Can metrics of acceleration provide accurate estimates of energy costs of locomotion on uneven terrain? Using domestic sheep (*Ovis aries*) as an example

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Abstract

Background: Locomotion is often a necessity for animal survival and can account for a large proportion of an individual's energy budget. Therefore, determining the energy costs of locomotion is an important part of understanding the interaction between an animal and its environment. Measures of animal acceleration, specifically 'dynamic body acceleration' (DBA) has proved to be a useful proxy of the energy cost of locomotion. However, few studies have considered the effects of interacting factors such as the animal's speed or changes to the terrain slope on the putative acceleration versus energy expenditure relationship and how this may affect the relationship between DBA and energy expenditure.

Methods: Here we conducted a methodological study to evaluate the ability of the metric 'vectorial dynamic body acceleration', VeDBA, obtained from tri-axial accelerometer data loggers, to act as a proxy for energy expenditure in non-uniform environments. We used indirect calorimetry to measure the oxygen consumption ($\dot{V}O_2$) of domestic sheep (*Ovis aries*) that were exposed to different ambient temperatures when immobile (resting) and that walked at various speeds (0.8 to 2.9 km h^{-1}) and slope angles ($-6°$ to $6°$) on a treadmill while simultaneously measuring tri-axial acceleration recorded at 40 Hz by body-mounted tags.

Results: The lower critical temperature of sheep was identified as 18 °C, and $\dot{V}O_2$ when they were immobile was 3.67 mL O_2 kg^{-1} min^{-1}. There were positive relationships between $\dot{V}O_2$, VeDBA, and speed of walking. However, VeDBA correlated less well with $\dot{V}O_2$ when the terrain slope either inclined or declined.

Conclusions: We advocate caution when using DBA metrics for establishing energy use in animals moving over uneven terrain and suggest that each study species or location must be examined on a case-by-case basis. Reliance upon the relationship described between acceleration and energy expenditure on horizontal-surface treadmills can lead to potential under- or over-estimates of energy expenditure when animals walk on uneven or inclined ground.

Keywords: Dynamic body acceleration, Energy expenditure, Locomotion, Oxygen consumption, Slope, Terrain, Thermoregulation, Indirect calorimetry, Accelerometry, Treadmill

Background

Locomotion occupies a pivotal position in animal ecology, because it is necessary in many species for, *inter alia*, food-finding, searching for mates and avoiding predators [1]. However, translocation of the body is

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energetically costly, which explains why locomotion accounts for a large proportion of the energy budget of many animals [2–4]. Studies on the energetics of animal locomotion often seek to determine how movement costs affect fitness and survival [5, 6] to understand or predict how individuals and populations can survive [7].

Measurement of body movement via tri-axial accelerometry was first considered in relation to energy expenditure by Cavagna, Saibene, and Margaria [8]. Meijer, Westerterp and Koper [9] proposed that acceleration-based metrics might relate to power use, while Terrier, Aminian and Schutz [10] related the mean of the integral of the vector magnitude of tri-axial accelerations measured by devices placed on human backs to oxygen consumption (VO_2) during locomotion. However, it was not until work by Wilson et al. [11] that an acceleration-based metric, 'dynamic body acceleration' (DBA), was formalized and quantified for moving animals, initially cormorants (*Phalacrocorax carbo*) walking on a treadmill. The positive linear relationship between DBA and VO_2 found by Wilson et al. [11] was echoed subsequently by a suite of authors working on several different taxa [12–20].

Despite the ease with which livestock can sometimes be studied compared to wild or non-domesticated animals, applications of tri-axial accelerometers to estimate the cost of locomotion of livestock are relatively rare (e.g., [21]). A study by Miwa et al. [22] measured DBA and heart rate in cattle, sheep and goats during locomotion of various intensities. While strong correlations were found, as the authors suggest, direct measurements of VO_2 would provide a better test of the relationship in ruminants between DBA and energy expenditure. The relationships between DBA and VO_2 derived from animals walking at various speeds on treadmills (e.g., [12, 13, 18, 23]) have faced criticism, as level treadmill-based estimates of energy expenditure may not reflect energy expenditure in the field [24]. Specifically, the natural environment is rarely uniform, and changes in terrain substrate have been shown to influence the energetic cost associated with locomotion. Soft substrates, such as snow [25] and sand [26], incur a greater energetic cost than harder substrates. Importantly, variation in terrain angle can affect energy expenditure; e.g., increased costs of locomotion with increasing steepness of incline have been reported in a number of animals [27–31] due to the mechanical work required to move the body against gravity [32]. Moreover, when the angle of the terrain decreases beyond level past a certain point, work is required to stabilise the body and control velocity of movement, which requires more energy than would be required when walking on a level surface or on shallow declines [29, 30, 33].

Much of the available data that defines relationships between VO_2 and acceleration in terrestrial animals was conducted on a flat surface, with fewer studies considering the effect of incline [19, 31] and even less considering decline [31]. In humans, the VO_2-acceleration relationship can be markedly different between flat and angled surfaces. Terrier, Aminian and Schutz [10] found that energy expenditure increased with increasing incline, and the relationships between acceleration and energy expenditure were only apparent when data from each incline were considered independently. Similarly, Halsey et al. [12] found that DBA measured using tri-axial accelerometry could be used to predict the energy costs of walking at different inclines on a treadmill, but the VO_2–DBA relationships were markedly different in each case. These different relationships need to be quantified, particularly for species that live typically in rugged terrains, such as sheep. Such terrains might vary in the steepness of slope from low (1–12°), to medium (13–25°) to high (>25°) [34, 35] and also in evenness (where 'uneven terrain' refers to regions, where changes in terrain angle are frequent and localised). In the current study we explore the effect of slope. Once terrain slope is known and an appropriate VO_2–DBA relationship for this slope has been determined, we can calculate the energetic cost of movement at this angle. Body pitch (θ) of the animal (the gradient of the spine in a quadruped) [36] could provide a useful continuous measurement of terrain slope. In other words, the slope of the terrain can be expected to be reflected in the pitch of the animal. As a baseline against which to compare the energy costs of specific movements or individual behaviours, we need to determine energy expenditure, while the animal is stationary. Species such as sheep spend a lot of time standing, i.e., in a locomotor stance but travelling at zero speed and effectively resting, and thus this behaviour will represent a considerable part of their overall energy expenditure.

In the present study, we aimed to examine the relationship between VO_2 and DBA using trained domestic sheep (*Ovis aries*) as a model quadruped, walking at different speeds and on different slope angles (both inclines and declines), to explore how VO_2 relates to acceleration under differing circumstances. Ultimately, this work can enable us to understand if estimates of oxygen consumption and a measure of acceleration determined under a controlled environment can be used with confidence to predict how the topography of the environment affects the energy costs of locomotion of free ranging animals. Sheep are common as livestock species across the world, with economic importance both in developed and developing countries. In the UK (population 32.7 million sheep, [37]) the industry was worth c. $1.3 billion in 2020 [38]. In Australia, the sheep

meat industry was worth an estimated \$4.5 billion in 2019–2020 [39] and in China this was an estimated \$106.7 in 2020 [40]. Sheep are kept in a variety of environments from lowland farms to rugged mountainous terrain and exposed to different farming practices, such as rotational grazing during which sheep are moved from one pasture to another and fleece shearing. Clearly fleece removal will affect body insulation, heat loss and thermoregulatory capabilities, including, presumably, an increase in lower critical temperature (LCT) - i.e., the temperature below which the animal expends additional energy to keep warm [41]. A secondary objective, therefore, was to determine the relationship between ambient temperature and VO_2 when sheep were stationary. Consequently, as well as contributing to a general framework examining the validity of DBA metrics as a proxy for energy expenditure, this work will introduce terrain as an energy modulator into perspective for a species of commercial importance. The specific aims were to: (i) investigate the relationship between resting VO_2 and ambient temperature; (ii) examine the effects of speed and angle of incline/decline upon VO_2; (iii) investigate the effects of speed, angle and pitch on VeDBA; (iv) define a relationship between VO_2 and VeDBA for sheep across various slope angles; (v) determine if the measurement of animal pitch (θ) can be used as a suitable proxy of terrain angle; and (vi) examine the effect of fleece shearing on VO_2.

Results

Resting VO_2 and the thermoneutral zone

Piecewise linear regression analysis indicated an inflection in VO_2 at 18 °C for sheep A, representing the lower critical temperature (LCT). Two inflection points were identified for sheep B at 12.3 °C and 18 °C (Fig. 1B). However, above 12.3 °C, VO_2 continued to decrease, by 22% to 3.33 mL kg^{-1} min^{-1} at 18 °C, beyond which VO_2 stabilised. The LCT was, therefore, identified as 18 °C for sheep B, which was consistent with sheep A (Fig. 1B). The mean resting VO_2 for the two sheep was 3.67 mL O_2 kg^{-1} min^{-1} when they were un-sheared (i.e., with a full fleece). When sheared, VO_2 decreased by 15% from a mean of 4.78 to 4.05 mL O_2 kg^{-1} min^{-1} as ambient temperature increased from 23.5 to 27 °C (Fig. 1B). There was no significant difference in the resting VO_2 of the sheep when they were not sheared compared with when they were sheared, at 27 °C ($X^2 = 2.106$, $df = 1$, $P = 0.147$, Fig. 1B). As per the VO_2 of non-sheared sheep, VO_2 did not change when temperature increased above 18 °C for sheared sheep.
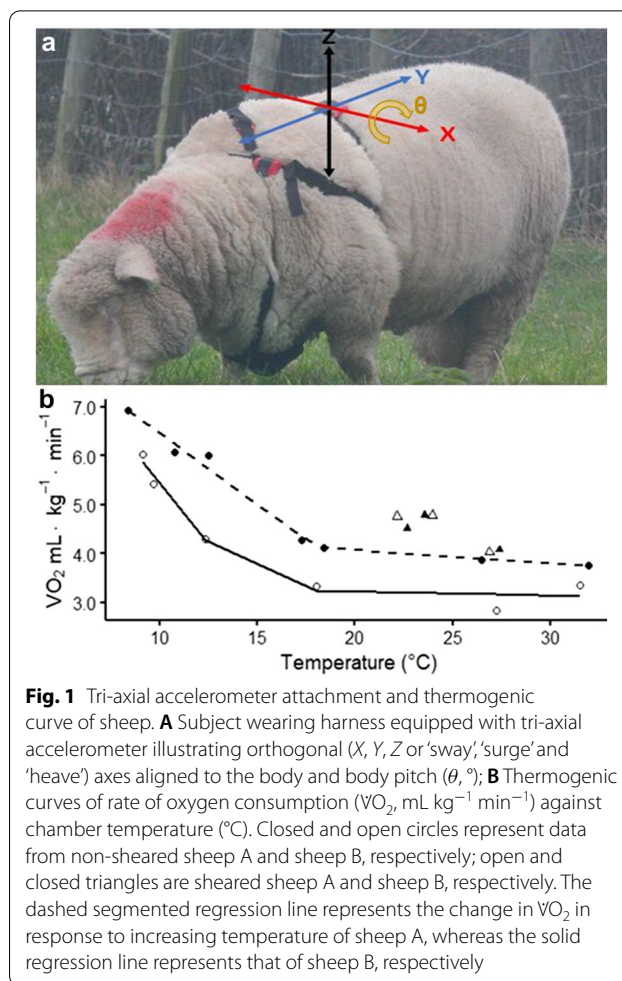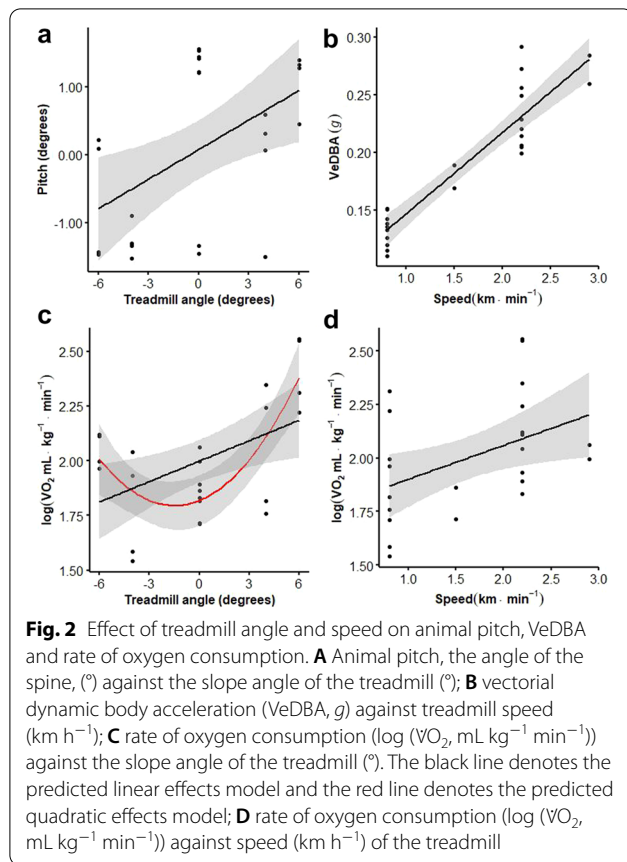

Fig. 1 Tri-axial accelerometer attachment and thermogenic curve of sheep. **A** Subject wearing harness equipped with tri-axial accelerometer illustrating orthogonal (*X*, *Y*, *Z* or 'sway', 'surge' and 'heave') axes aligned to the body and body pitch (θ, °); **B** Thermogenic curves of rate of oxygen consumption (VO_2, mL kg^{-1} min^{-1}) against chamber temperature (°C). Closed and open circles represent data from non-sheared sheep A and sheep B, respectively; open and closed triangles are sheared sheep A and sheep B, respectively. The dashed segmented regression line represents the change in VO_2 in response to increasing temperature of sheep A, whereas the solid regression line represents that of sheep B, respectively

VO_2 and locomotion

There was a significant positive relationship between animal pitch and slope angle ($X^2 = 4.949$, $df = 1$, $P = 0.026$). VO_2 increased significantly with both increasing slope angle (Fig. 2C) and treadmill speed ($X^2 = 6.299$, $df = 1$, $P = 0.012$ and $X^2 = 4.534$, $df = 1$, $P = 0.033$, respectively) (Fig. 2D). Inclusion of a quadratic rather than a linear function improved the fitted relationship ($r^2 = 0.33$ versus $r^2 = 0.17$, respectively) (Fig. 2C).

Factors affecting VeDBA

VeDBA increased significantly with increasing treadmill speed ($X^2 = 67.75$, $df = 1$, $P < 0.001$) (Fig. 2B). However, there was no significant change in VeDBA with treadmill slope angle at any of the measured speeds ($X^2 = 0.348$, $df = 1$, $P = 0.55$) or pitch of the animal's body ($X^2 = 2.3067$, $df = 1$, $P = 0.129$).

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Fig. 2 Effect of treadmill angle and speed on animal pitch, VeDBA and rate of oxygen consumption. **A** Animal pitch, the angle of the spine, (°) against the slope angle of the treadmill (°); **B** vectorial dynamic body acceleration (VeDBA, *g*) against treadmill speed (km h^{-1}); **C** rate of oxygen consumption (log ($\dot{V}O_2$, mL kg^{-1} min^{-1})) against the slope angle of the treadmill (°). The black line denotes the predicted linear effects model and the red line denotes the predicted quadratic effects model; **D** rate of oxygen consumption (log ($\dot{V}O_2$, mL kg^{-1} min^{-1})) against speed (km h^{-1}) of the treadmill

Relationship between $\dot{V}O_2$ and VeDBA

The GLMM model (Table 1, Model 1a), which incorporated data obtained from all slope angles and all speeds, revealed no significant relationship between $\dot{V}O_2$ and VeDBA ($X^2 = 2.06$, $df = 1$, $P = 0.107$). Therefore, in subsequent analyses each slope angle was considered independently. The only significant relationship between $\dot{V}O_2$ and VeDBA was noted when the treadmill was level (0°), Spearman $r_s^2 = 0.88$, $P = 0.007$, (Fig. 3). The least-squares relationship was defined:

$$\dot{V}O_2\left(mL\,O_2\,kg^{-1}\,min^{-1}\right) = 13.72\,VeDBA(g) + 3.67 \tag{1}$$

(Spearman $r_s^2 = 0.77$). No significant relationships were noted between $\dot{V}O_2$ and VeDBA for treadmill gradients of 4°, 6°, $-4°$ and $-6°$ ($r_s = 1$, 0.8, 0.6, 0.8, $P = 0.08$, 0.33, 0.42, 0.33, respectively). Small sample size effectively precluded statistical power for significance (cf. $r_s = 1$, above). Grouping data into incline walking (4° and 6°), level walking (0°) and decline walking ($-4°$ and $-6°$) resulted in significant relationships between $\dot{V}O_2$ and VeDBA for both incline walking and level walking ($r_s = 0.86$, $P = 0.01$,

$r_s = 0.88$, $P = 0.007$, respectively), although there was variation observed around the relationship defined for incline walking (Fig. 3). The relationship defined for incline walking was:

$$\dot{V}O_2\left(mL\,O_2\,kg^{-1}\,min^{-1}\right) = 36.31\,VeDBA(g) + 3.67 \tag{2}$$

(Least squares regression, Spearman $r_s^2 = 0.74$). There was no significant relationship between $\dot{V}O_2$ and VeDBA for decline walking ($r_s^2 = 0.64$, $P = 0.096$).

Discussion

This study sought to understand the relationships between DBA recorded by body-mounted accelerometer tags, and simultaneously measured energy expenditure using domestic sheep as a model quadruped. It also sought to determine whether DBA–$\dot{V}O_2$ relationships differed according to speed of travel and incline of the terrain. Furthermore, the study aimed to evaluate the potential use of animal pitch as a useful proxy for monitoring the incline of the terrain.

Relationship between DBA and $\dot{V}O_2$

A positive relationship between DBA and energy expenditure has been reported previously in a range of species (e.g., [11, 13, 16, 17, 19, 22]). Results of the current work are in broad agreement with these studies, with a positive relationship noted between DBA and $\dot{V}O_2$ when subjects walked on a level surface. However, no significant relationship emerged when data from level, positive and negative treadmill angles were included together in the analysis. Terrier, Aminian and Schutz [10] also found no relationship between acceleration metrics and energy expenditure for humans when multiple gradients were examined. They did, however, note that significant relationships existed when gradients were analysed separately. Similarly, when data from the current study were grouped into "incline", "level" or "decline" walking, relationships between DBA and $\dot{V}O_2$ showed that "incline" walking was more energetically costly than "level" walking and "decline" walking. The fact that there were fewer data in the current study for sheep walking on slopes, compared to sheep walking on the level, may have inhibited our ability to determine DBA-energy expenditure relationships for each angle or indeed determine an overall relationship for $\dot{V}O_2$ which incorporates VeDBA and terrain slope.

The variation observed in the $\dot{V}O_2$–VeDBA relationship defined for "incline" walking does not provide confident estimates of $\dot{V}O_2$ with slope. Importantly, the use of VeDBA as a proxy for energy expenditure based upon a

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Table 1 Structure of the various generalised linear mixed statistical models

	Dependent variable	Model structure	Random factor	AIC
Model 1a	**Oxygen consumption ($\dot{V}O_2$)-Intercept**	**VeDBA**	**ID**	**10.85***
Model 1b (null model)	Oxygen consumption ($\dot{V}O_2$)-Intercept		ID	11.45
Model 2a	Oxygen consumption ($\dot{V}O_2$)-Intercept	Speed:Pitch + Speed:Slope + Slope + Speed + Pitch	ID	13.79
Model 2b	Oxygen consumption ($\dot{V}O_2$)-Intercept	Speed:Slope + Slope + Speed + Pitch	ID	12.16
Model 2c	Oxygen consumption ($\dot{V}O_2$)-Intercept	Speed:Pitch + Slope + Speed + Pitch	ID	11.89
Model 2d	Oxygen consumption ($\dot{V}O_2$)-Intercept	Slope + Speed + Pitch	ID	10.16
Model 2e	Oxygen consumption ($\dot{V}O_2$)-Intercept	Speed + Pitch	ID	11.56
Model 2f	Oxygen consumption ($\dot{V}O_2$)-Intercept	Slope + Pitch	ID	12.64
Model 2g	**Oxygen consumption ($\dot{V}O_2$)-Intercept**	**Speed + Slope**	**ID**	**8.46***
Model 2h	Oxygen consumption ($\dot{V}O_2$)-Intercept	Speed	ID	12.76
Model 2i	Oxygen consumption ($\dot{V}O_2$)-Intercept	Slope	ID	10.99
Model 3a	VeDBA	Speed:Slope + Pitch:Speed + Speed + Slope + Pitch	ID	− 123.80
Model 3b	VeDBA	Pitch:Speed + Speed + Slope + Pitch	ID	− 123.11
Model 3c	VeDBA	Speed:Slope + Speed + Slope + Pitch	ID	− 123.79
Model 3d	VeDBA	Speed:Slope + Pitch:Speed + Slope + Pitch	ID	− 57.77
Model 3e	VeDBA	Speed:Slope + Pitch:Speed + Speed + Pitch	ID	− 125.45
Model 3f	VeDBA	Speed:Slope + Pitch:Speed + Pitch	ID	− 59.69
Model 3g	**VeDBA**	**Speed:Slope + Pitch:Speed + Speed**	**ID**	**− 125.44***

In the table, ':' denotes an interaction and '+' denotes a main effect between two independent variables. An asterisk, *, denotes the final model used as defiled buy the lowest Akaike Information Criteria (AIC) value. The intercept was set at a value of 3.67 mL O_2 kg^{-1} min^{-1} (measured resting $\dot{V}O_2$). For each model, a Gamma error family was used

single relationship between $\dot{V}O_2$ and VeDBA, using Eq. 1 can result in large variation in predictions of the associated energy expenditure, ranging in this case from underestimates of $22.4 \pm 12.7\%$ for the 4° incline, $46.7 \pm 2.3\%$ for a 6° incline, and $16.2 \pm 7.11\%$ for a 6° decline when using VeDBA measurements on these incline. Where the decline angle is 4°, VeDBA could overestimate the cost of locomotion, depending on the speed of movement exhibited ($6.8 \pm 9\%$ error, Eq. 1).

Terrier, Aminian and Schutz [10] suggested that a solution for poor predictive capacity of power (watts) from acceleration metrics in the case of incline movement was to assess slopes by independent methods (such as altimeters or differential GPS [42, 43]) so that slope-specific acceleration proxies could be applied. Certainly, the rapid development of sensors within animal-attached loggers such as altimeters (e.g., [44]) or logger pitch angle is making this increasingly feasible.

Factors affecting $\dot{V}O_2$

The positive relationship observed in the current study between $\dot{V}O_2$ and walking speed is in agreement with Clapperton, [27] who examined $\dot{V}O_2$ of sheep walking at similar inclines (Fig. 4). Dailey and Hobbs [29] examined the $\dot{V}O_2$ of sheep and goats walking up much steeper inclines (25°). Their $\dot{V}O_2$ values were higher,
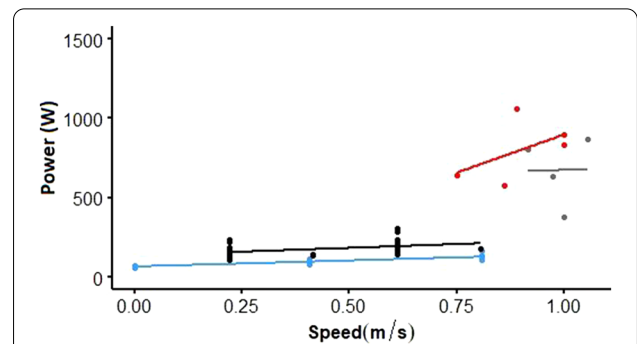
which is expected as increased energy is required to move their mass against gravity [32]. Interestingly, in the current study which incorporated decline walking, once slope angle decreased to less than −4°, energy expenditure increased (Fig. 2C). A similar trend was found by Minetti et al. [33] in humans and in a number of animals by Halsey and White [30]. This suggests that steep downhill walking incurs greater costs than walking on a level surface, which may be due, for example, to the energy required for controlling velocity and for gait stabilization [45]. In support of this, the use of a quadratic rather than a linear function to examine the relationship between $\dot{V}O_2$ and treadmill angle supports the notion that energy expenditure may increase with increasing downhill gradient (Fig. 2D). Nevertheless, the efficacy of using either a linear or quadratic relationship should be interpreted with caution for $n = 2$ subjects. We only measured two animals was for the following two reasons: The first was logistical. It took 6 weeks to tame and train sheep from lambs to adults that would walk on the treadmill at will. It also took a further 11 months to collect data during which only one measurement could be collected per animal per day. Second, we were interested in exploring the concept of DBA–$\dot{V}O_2$ relationship variation with terrain slope and walking speed, rather than determining species-specific values per se. Therefore, while inclusion of

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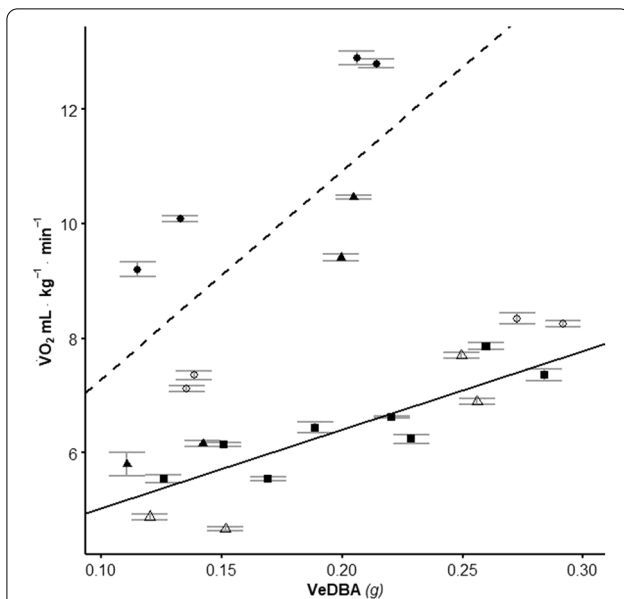
Fig. 3 Oxygen consumption (VO_2 mL kg^{-1} min^{-1}) against vectorial dynamic body acceleration (VeDBA, g) of sheep walking on a treadmill. Data include sheep walking at varying speeds on each slope angle. Black closed squares represent data of two sheep walking at 0° slope incline; the solid line represents the relationship between VO_2 and VeDBA at 0° slope incline. Similarly, black closed triangles represent 4° slope incline and closed circles represent 6° slope incline; the dotted line represents the relationship between VO_2 and VeDBA at slope incline angles (both 4° and 6°); open triangles represent 4° slope decline and open circles 6° slope decline, respectively

Fig. 4 Power in watts (W) against treadmill speed (m/s) from oxygen consumption data collected on sheep and goats. Blue data points and blue line represent data from sheep in the current study on treadmill inclines and decline angles (− 6 to 6°). Black data points and line represent data on sheep on treadmill inclines of 0°, 2.6° and 5° obtained from Clapperton [27]. The red points and red line represent similar data from sheep, and the grey points and grey line represent data from goats, both of which were walking at a 25° treadmill incline, obtained from Dailey and Hobbs [25]

data from additional subjects would provide more information yielding more accurate energy expenditure values for sheep of that breed in general, it would probably not change the qualitative observation that VO_2 and DBA vary with terrain slope and speed.

The lower critical temperature (LCT) of sheep with a full fleece in the current study was determined as 18 °C. This is similar to the 15–20 °C previously described [46, 47]. Above the LCT, resting VO_2 of non-shorn sheep was determined at 3.67 mL kg^{-1} min^{-1}. While the current study did not seek to determine the upper limits of the thermoneutral zone, previous studies have suggested that the upper critical temperature of sheep lies between 25 and 40 °C [48, 49]. The highest temperature to which the sheep were exposed in the current study was 32 °C. In terms of the VO_2 of shorn sheep, although we did not identify the lower critical temperature, work by Blaxter et al. [41] reported a value of c. 23 °C. Here the VO_2 values of shorn sheep were not different to the VO_2 values of the sheep with a full fleece at temperatures above 18 °C but VO_2 was higher at lower temperatures suggesting that there are indeed thermoregulatory costs associated with fleece removal.

Animal pitch as a proxy for terrain angle

Although a positive relationship was apparent between measurements of pitch and angle of the treadmill (equivalent to terrain slope), large variations in animal pitch (28%) occurred, predominantly during decline locomotion. Thus, the pitch of an animal's body may not necessarily be an accurate representation of the terrain slope. Differences in terrain slope and animal pitch may be due to postural changes by the animal attempting to stabilise and control downhill movement. Presumably this is akin to a human maintaining an upright posture when walking up or downhill [50]. Biewener [51] reported that animals may adopt a more upright posture of the limbs to reduce the stresses of downhill walking on muscles and joints, which may lead to a reduced downward body angle. As the current data were obtained across multiple locomotion speeds, it is possible that the variation in animal pitch may also be due to such changes in gait [52]. Thus, although a measure of terrain slope can be estimated using animal pitch angle, we also suggest that additional methods, such as altimeters or GPS data [43], can provide additional information on whether an animal travels up or down an incline and subsequently be used to inform an estimate of energy expenditure in the field (e.g., [19]).

While current results describe associations between DBA and VO_2, we understand that these are not representative of the species. However, results highlight significant variation between individuals and under different conditions (speed, terrain slope). Hence, we demonstrate that DBA–VO_2 relationships depend on a variety

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of environmental conditions. Field studies using DBA as a proxy for energy expenditure should also consider other aspects of the terrain, such as the penetrability of the substrate on which the animal is moving, and terrain evenness (i.e., regions, where changes in terrain angle are frequent and localised, to the extent that perhaps the forelimbs might even be on ground that is at a different angle than the ground on which the hind limbs stand) and explore how these may influence the relationship between acceleration metrics and energy expenditure.

Conclusions

We recorded simultaneous measurements of oxygen consumption (VO_2) by indirect calorimetry and body movement by tri-axial accelerometry to determine whether the metric 'vectorial dynamic body acceleration' (VeDBA) could be used as a proxy for instantaneous energy expenditure. Results showed that VeDBA increased linearly with VO_2 as walking speed increased, and that this relationship varied with both incline and level terrain slope. Therefore, we recommend that future attempts to determine VO_2–VeDBA relationships should examine a variety of incline and decline terrain slopes according to individual circumstance. We also show that although animal pitch could be used as a broad measure of terrain slope, this relationship was variable, especially for locomotion on a decline, presumably because subjects make adjustments in their posture.

Methods

Animals and study site

The experiments were conducted on a working farm near the town of Carnlough, Co. Antrim, Northern Ireland. The apparatus was set up inside a barn (c. 5×10 m floor space, 4 m height), which was exposed to natural lighting and ambient temperature with one whole side of the building remaining open. Two non-reproductive adult (1–2 years) Border-Cheviot ewes (66.47 ± 2.32 kg, sheep A and sheep B), which had been habituated to human contact since birth, were used. Prior to measurements of VO_2 (see below), sheep initially underwent a 6-week training period which included becoming accustomed to a metabolic chamber and being exercised on a treadmill for 15 min at a time at 0.8, 1.5, 2.2 and 2.9 km h^{-1} twice per day, three times a week. During this period, VO_2 measurements were not taken. Sheep were provided with c. 5 g of concentrate pellets (Thompsons Feed Innovation, Belfast) as a food reward when they entered the chamber and walked on the treadmill. Between training sessions, sheep were held in a field (0.12 ha) adjacent to the barn, where grass and water were available ad libitum.

Indirect calorimetry setup

Measurements of VO_2 were determined using indirect calorimetry. A metabolic chamber ($100 \times 130 \times 50$ cm) made from clear Perspex® sheeting was mounted upon a treadmill (running area: 33×114 cm; Vfit, DOG Jogger™ Canine Rehabilitation Treadmill, West Yorkshire, UK). Ambient air was drawn from outside through plastic tubing (3 cm diameter, 100 cm length), to a pump (3.15 CFM 120 VAC, THOMAS, Munich, Germany). The air was then pushed from the pump through plastic tubing (1.5 cm diameter, 70 cm length), through a flowmeter ($\pm 1.25\%$ FSD, CT Platon NG series Flow Meter 10–100 L min^{-1} N_2, Hampshire, England) and then through a copper coil (1.5 cm diameter, 150 cm length) which was placed inside a cabinet (PTC-1 Cabinet, Sable Systems Europe©, Berlin, Germany) controlled by a Pelt-5 temperature controller (Sable Systems Europe©, Berlin, Germany) which either warmed or cooled the air flowing through the coil to the desired temperature before entering the chamber at positive pressure (Fig. 5A). Within the metabolic chamber, air was mixed by five inbuilt electric
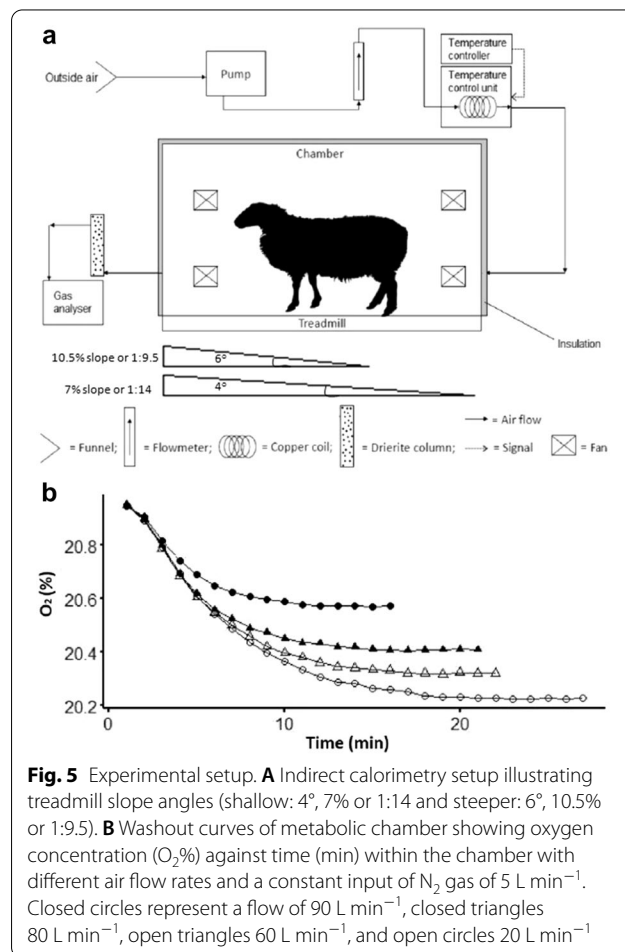
Fig. 5 Experimental setup. **A** Indirect calorimetry setup illustrating treadmill slope angles (shallow: 4°, 7% or 1:14 and steeper: 6°, 10.5% or 1:9.5). **B** Washout curves of metabolic chamber showing oxygen concentration ($O_2\%$) against time (min) within the chamber with different air flow rates and a constant input of N_2 gas of 5 L min^{-1}. Closed circles represent a flow of 90 L min^{-1}, closed triangles 80 L min^{-1}, open triangles 60 L min^{-1}, and open circles 20 L min^{-1}

fans. Temperature and pressure were measured inside the chamber using a digital thermometer and barometer (resolution $\pm 0.1°/\pm 0.1$ hpa, Sunroad FR500, EverTrust™ UK). A subsample of air was drawn from inside the chamber at 300 mL min^{-1} and passed through a drying column (Drierite, Fisher Scientific, Leicestershire, England) before entering a gas analyser (Foxbox Field Gas Analysis System, Sable Systems Europe©, Berlin, Germany) which measured O_2 concentration (O_2%). Prior to experiments, the gas analyser was factory-calibrated by Sable Systems Europe© (Berlin, Germany).

The time taken for gases to reach equilibration within the system was determined by a series of Nitrogen (N_2) gas injections [13, 53], which were undertaken prior to animal measurements. For these experiments, N_2 was injected at various rates (L min^{-1}) into the chamber and the time for O_2% within the chamber to reach equilibration was measured at flow rates of air entering the chamber ranging from 20 to 90 L min^{-1} (Fig. 5B).

Measurements of resting $\dot{V}O_2$ and variation in $\dot{V}O_2$ with ambient temperature

Experiments were conducted between October 2016 and August 2017 during this time ambient temperatures ranged between -3 and 24 °C. Sheep were weighed (large animal scales, Tree LC–VS 180, ± 0.05 kg, LW measurements LLC©, California) prior to measurements of oxygen concentration, which were determined between 11:00 and 16:00, during the normal period of activity for these animals [54]. Before an individual was placed inside the chamber, the chamber conditions (temperature, air flow) were allowed to stabilise by running the pumps and the analyser for 60 min. Thereafter, the subject animal was encouraged to enter the chamber via a sliding door at the rear (Fig. 5A). This was then closed, secured, and the time the animal entered the chamber was recorded. Measurements of O_2%, ambient pressure and temperature commenced as soon as the animal was observed to be stationary inside the chamber and were then taken every 5 min until the concentration of oxygen within the chamber was deemed to have 'settled'. This was determined as a change of less than 0.1% O_2 over a 5-min period [55]. This process took approximately 20 min. Once stable conditions had been reached, the O_2% within the chamber, temperature and pressure were recorded every 60 s for a further 5 min to enable 5 min of stable recordings to be taken with the animal at rest. If the animal became agitated at any time during the proceedings, or did not rest, measurements were abandoned and attempted on another day. Once measurements were complete, the animal was promptly removed from the respirometry chamber and the door of the chamber closed and

secured. Measurements of O_2% within the chamber were once again measured every 5 min until the O_2% inside the chamber increased to a constant reading (e.g., to an ambient concentration of 20.95%), which took 15–20 min. At this point, measurements increased in frequency to one every 60 s. When O_2% changed < 0.1% over a further 5-min period, the system was deemed to have stabilised. An average of these five measurements was then taken to determine the drift in O_2% from the original span of 20.95%. This protocol was repeated for a range of temperatures between 8 and 32 °C for both subjects. One measurement was collected per temperature per animal per day. Towards the end of the study (July–August 2017), sheep were shorn (normal practice on the working farm) and $\dot{V}O_2$ measurements over a range of ambient temperatures were repeated. Note that we did not conduct experiments in which the subjects were walking on shorn sheep. As these latter measurements were conducted during the summer, the higher ambient temperatures (21–24 °C) precluded lower temperatures being achieved within the chamber.

Calculation of $\dot{V}O_2$

Oxygen drift

To correct for drift in the oxygen analyser, the final O_2% was subtracted from the initial span of 20.95% to determine the difference in oxygen percentages. This was then divided by the time difference between the initial span and final recorded O_2% to determine drift per minute, assuming a linear drift over time [56]. The corrected O_2% for each reading was determined as follows:

$$\text{Correct } O_2\% = O_2\% + \text{drift per minute} \times t \qquad (1)$$

where O_2% is the percentage O_2 recorded, t is time between initial span and the reading obtained.

Rate of oxygen consumption ($\dot{V}O_2$)

To determine $\dot{V}O_2$, first flow rate of air through the chamber was corrected to standard volume (V_{std}), (standard temperature (273.15 K) and pressure (760 mmHg) as in Winberry, [57]), as follows:

$$\dot{V}_{std} = \left(\dot{V}_i\right) * (T_{std}/T_a) * (P_{std}/P_a) \qquad (2)$$

where V_i is the volume of gas (L min^{-1}) sampled at the measured atmospheric pressure (P_a) and temperature (T_a). T_{std} (K) and P_{std} (mmHg) are standard temperature (273.15 K (0 °C, 32F)) and standard pressure (100 kPa, 1 bar), respectively [57]. V_{std} was then converted to mL min^{-1}.

$\dot{V}O_2$ (mL min^{-1}) was then calculated following Withers [58] as

$$\dot{V}O_2 = \dot{V}_{std} \times (F_i - F_e)/(1 - F_e) \qquad (3)$$

where F_i is the fractional percentage of oxygen flowing into the chamber and F_e is the fractional percentage of oxygen exiting the chamber. VO_2 was then divided by animal body mass to return mass-specific rate of oxygen consumption (VO_2; mL kg^{-1} min^{-1}).

Tri-axial accelerometer loggers

Daily diary 'DD' loggers (Wildbyte Technologies Ltd, Swansea, UK) were secured to the sheep via a harness (detailed below) prior to them entering the metabolic chamber. These devices comprised a 12-bit resolution, tri-axial accelerometer (± 16 g), which recorded acceleration on three orthogonal axes ('surge'—forwards and backwards; 'heave'—up and down and 'sway'—side to side, [36]) at 40 Hz. Devices were housed inside vacuum-formed plastic cases ($4.5 \times 1.8 \times 4$ cm) and were powered using a 3.7 V lithium-ion rechargeable battery. Data recorded by the device were saved to an onboard 32 GB MicroSD card, (SanDisk®, Western Digital Technologies, Inc). The device and battery were wrapped in waterproof, 19 mm PVC insulation tape to prevent water ingress.

Attachment of accelerometer loggers to sheep

Accelerometers were secured to a nylon ram harness (Nettex©; Kent, England) via Tesa®; tape (No. 4651; Tesa AG, Hamburg, Germany) and bound by four cable ties (300×4.5 mm) to minimise rotation or movement of the device not caused by animal activity. The harness was then fitted to the subject (Fig. 1A). Sheep remained stationary while being instrumented with the equipped harness and did not require restraint. The total weight of the device, batteries and harness was 460 g. Attachment of the logger and harness took less than 5 min per animal. Devices were positioned on the dorsal mid-line of each animal, behind the shoulders. Care was taken to ensure each accelerometer was orientated with the Y axis representing forward and backward movement, the Z axis representing up and down movement and the X axis representing side to side movement (Fig. 1A).

Treadmill measurements

Measurements were conducted on one animal at a time. The temperature inside the chamber for these measurements was above the lower critical point for each animal. During this experiment, each sheep was initially placed on a level treadmill and subjected to four different speeds: 0.8, 1.5, 2.2 and 2.9 km h^{-1}. As above, only one speed was measured on any one occasion, i.e., each session on the treadmill resulted in one data point. Speeds were chosen based on previous data of sheep walking

with no changes in gait [27, 59]. Thereafter, experiments were conducted with the treadmill set at inclines of 4° and 6°, and declines of 4° and 6°, at two speeds, which were 0.8 and 2.2 km h^{-1}. These gradients, at 4° and 6°, are equivalent to slopes of 7% or 1 in 14.3, and 10.5% or 1 in 9.5, respectively (Fig. 5A) and are the equivalents of walking on a gently sloping field. Once a sheep was inside the chamber, the treadmill was switched on and set to the desired speed. As per the description of the measurements of resting VO_2 above, measurement of O_2%, ambient pressure and chamber temperature were conducted every 5 min until the O_2% within the chamber changed less than 0.1% over 5 min. At this point, the O_2%, temperature and pressure were recorded every 60 s for a further 5 min. After this, the treadmill was safely stopped, and the animal was removed from the chamber. Again, as per the resting VO_2 determination described above, measurements of O_2% within the chamber were measured every 5 min until the O_2% inside the chamber increased to a constant reading (ambient O_2% of c. 20.95%). After this, measurements increased to every 60 s. When O_2% changed < 0.1% over a further 5-min period, the system was deemed to have stabilised. An average of these five measurements was then taken as described above to determine the drift in O_2% from the original span.

Calculation of vectorial dynamic body acceleration (VeDBA)

Acceleration data were measured during the 5-min steady-state period when the sheep was inside the treadmill, from which the measure vectorial dynamic body acceleration (VeDBA) values were derived. We used VeDBA as a proxy for activity-related energy expenditure, rather than other measures, such as overall dynamic body acceleration (ODBA) [11]. The effectiveness of different measures of DBA has been debated previously (e.g., [17]). ODBA and VeDBA, are highly correlated, and both provide reliable estimates of VO_2. However, we used VeDBA, because it has been suggested to be the preferred metric if device orientation cannot be maintained or be consistent [17], and therefore, the results should be applicable to a wide range of studies. For the calculation of VeDBA, the element of measured acceleration that is static acceleration was estimated by taking a running mean with a 2-s window of the raw acceleration data for each axis [36]. Dynamic acceleration was then calculated by subtracting the static acceleration from the raw acceleration for each axis [36]. VeDBA was then calculated according to Qasem et al. [17] as

$$VeDBA = \sqrt{(A_x^2 + A_y^2 + A_z^2)} \qquad (5)$$

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where A_x is dynamic acceleration on the X axis ('sway'), and A_y and A_z are the dynamic accelerations of the Y ('surge') and Z ('heave') axes, respectively.

Calculation of animal pitch

The pitch of the animal, i.e., the angle of the tag placed on dorsal mid-line, behind the shoulders relative to the horizontal plane, θ (Fig. 1a) was calculated according to Collins et al. [60]:

$$\theta = \mathrm{Arctan}(\mathrm{stat}A_y/(\mathrm{stat}A_x^2 + \mathrm{stat}A_z^2)^{1/2}) * (180/\pi) \tag{6}$$

where $\mathrm{stat}A_y$ is static acceleration recorded on the Y ('surge') axis and $\mathrm{stat}A_x$ and $\mathrm{stat}A_z$ are static acceleration recorded on the X ('sway') and Z ('heave') axes, respectively.

Statistical analyses

Analyses were conducted using R version 3.4.4 [61]. To define the lower critical point, when sheep where stationary and exposed to different ambient temperatures, broken-stick linear regression models were fitted using the "segmented" package [62]. This enabled inflection points for the relationship between VO_2 and chamber temperature to be identified [63] for each sheep. Differences in VO_2 before and after shearing were assessed with a general linear mixed model (GLMM) that included individual identity (i.e., sheep A and sheep B) as a random factor, using the "lme4" package [64]. This package was also used to conduct another GLMM (Table 1), with log transformed VO_2 as the dependent variable and VeDBA as the main effect and identity as a random factor. Further separate GLMMs with log transformed VO_2 and VeDBA as the dependent variables were conducted to determine how VO_2 and VeDBA varied depending on interactions between speed and pitch, and speed and slope. Speed, pitch, and slope were included in these two models as main effects, with individual identity as a random factor. Stepwise deletion refined each model, and the best model was selected based on the Akaike information criterion (AIC) (Table 1). Residuals and fitted values of the final models were checked via histograms and scatterplots to ensure model assumptions were met. VO_2 was log-transformed to generate normal residuals for the models. The relationship between VO_2 and treadmill angle was also explored using a quadratic relationship to examine whether there was any evidence for VO_2 increasing at steeper gradients [29], both for inclines and declines. Due to limited data for each slope, Spearman's rank correlations were used to assess relationships between VO_2 and VeDBA at each individual treadmill angle using data obtained from walking at all speeds. Results were deemed to be significant if $P < 0.05$. Figures were created using the "ggplot2" package [65].

Declarations

Ethics approval and consent to participate

Ethical approval was granted by the School of Biological Sciences Ethical Review Committee, Queen's University Belfast and the UK Home Office. The owner of the animals, Mr H. McKay, consented to the research.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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