $\eta_p^2=0.39$, $P<0.001$ U, $F_{1,67}=1250.36$, $\eta_p^2=0.95$, $P<0.001$	-0.021 ^b	-0.021 ^b	0.000	0.011
f_{stride}	$F_{2,65}=0.77$ $P=0.466$	Grad, $F_{2,67}=7.68$, $\eta_p^2=0.19$, $P=0.001$ Goose, $F_{4,67}=9.01$, $\eta_p^2=0.35$, $P<0.001$ U, $F_{1,67}=3531.67$, $\eta_p^2=0.98$, $P<0.001$	0.090 ^b	0.086 ^b	-0.004	0.052

Walking speed (U) is a covariate in each ANCOVA.

\dot{V}_{O_2} , rate of oxygen consumption; RER, respiratory exchange ratio; t_{stance} , stance time; t_{swing} , swing time; l_{stride} , stride length; f_{stride} , stride frequency; GLM, general linear model; LSD, least significant difference.

^aThe interaction term was not statistically significant and therefore was not included in the final ANCOVA.

^bThe mean difference between the gradients ±95% CI did not encompass zero. Therefore, the gradient means are statistically different at $\alpha=0.05$.

^cThe independent variable U was \log_{10} transformed.

studies that have found the slope of the relationship between \dot{V}_{O_2} and U increases with incline angle and decreases with decline angle (e.g. Chassin et al., 1976; Clapperton, 1964; Cohen et al., 1978; Margaria et al., 1963; Raab et al., 1976; Taylor et al., 1972; White and Yousef, 1978; Wunder and Morrison, 1974; Yousef et al., 1972). The findings of the current study, then, pose two significant questions. First, why do the barnacle geese not save metabolic energy when walking downhill, and second, why is the incremental increase in \dot{V}_{O_2} with U independent of gradient?

As highlighted by Gillis and Biewener (Gillis and Biewener, 2002), there is a dearth of studies providing comparative whole-limb kinematics data where animals are encouraged to walk on gradients (less for declines than inclines) and on level ground at similar speeds, and even fewer studies where metabolic data were collected simultaneously (Table 2). Consequently, making specific statements about the effects of gradients on limb kinematics may be premature. Nevertheless, it is possible to identify some broad trends (Table 2) and the data do hint at why in barnacle geese the metabolic costs are the same for decline as for level walking. The changes in barnacle goose kinematics when on a decline, compared with locomoting on level ground, are similar to those of the squirrel monkey (Vilensky et al., 1994) and the Sprague–Dawley rat (Gillis and Biewener, 2002) with no change in duty factor; increasing f_{stride} and decreasing l_{stride} and t_{swing} . In contrast, however, the barnacle goose also showed reduced t_{stance} . In humans, where both kinematics and metabolic data were collected, a negative gradient did not affect f_{stride} or l_{stride} , but \dot{V}_{O_2} was reduced (Snyder and Farley, 2011). Similar

kinematic changes were induced by declines in the cat (Carlson-Kuhta et al., 1998; Smith et al., 1998), turkey *Meleagris gallopavo* (Gabaldón et al., 2004) and iguanian lizard *Dipsosaurus dorsalis* (Jayne and Irschick, 1999), although concomitant metabolic data were not collected. A shorter, faster ('choppy') stride suggests that the barnacle geese were trying to get their feet down quickly to brake and avoid falling. Losing kinematic energy at each footfall requires negative work from the muscles, and the more energy absorbed (increased braking effort) the more metabolically costly it is to the organism. The assistance of gravity in helping move the CoM is probably negated by this need to brake and the increase in f_{stride} (\dot{V}_{O_2} tends to increase with f_{stride}).

These geese are the only species so far to show an increase in duty factor and a decrease in t_{swing} during incline locomotion. An animal must produce more force to ascend an incline at a similar speed to that on the level and it appears that how this is achieved may vary both inter- and intra-specifically (Table 2). The barnacle goose, rat (Gillis and Biewener, 2002) and some horses (van Oldruitenborgh-Ooste et al., 1997) require an increased t_{stance} to generate the additional force, whereas other species appear to generate more force within the same t_{stance} (Table 2). The barnacle goose maintains l_{stride} and f_{stride} at level walking intensities when ascending and so compensates for the increase in t_{stance} by decreasing t_{swing} , which contributes to an increase in duty factor. The muscles responsible for swinging the limb are accountable for 26% of the energy used in guinea fowl *Numida meleagris* (Marsh et al., 2004). Swinging the leg through more rapidly must increase the work done

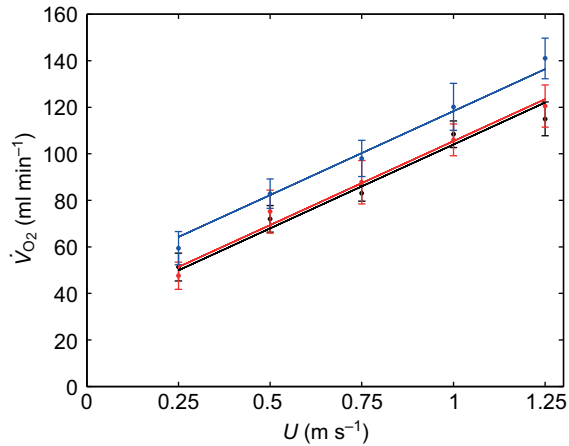


Fig. 1. Rate of oxygen consumption (\dot{V}_{O_2}) plotted against velocity (U) for level, decline and incline walking. The data points represent the values averaged across all five geese and the lines of best fit were derived from the general linear model (GLM) and are $\dot{V}_{O_2}=46.21+72.12U$ (incline, blue symbols and line), $\dot{V}_{O_2}=31.89+72.12U$ (level, black symbols and line) and $\dot{V}_{O_2}=33.32+72.12U$ (decline, red symbols and line).

by the swing phase muscles and hence contributes to the increased metabolic cost. Although perhaps indicating a change in fuel source composition with increasing speed, the RER values suggest that, at the whole-animal level, the geese were always operating aerobically and gradient did not affect this.

Data available on the effects of gradient on leg kinematics where speed was also controlled are few, but suggest that differences between species persist and are probably driven by leggedness (bipedal or quadrupedal), ecological niche and postural effects (Table 2). The relative proportions of different leg muscles and their architecture are also likely to lead to species-specific responses to locomoting on gradients. Cats adopt a more crouched posture when negotiating both negative and positive gradients (Carlson-Kuhta et al., 1998; Smith et al., 1998) and lizard limbs are flexed more when

travelling downhill (Jayne and Irschick, 1999) than when walking on the level. This reduction in effective limb length reduces hip height. Consequently, the lever arm between the foot and the CoM is reduced when going uphill. Similarly, crouching on a decline reduces the lever arm between the braking foot and CoM, which reduces the moment around the foot and presumably reduces the costs of braking. Although not measured directly, the geese did not appear to adjust their effective limb lengths on the gradients and a lack of plasticity in posture may be a contributor to the lack of a reduction in \dot{V}_{O_2} for the geese walking downhill. The coefficient of friction may also be important in determining running performance uphill and downhill (Lammers et al., 2006). This must be dependent upon foot morphology and a webbed foot in geese may not provide the same level of traction as the clawed foot of a galliform. Posture will also determine how the foot is weighted and, consequently, influence traction. The timing and direction of weighting relative to the ground, and how much of the foot is in contact with the ground throughout stance, will affect traction.

Previous studies have found the slope of the line of best fit between \dot{V}_{O_2} and U to increase with gradient (Armstrong et al., 1983; Bamford and Maloij, 1980; Chassin et al., 1976; Clapperton, 1964; Cohen et al., 1978; Eaton et al., 1995; Farley and Emshwiller, 1996; Full and Tullis, 1990; Margaria et al., 1963; McDonough et al., 2002; Raab et al., 1976; Robbins et al., 1979; Taylor et al., 1972; Warncke et al., 1988; White and Yousef, 1978; Wunder and Morrison, 1974; Yousef et al., 1972) and the established way of calculating the efficiency of moving along gradients for comparison between different species is dependent upon there being a difference (Taylor et al., 1972).

The net metabolic cost of lifting 1 kg of body mass 1 m in J is calculated using:

$$\frac{S - S_0}{\sin \theta} \times 20.1, \quad (3)$$

where S is the slope of the line of best fit between \dot{V}_{O_2} and U for gradient exercising, S_0 is the same for locomoting on a level substrate, θ is the angle of incline and 20.10 is the thermal

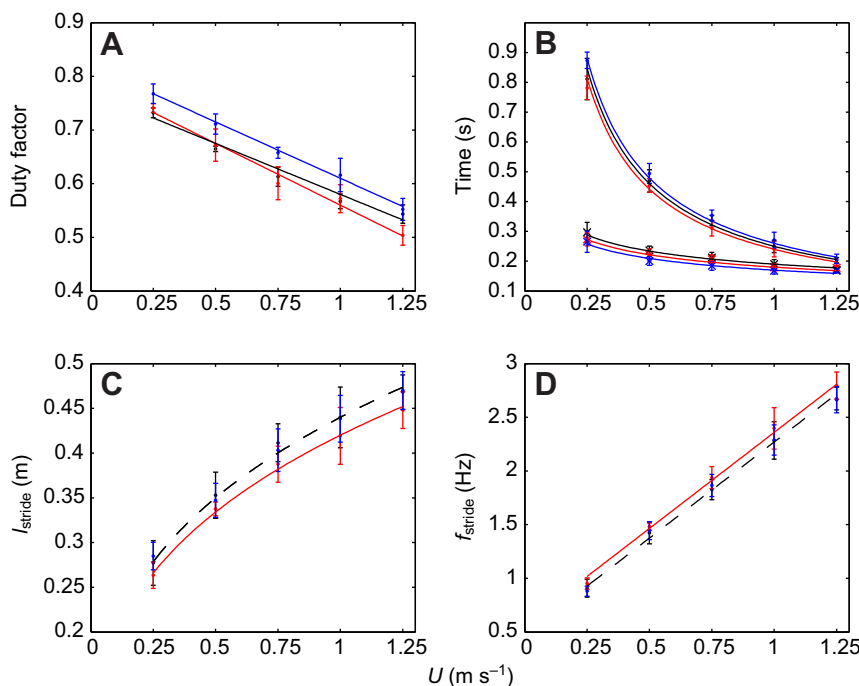


Fig. 2. Kinematics variables (A) duty factor, (B) stance time (t_{stance} , filled circles) and swing time (t_{swing} , crosses), (C) stride length (l_{stride}) and (D) stride frequency (f_{stride}) plotted against velocity (U) for incline (blue symbols), level (black symbols) and decline (red symbols) walking. The data points represent the values averaged across all five geese and the lines of best fit were derived from the GLMs, and are (A) duty factor: $y=0.82-0.21U$ (incline, blue line), $y=0.77-0.19U$ (level, black line) and $y=0.79-0.23U$ (decline, red line), (B) stance: $y=0.26U^{-0.88}$ (incline, blue line), $y=0.25U^{-0.88}$ (level, black line) and $y=0.24U^{-0.88}$ (decline, red line), swing: $y=0.17U^{-0.30}$ (incline, blue line), $y=0.19U^{-0.30}$ (level, black line) and $0.18U^{-0.30}$ (decline, red line), (C) l_{stride} : $y=0.44U^{0.33}$ (incline, black dashed line), $y=0.44U^{0.33}$ (level, black dashed line) and $0.42U^{0.33}$ (decline, red line), and (D) f_{stride} : $y=0.49+1.79U$ (incline, black dashed line), $y=0.48+1.79U$ (level, black dashed line) and $y=0.57+1.79U$ (decline, red line). Because of the non-linear nature of the relationship between t_{stance} , t_{swing} and l_{stride} and U , statistical analyses were conducted on \log_{10} -transformed data (Table 1). Note that where lines of best fit are not discernable either statistically or graphically between two different gradients a single black dashed line is plotted to represent both.

Table 2. Trends in the metabolic cost and kinematic responses to locomoting on a decline or incline compared with on a level surface for a range of species

Species	Decline					Metabolic cost	Incline					Metabolic cost
	Duty factor	t_{stance}	t_{swing}	l_{stride}	f_{stride}		Duty factor	t_{stance}	t_{swing}	l_{stride}	f_{stride}	
Barnacle goose	=	–	–	–	+	=	+	+	–	=	=	+
Turkey	=			= ^a	=		=			= ^a	=	
Human				= ^a		–				= ^a		+
Squirrel monkey		=	–	– ^a	+		= ^b	=	=	= ^a	=	
Iguanian lizard	=			=	=		=			–	+	
Rat ¹	=	=	–	– ^a	+		=	+	=	+ ^a	–	
Rat ²										+ ^a	–	
Cat ¹	=			– ^a	+		=			– ^a	+	
Cat ²										= ^a	=	
Horse ¹								+		+ ^a	–	
Horse ²										=	=	+
Horse ³							= ^b	=	=	=	=	
Horse ⁴										= ^a	=	
Horse ⁵								=		+ ^a	–	
Cockroach										= ^a	=	+

Increases in the variable relative to locomoting on a level surface are represented by '+', decreases by '–' and no obvious change by '='. An empty cell means that no data are available.

Data sources are: barnacle goose (this study), turkey (Gabaldon et al., 2004), human (Snyder and Farley, 2011), squirrel monkey (Vilensky et al., 1994), iguanian lizard (Jayne and Irschick, 1999); Sprague–Dawley rat¹ – data refer to slow walking only (faster gaits were not reported) and trends are estimated from histograms (see their fig. 2) and may not be real as no *post hoc* tests were conducted (Gillis and Biewener, 2002), Sprague–Dawley rat² (Roy et al., 1991), cat¹ (Carlson-Kuhta et al., 1998; Smith et al., 1998), cat² (Pierotti et al., 1989), horse¹ (Sloet van et al., 1997), horse² (Eaton et al., 1995), horse³ (Kai et al., 1997), horse⁴ (Robert et al., 2000), horse⁵ (Hoyt et al., 2000) and cockroach *Periplaneta americana* (Full and Tullis, 1990).

^aBased on the f_{stride} data. The animals were exercised at the same speed on each gradient. Therefore, a change in f_{stride} must mean the opposite change to l_{stride} .

^bExtrapolated from there being no change in t_{stance} or t_{swing} .

equivalent for conversion of \dot{V}_{O_2} into J (Taylor et al., 1972). Efficiency (η) is mechanical work/energy expended or energy recovered/mechanical energy stored for uphill and downhill locomoting, respectively.

The incremental cost (\dot{V}_{O_2}) of walking on gradients in the barnacle goose, however, was independent of gradient angle. An increasing slope of \dot{V}_{O_2} against U with increasing gradient is expected, if the change in cost is due solely to the external mechanical work associated with increasing the animal's potential energy ($E_p = MgU \sin \theta$, where M is body mass, g is the acceleration due to gravity and θ is the angle of the gradient with the horizontal). Only if η is constant across all speeds would a similar pattern in \dot{V}_{O_2} variation with U to that predicted for E_p be expected. Alternatively, if η increased with U on inclines and decreased with U on declines then similar slopes between \dot{V}_{O_2} and U for each gradient could result. Such speed and gradient variations in η were previously found for sheep (Clapperton, 1964).

The previous findings of increasing incremental costs with increasing gradient may be a real effect or alternatively a consequence of fitting separate regression lines to the data for each gradient and not using ANCOVA to test whether the slopes of these lines really (statistically) differ. For example, fitting independent linear regression analyses to the \dot{V}_{O_2}/U relationships for each gradient here indicated an increased slope for incline walking. Another confounding factor could stem from the fact that most studies have not determined the gaits used by the study animals and have included all the data together, which may have skewed the line(s) of best fit. The relationship between \dot{V}_{O_2} and U is not necessarily linear (Nudds et al., 2009) and the nature of it may also differ between gaits (Nudds et al., 2011). There is also evidence that the effect of gradient upon limb kinematics is gait dependent. When galloping on an incline, horses increase their f_{stride} and increase

the work per stride for a given U when compared with similar speeds during galloping on level ground (Parsons et al., 2008). When trotting, however, they only increase the work per cycle, leaving f_{stride} unchanged (Wickler et al., 2005). Alterations in joint angles and joint articulation angles are also dependent upon U as well as gradient (Carlson-Kuhta et al., 1998; Jayne and Irschick, 1999; Lammers et al., 2006; Leroux et al., 1999; Schmidt and Fischer, 2011; Smith et al., 1998; Vilensky et al., 1994). Perhaps, if only walking gaits were analysed in previous studies the slope of the relationship between \dot{V}_{O_2} and U would also be independent of gradient, as found for the barnacle goose.

The lack of variation in the incremental increase in \dot{V}_{O_2} with U between gradients means a comparison with other studies to determine whether the increased costs experienced by the geese are relatively higher is not possible. Therefore, only one of the two original predictions is supported, i.e. the geese saved less energy descending when compared with walking on the level than previous species studied. In addition, they showed a novel kinematic response to gradients. Raab and colleagues argued that there is little difference in the mechanical aspects of grade locomotion between bipeds and quadrupeds as indicated by similar net metabolism values at similar grades for humans and dogs (Raab et al., 1976). In subsequent work involving a broader range of species, Gillis and Biewener began to identify general patterns among species, but also postulate that species-specific differences in kinematic responses to gradients were likely (Gillis and Biewener, 2002). For the first time, the present study has identified no energy saving for decline running and an increase in duty factor when ascending a gradient. The barnacle goose data reported here therefore add important novel information to existing data and reinforce the notion that a suite of factors (such as ecological niche, posture, gait, leggedness and foot morphology) are likely to affect an organism's ability to negotiate gradients.

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