The Journal of Experimental Biology 203, 301–310 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2356

POSTPRANDIAL HEAT INCREMENT DOES NOT SUBSTITUTE FOR ACTIVE THERMOGENESIS IN COLD-CHALLENGED STAR-NOSED MOLES (*CONDYLURA CRISTATA*)

KEVIN L. CAMPBELL*, IAN W. MCINTYRE AND ROBERT A. MACARTHUR

Department of Zoology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

*Present address: Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4
(e-mail: campbelk@zoology.ubc.ca)

Accepted 1 November; published on WWW 22 December 1999

Summary

The postprandial increase in metabolic rate associated with consuming, assimilating and excreting a meal is often termed the heat increment of feeding (HIF). The metabolic heat production of star-nosed moles, *Condylura cristata*, held at thermoneutrality was monitored for 4 h following a single 10 min session of feeding on a ration consisting of 0 g (controls), 3.5 g or 10 g of earthworms. Coefficients for metabolizable energy digestibility and digesta passage rate of earthworms fed to *C. cristata* were also determined. We then tested whether feeding-induced thermogenesis substitutes partially or completely for thermoregulatory heat production in these animals exposed to sub-thermoneutral air temperatures (9–24 °C). A single feeding on earthworms had both short- and long-term effects on the metabolic rate and respiratory exchange ratio of *C. cristata*. The observed short-term (0–65 min) rise in metabolic rate, assumed to be associated primarily with the physical costs of nutrient digestion, absorption and excretion, was similar to the calculated mean retention time (66.7±7.8 min; mean ± S.E.M., *N*=5) of this species. This component of the HIF represented 2.9 % of the food energy ingested by moles fed a single 3.5 g (13.21 kJ) meal of earthworms and 1.4 % of the food energy ingested by moles fed a single 7.5 g (28.09 kJ) meal of earthworms. At all test temperatures, resting metabolic rate typically remained above fasting levels for 1–4 h following ingestion of the high-protein earthworm diet. This protracted rise in metabolic rate, presumably associated with the biochemical costs of amino acid oxidation/gluconeogenesis and ureagenesis, averaged 12.8 % of the metabolizable energy and 8.7 % of the gross energy intake. Despite the potential thermoregulatory benefit, we found no evidence that biochemical HIF substitutes for facultative thermogenesis in star-nosed moles exposed to low air temperatures.

Key words: heat increment of feeding, metabolism, energetics, digesta passage rate, digestibility, star-nosed mole, *Condylura cristata*, Insectivora, thermoregulation, earthworm.

Introduction

Indigenous to moist organic soils bordering on creeks, lakes and wetlands, the star-nosed mole (*Condylura cristata*) is one of the most distinctive mammals anywhere on Earth. Extensively distributed throughout eastern Canada and northeastern USA, star-nosed moles are reported to inhabit subarctic regions as far north as the fifty-fifth parallel (Peterson and Yates, 1980). Active year-round, *C. cristata* is chronically exposed to low ambient temperatures that may persist for six or more months a year over much of the species' range. In fact, star-nosed moles have been observed actively tunnelling through snow (Tenny, 1871; Merriam, 1884a) and even swimming and diving in ice-covered streams during winter (Merriam, 1884b; Seton, 1909; Hamilton, 1931). The small body size (40–70 g), northern distribution and conspicuous semi-aquatic habits of *C. cristata* (Peterson and Yates, 1980) suggest that this species may be among the most thermally challenged members of the Order Insectivora. Considering the varied and potentially stressful thermal environments encountered by *C. cristata* in nature, surprisingly little data exist on the energetics and thermal biology of these peculiar animals, either in air or in water (Campbell et al., 1999). From this perspective, the strategies employed by star-nosed moles to limit or prevent body cooling at low temperatures are of particular interest. One potential mechanism that could augment or substitute for primary metabolic heat production at low air temperatures is the heat increment of feeding or HIF (MacArthur and Campbell, 1994; Chappell et al., 1997; Hawkins et al., 1997).

The 'apparent' HIF, defined as the unavoidable liberation of heat that ensues from feeding, can be partitioned into two components. The first is associated with the mechanical costs of feeding (hereafter termed 'mechanical HIF') and includes

the cumulative effects of chewing, swallowing, digesting, absorbing and excreting foodstuffs (Carefoot, 1990). The second component ('biochemical HIF') arises from the post-absorptive biochemical transformations of carbohydrates, lipids and proteins to produce ATP (Carefoot, 1990). Because of the high biochemical costs of amino acid oxidation/gluconeogenesis and ureagenesis, it has been estimated that the oxidation of amino acids liberates 23 % more heat per net ATP yield than carbohydrate metabolism and 20 % more than lipid oxidation (Jungas et al., 1992). Given the appreciable metabolic costs of protein anabolism and amino acid catabolism, it is not surprising that the magnitude of the HIF response is larger for animals on high-protein diets than for those fed primarily carbohydrate- or lipid-based diets (Janes and Chappell, 1995; Rosen and Trites, 1997). Moreover, a strong positive correlation exists between the amino acid concentration of the diet and the magnitude of the biochemical HIF response (Carefoot, 1990).

The possibility that HIF may substitute for active thermogenesis in endotherms exposed to sub-thermoneutral air temperatures, thereby reducing active thermoregulatory costs, was first proposed by Rubner (1910). However, data obtained thus far are equivocal. Several researchers have suggested that HIF may largely supplant facultative thermogenesis (Rubner, 1910; Šimek, 1976; Chappell et al., 1997), while others reported only partial substitution (Biebach, 1984; Masman et al., 1988) or failed to detect thermal substitution altogether (Platt, 1974; Klaassen et al., 1989). Some of these discrepancies may have arisen from differences in the level of activity (and thus heat production) between fed and fasted animals or were due to variable thermal environments and periods of fasting prior to experimentation. Moreover, comparisons of metabolic rate between fed and fasting states were typically based on changes in rates of oxygen consumption ($\dot{V}_{O_2}$) rather than on actual differences in metabolic heat production. Measurements of $\dot{V}_{O_2}$ tend to overestimate realized heat production, with the degree of error varying according to substrate utilization (Gessaman and Nagy, 1988). Consequently, previous studies may not have enabled detection of subtle differences (or lack thereof) in the metabolic rates of pre- and postprandial animals exposed to sub-thermoneutral temperatures. Data are also generally lacking for those species that could derive the greatest thermal benefit: small-bodied endotherms that consume relatively large, high-protein meals and inhabit thermally stressful microenvironments (Costa and Kooyman, 1984). Given its semi-aquatic habits, intrinsically high basal rate of metabolism (Campbell et al., 1999) and voracious appetite (Schmidt, 1931) for high-protein aquatic and terrestrial invertebrates (French et al., 1957), the star-nosed mole offers an ideal candidate for such investigation.

The objectives of this study were threefold. First, to obtain baseline information on the energy exchange of star-nosed moles fed an earthworm-based diet, we determined coefficients for assimilation efficiency, metabolizable energy (ME) intake and digesta passage rate. Our second aim was to determine the magnitude and duration of HIF in star-nosed moles fed a natural diet of earthworms. In conjunction with this goal, we tested whether meal mass influenced the postprandial metabolic rate ($J\,g^{-1}\,h^{-1}$), the time course of elevated metabolic rate or the time course and deviation of the respiratory exchange ratio (RER) from fasting levels. Our final objective was to test whether the HIF response to a single 10 min session of feeding substitutes partially or fully for facultative thermogenesis of star-nosed moles exposed to sub-thermoneutral air temperatures.

Materials and methods

Study animals

Eight star-nosed moles *Condylura cristata* (Illiger, 1811) were captured in Sherman live-traps 2 km north of Piney, Manitoba, Canada (49°06′N, 95°59′W), under Manitoba Wildlife Scientific Permit no. WSP 97004. Following capture, moles were transported to the Animal Holding Facility, University of Manitoba. Details regarding animal holding chambers and maintenance diet are reported elsewhere (Campbell et al., 1999). Moles were acclimated to holding conditions for at least 4 weeks prior to initiating experiments. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care under the authorization of a University-approved animal research protocol. Subsequent to this study, star-nosed moles were used in two parallel studies examining various aspects of their diving behaviour and physiology. Following completion of these trials, moles were killed with an overdose of Halothane anaesthetic (M. T. C. Pharmaceuticals), and blood and tissue samples were obtained for biochemical assays (I. W. McIntyre, unpublished data).

Digestion trials

In vivo digestion estimates were derived for five moles confined to rodent metabolic cages (Nalgene Co., Rochester, NY, USA) using the total balance trial method (Robbins, 1993). Preliminary trials indicated that moles became sluggish and reduced their food intake after 24–36 h of placement in these cages. Accordingly, trials were restricted to 24 h and consisted of a 12 h pretrial period followed by a 12 h faecal and urine collection period in a separate, clean metabolic cage. Water was provided *ad libitum*, and trials were conducted at 20 °C. Prior to both digestibility and digesta passage rate experiments (see below), moles were fed earthworms (*Aporrectodea turgida*) exclusively for at least 24 h.

Earthworms were collected from the site of mole capture and placed in soil (also from the trapping site) on the day prior to each test session. At 4 h intervals, approximately 10–15 g of worms were rinsed in fresh water, patted dry, weighed to the nearest 0.01 g and offered to each mole. A separate sample of earthworms (3–5 g; ration sample) was treated in a similar manner and then frozen at −20 °C for subsequent analyses. Faeces were collected following the 12 h trial period and similarly frozen. To prevent the loss of urinary nitrogen as ammonia, 2–3 drops of concentrated HCl were added to urine

collection vials prior to each measurement interval (Robbins, 1993). The mass and total volume of urine produced were measured every 4 h. Following digestion trials, metabolic chambers were rinsed thoroughly with distilled water and the washings pooled with urine and frozen at −20 °C. Uneaten worms were also collected, rinsed and frozen.

Ration samples, uneaten worms, faeces and urine were lyophilized separately to constant mass. The gross energy (GE) content for each of these components was obtained by duplicate measurement in an adiabatic oxygen bomb calorimeter (Parr 1241 calorimeter, Parr Instrument Co., Moline, IL, USA) using benzoic acid as a standard. Urine samples were mixed with a known mass of mineral oil to ensure complete combustion. Coefficients for apparent dry matter, apparent digestible energy and apparent ME digestibility were calculated after Robbins (1993). A portion of each ration sample was also sent to a feed analysis laboratory (Norwest Labs, Winnipeg, Manitoba, Canada) for protein and lipid determinations.

Digesta passage rate

The gut contents of earthworms consist largely of inorganic soil particles (French et al., 1957). Thus, to quantify the flow of inorganic matter and thereby estimate the rate of passage of digesta through the gut of star-nosed moles, animals were presented with 3–5 earthworms (1–2 g) injected with Microtracer iron/nickel alloy particles (Micro Tracers Inc., San Francisco, CA, USA). In all cases, earthworms were consumed within 2 min. Following this initial feeding, moles were briefly allowed access to a small container furnished with 5–10 cm of water to rinse off any alloy grains adhering to their forefeet, then transferred to an empty 38 l plastic container where they were closely monitored for 6 h. For the duration of each passage-rate trial, moles were provided with freshly collected earthworms and water *ad libitum*.

Defecation times were recorded to the nearest minute, and all faeces were collected and stored at −20 °C. Subsequently, faecal samples were ashed individually at 550 °C for approximately 15–20 min to eliminate organic matter. The alloy marker was recovered from the ash trailings by filtration through Whatman no. 1 filter paper (12.5 cm). The number of particles per defecation was determined by transferring individual dried filter papers to a clean glass plate containing 2–3 ml of acid reagent (25 g of tartaric acid dissolved in 100 ml of water and 100 ml of hydrochloric acid). The paper was then dried (20–30 min), lightly sprinkled with a 1:1 (v/v) mixture of dimethylglyoxime (1 % alcoholic solution):ammonium hydroxide, and dried as before. This step converts nickel particles into discrete red specks of nickel dimethylglyoxime, which are easily identified and counted under a dissecting microscope.

Mean retention time (t_m) was calculated following the procedure of Warner (1981):

$$t_m = \sum m_i t_i / \sum m_i , \quad (1)$$

where m_i is the amount of marker excreted at the ith defecation time (t_i) after dosing. The mean times to excretion of 50 % (t_{50}) and 90 % (t_{90}) of the faecal marker and the time to first appearance (t_0) of the marker were also calculated.

Heat increment of feeding

Effect of meal size

One control and two feeding trials were conducted on seven moles. Trial order was randomized for each animal and at least 7 days separated successive tests on the same individual. To ensure that all animals were fasted for precisely 8 h prior to testing, moles were fed a final meal between 23:45 and 24:00 h on the night preceding each trial. At 08:00 h on the following morning, moles were weighed to the nearest 0.01 g, then transferred to a small feeding container and presented either with no food (controls) or with 3.5 g or 10 g of fresh earthworms that had been collected the evening prior to each trial. Following feeding, animals were again weighed and transferred to a respirometry chamber consisting of a modified 0.95 l paint can with a flat black interior fitted with inlet and outlet ports and furnished with 3–4 mm of dry-sterilized soil. A maximum of 2 min elapsed between the end of feeding and the initiation of metabolic measurements, which lasted for 4 h. The chamber was installed in a controlled-temperature cabinet set at 26 °C, which is near the lower limit of the thermoneutral zone of this species (25 °C; Campbell et al., 1999). The metabolic response of each mole was measured using negative-pressure, open-circuit respirometry as described by Campbell et al. (1999). Briefly, dry CO_2-free air was drawn through the chamber and metered at a rate of approximately 900 ml min^{-1} using a combination pump/mass flowmeter (TR-SS1 gas analysis subsampler, Sable Systems Inc., Henderson, NV, USA). A 250 ml subsample of dry exhaust gas was then routed continuously through a Beckman LB-2 carbon dioxide analyzer, passed through a soda lime/Drierite column, and then drawn through the M-22 sensor of an Applied Electrochemistry S3-A oxygen analyzer. Air flow rate, chamber temperature and fractional O_2 and CO_2 contents of expired air were recorded every 5 s using Datacan V data-acquisition software (Sable Systems Inc.). The delay in response time of the two gas analyzers (20 s) was corrected for when calculating the respiratory exchange ratio RER (see below). Following respirometry trials, moles were again weighed and the mean mass used for metabolic analyses (see below).

Thermogenic substitution

To test the hypothesis that HIF substitutes partially or completely for thermoregulatory heat production, metabolic responses of fasted and fed moles were determined at five, randomly assigned sub-thermoneutral air temperatures (9, 15, 18, 21 and 24 °C). The experimental protocol followed that described above except that, for each mole, both control and HIF trials at a given test temperature were conducted on the same day. In control trials, animals were fasted for 8 h, sham-fed for 10 min, and the metabolic rate of the fasted moles was monitored for 4 h (08:10–12:10 h). Immediately following this trial, moles were allowed 10 min in which to consume up to

10 g of worms. The metabolic rate of these fed moles was then monitored for an additional 4 h period (12:20–16:20 h).

For each feeding trial described above, 3–5 g of earthworms was set aside for dry matter, energy and ash determinations. A sample of worms from all 'effect of meal size' and 'thermogenic substitution' trials was homogenized separately and analyzed in duplicate for protein and lipid content. Following each feeding trial, uneaten earthworms (if any) were collected, weighed and dried to constant mass to calculate the intake of fresh and dry matter, gross energy and metabolizable energy.

Treatment of metabolic data

To evaluate the effects of meal size and ambient temperature (T_a) on the HIF response, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (ml g^{-1} h^{-1}) were calculated for each trial using Datacan V software (for details, see Campbell et al., 1999). For fasted moles, the RER calculated for each sampling period was used to convert $\dot{V}_{O_2}$ into units of heat production (H; J g^{-1} h^{-1}) as:

$$H = (16.218 + 4.716\mathrm{RER})\dot{V}_{O_2}, \quad (2)$$

assuming energy equivalents of 20.93 J ml^{-1} O_2 for glucose and 19.55 J ml^{-1} O_2 for lipid oxidation (Table 17 of Jungas et al., 1992) and that protein catabolism was negligible (Gessaman and Nagy, 1988). However, ingested amino acids in excess of those required for protein synthesis cannot be stored as amino acids. Surplus molecules must therefore be partially oxidized in the liver and then either converted to glucose (primarily) and acetoacetate or catabolized completely in the peripheral tissues to CO_2 and urea (Jungas et al., 1992). After accounting for bond energy excreted as urea, the net heat liberated from the oxidative, gluconeogenic and ureagenic pathways of representative amino acids in humans (17.71 J ml^{-1} O_2; Jungas et al., 1992), and presumably other ureotelic mammals, is substantially less than for the oxidative degradation of ingested carbohydrate and lipid molecules. Consequently, equation 2 would tend consistently to overestimate the postprandial rate of metabolism of animals consuming a diet rich in protein. Thus, with the assumption that the catabolism of these fuels by postprandial moles is proportional to their concentration in the diet (Gessaman and Nagy, 1988), $\dot{V}_{O_2}$ was converted to J g^{-1} h^{-1} as:

$$H = (17.71P + 20.93C + 19.55L)\dot{V}_{O_2}, \quad (3)$$

where P, C and L are the mean percentage contents of protein, carbohydrate and lipid of the ration, respectively. These values were obtained from Table 1 after correcting for the ash content of the earthworms fed to our study animals.

For the sham, 3.5 g and 10 g feeding trials, resting rates of metabolism and corresponding RERs were calculated at 5 min intervals throughout each 4 h trial. Similar to the findings of Chappell et al. (1997) for house wren (*Troglodytes aedon*) chicks, the metabolic rate tracings of star-nosed moles exhibited distinctive spikes arising from animal activity. These spikes were deleted and replaced by linearly interpolated data calculated from rates of metabolism immediately preceding and following each period of activity (Janes and Chappell, 1995; Chappell et al., 1997).

For both the fasted and fed portions of the 'effect of meal size' and 'thermogenic substitution' trials, the lowest metabolic rate (J g^{-1} h^{-1}) maintained over 3 min was determined for each 30 min interval using the Datacan 'nadir' function. In most cases, low, stable metabolic rate readings were obtained from study animals during each 30 min interval. In 15 of the 568 measurement intervals, we were unable to obtain stable recordings of minimal metabolic rate lasting 3 min. In these cases, metabolic rate was taken as the average of the 30 min periods immediately preceding and immediately following the interval in question. Finally, the resting metabolic rate (RMR) was calculated for each trial as the average of the three lowest steady-state rates of metabolism lasting at least 5 min in each case.

Body temperature

To evaluate body temperature (T_b) dynamics of fed and fasted moles, a limited number of abdominal T_b measurements were obtained telemetrically from a single mole implanted with a 1.3 g model X-M transmitter (Mini-Mitter Inc., Sunriver, OR, USA) as part of another study (Campbell et al., 1999). Surgical procedures are described elsewhere (Campbell et al., 1999). Briefly, the mole was weighed to the nearest 0.01 g and anaesthetized in stages with Ketamine hydrochloride (25–35 mg kg^{-1} intraperitoneally). Following induction of a surgical plane of anaesthesia, the abdominal incision site was scrubbed with 70 % isopropyl alcohol and a 1 cm midline incision made in the linea alba. The sterilized transmitter was inserted into the abdominal cavity, and the wound was closed in layers using absorbable sutures and treated with a local anaesthetic (Lidocaine gel). Following surgery, the mole was transferred to a clean 38 l plastic container furnished with a nest box and dry paper towels and provided with fresh food and water. The mole was returned to its holding quarters the following day and allowed a 7 day recovery period (see Campbell et al., 1999). The experimental protocol for this animal followed that described for the 'thermogenic substitution' trials, with trials initiated approximately 90 days post-operatively. Unfortunately, owing to battery failure, we were able to obtain T_b measurements for one trial only (9 °C).

Statistical treatment of data

Mean metabolic rates of fasting and fed animals for each 30 min interval at each test temperature were compared using one-tailed paired *t*-tests. To test whether HIF substitutes fully or partially for active thermogenesis, slopes and intercepts derived for the relationship between RMR and T_a were compared for fed and fasted moles using a one-way analysis of covariance (ANCOVA) with feeding state as the covariate. In all comparisons, significance was set at the 5 % level. Values are presented as means ±1 S.E.M.

Results

Digestion trials

The mean gross energy (GE) content of earthworms fed to star-nosed moles was 18.43±0.59 kJ g^{-1} dry matter (N=4

Table 1. *Mean dry matter content and composition of earthworms (*Aporrectodea turgida*) fed to star-nosed moles during digestibility, passage rate and heat increment of feeding trials*

		Heat increment of feeding trials	
	Digestibility and passage rate trials	Effect of meal size	Thermogenic substitution
Dry matter (%)	20.73	20.14	21.40
Gross energy (kJ g^{-1} dry matter)	18.43	18.68	17.54
Ash (%)[a]	21.73	22.55	28.42
Protein (%)[a]	56.35 (72.00)[b]	58.95 (76.11)	58.80 (82.15)
Lipid (%)[a]	3.00 (3.83)	3.50 (4.52)	2.60 (3.63)
Carbohydrate (%)[a]	18.92 (24.17)	15.00 (19.37)	10.18 (14.22)

[a]Data are expressed as a percentage of dry matter content.
[b]Values in parentheses denote percentage composition on an ash-free basis.

Table 2. *Body mass, dry matter intake and apparent digestibility coefficients of five star-nosed moles fed exclusively on earthworms*

	Mole 1	Mole 2	Mole 4	Mole 5	Mole 7	Mean
Mean body mass (g)	57.24	52.62	66.18	66.74	54.02	59.36±3.35
Mass change (g)	5.34	0.56	–0.99	0.67	–5.77	–0.04±1.99
Dry matter intake (g 12 h^{-1})	7.87	4.25	4.27	7.90	5.49	5.96±0.92
Digestibility (%)						
Dry matter	66.53	70.25	57.09	64.85	55.90	62.92±3.10
Ash-free dry matter	80.02	81.96	77.43	80.61	73.53	78.71±1.66
Digestible energy	80.65	83.00	79.50	79.83	70.94	78.79±2.30
Metabolizable energy	73.47	75.08	68.40	69.12	53.76	67.96±4.22

Values are means ± 1 S.E.M.

samples; Table 1). The intake of GE by star-nosed moles fed this diet averaged 108.9±17.0 kJ over the 12 h trial. Apparent dry-matter, digestible energy and metabolizable energy (ME) coefficients of captive star-nosed moles averaged 62.92±3.10, 78.79±2.30 and 67.96±4.22 %, respectively (Table 2).

Digesta passage rate

The rate of passage of particulate alloy markers through the alimentary canal was measured in five moles held at a T_a of 25 °C. The cumulative rate of excretion of marked faeces followed a sigmoidal distribution in all animals (Fig. 1). On average, the marker first appeared at 15 min post-ingestion (range 8–21 min). Mean retention time was 66.7±7.8 min, with 90 % of the marker excreted after approximately 138 min (Fig. 1).

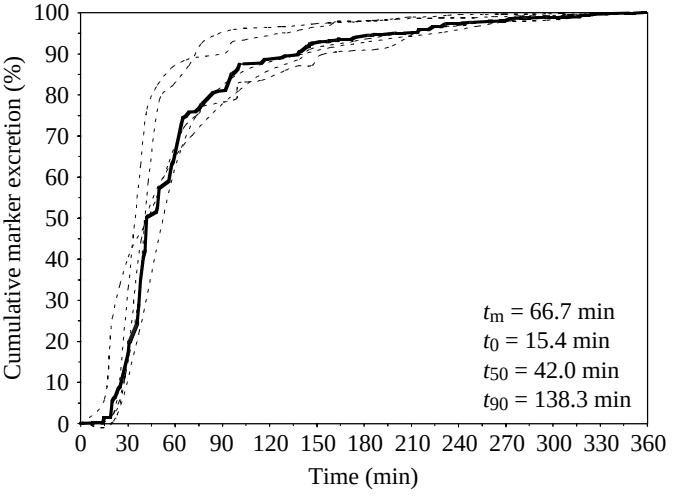

Fig. 1. Rate of passage of digesta in five star-nosed moles held at 25 °C and fed freshly collected earthworms (*Aporrectodea turgida*). The solid line indicates the mean cumulative percentage excretion of the particulate tracer; the dotted lines denote excretion patterns of individual animals. t_m, mean retention time (min); t_0, time to first appearance of marker; t_{50}, time to 50 % excretion of marker; t_{90}, time to 90 % excretion of marker.

Heat increment of feeding

A single 10 min bout of feeding on earthworms appeared to induce both short-term (0.5–1 h) and long-term (1–4 h) effects on the metabolic rate of star-nosed moles (Fig. 2; see Fig. 4). We assumed that transient changes in metabolic rate following the initiation of metabolic trials resulted from the physical costs associated with the transport of food through the

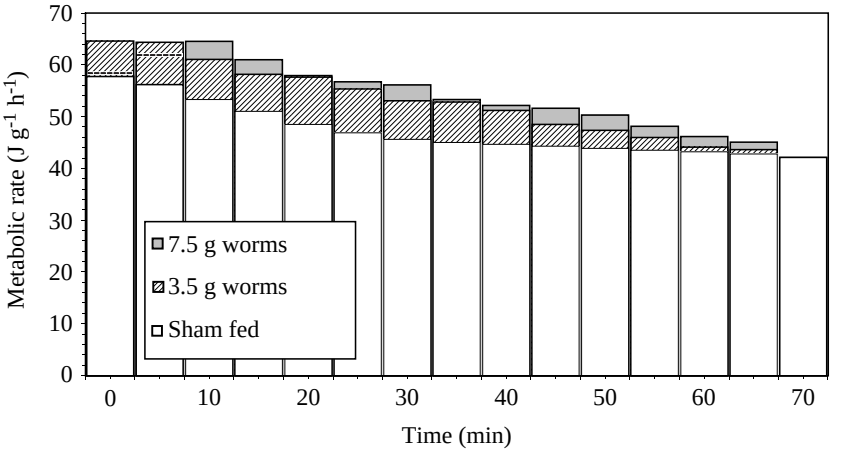

Fig. 2. Acute metabolic rates of fasted (sham-fed; open columns) and fed (hatched and shaded columns) star-nosed moles at 26 °C following a single 10 min session of sham feeding (no food) or of feeding on a 3.5 g or 7.5 g meal of earthworms. All animals (*N*=6 in each case) were fasted for 8 h prior to initiating sham and feeding trials. Values are means.

alimentary tract ('mechanical HIF') and were not greatly influenced by the catabolism of amino acids from the ingested meal. Thus, metabolic rate values of fed moles assumed to reflect mechanical HIF (see below) were calculated using equation 2. All other metabolic data presented for fed animals were estimated using equation 3.

Effect of meal size

The intake of earthworms by star-nosed moles offered the 3.5 g meal averaged 3.51±0.09 g (13.21 kJ) and represented 6.5 % of mole body mass; for moles offered the 10 g meal, the intake averaged 7.54±0.48 g (28.09 kJ) and represented 15.0 % of body mass. We collected metabolic data following the control, 3.5 g and 10 g feeding trials for seven moles (21 trials in total). Because one animal refused to remain calm in the respirometry chamber following the control and 10 g feeding trial, metabolic measurements for this animal were omitted from analyses.

In all cases, fed and fasted star-nosed moles exhibited a short-term (approximately 30–60 min) increase in resting metabolic rate following the commencement of trials, with the largest gain occurring within the first 5–15 min (Fig. 2). This transient increase in metabolic rate above baseline values averaged 0.344, 0.725 and 0.721 kJ for the control, 3.5 g and 10 g feeding trials, respectively. We subtracted the increase in metabolic rate for the control trials from those observed in feeding trials to estimate the physical costs of food assimilation and transport in fed moles. For the 3.5 g and 7.5 g meals, the calculated mechanical HIF, expressed as a percentage of GE intake, averaged 2.9 and 1.4 %, respectively.

The cumulative (mechanical + biochemical) HIF following ingestion of the 3.5 g meal averaged 1.35 kJ or 10.2 % of the GE intake, while the HIF following ingestion of the 7.5 g meal averaged 1.77 kJ or 6.3 % of the GE intake. Over the 4 h trial, the mean RMRs following ingestion of these meals were 9.7 % (3.5 g meal) and 14.2 % (7.5 g meal) higher than that observed in fasting moles. The time course of the metabolic response was positively correlated with meal mass and was similar to that observed for RER (see below). Most of the HIF response of moles consuming the 3.5 g meal was completed within

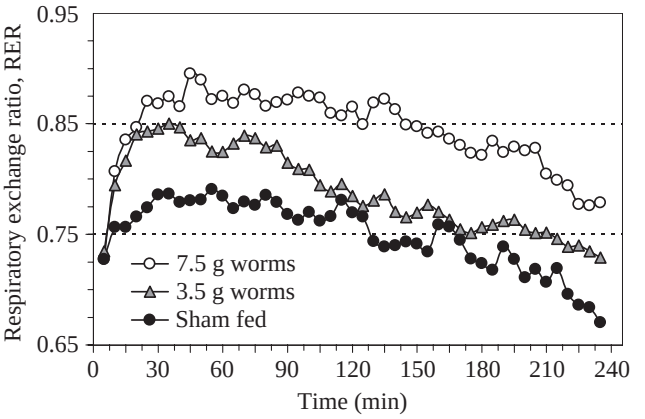

Fig. 3. Mean respiratory exchange ratios of fasted and fed star-nosed moles held at 26 °C following a single 10 min session of sham feeding (no food) or of feeding on a 3.5 g or 7.5 g meal of earthworms. All animals (*N*=6 in each case) were fasted for 8 h prior to initiation of sham and feeding trials.

120 min of ingestion, while the metabolic rate of moles fed the 7.5 g meal remained elevated above fasting levels for nearly the entire 4 h trial.

The RER of star-nosed moles increased rapidly above fasting values following ingestion of earthworms and plateaued at a value close to 0.85 (Fig. 3). By approximately 120 min post-feeding, the RER of moles offered the 3.5 g ration differed little from that exhibited by fasted animals. The RER of moles fed the 7.5 g meal remained elevated above fasting levels for the duration of the trial; however, it began to drop markedly approximately 200 min after ingestion of the earthworm diet (Fig. 3).

Thermogenic substitution

Metabolic data were obtained from five moles held at T_a values ranging from 9 to 24 °C. For all trials, including control runs on fasted animals, moles tended to exhibit a progressive increase in metabolic rate during the first 30 min of metabolic testing. Following the initial 30 min period of each trial, the metabolic rate of fasted star-nosed moles varied little with time

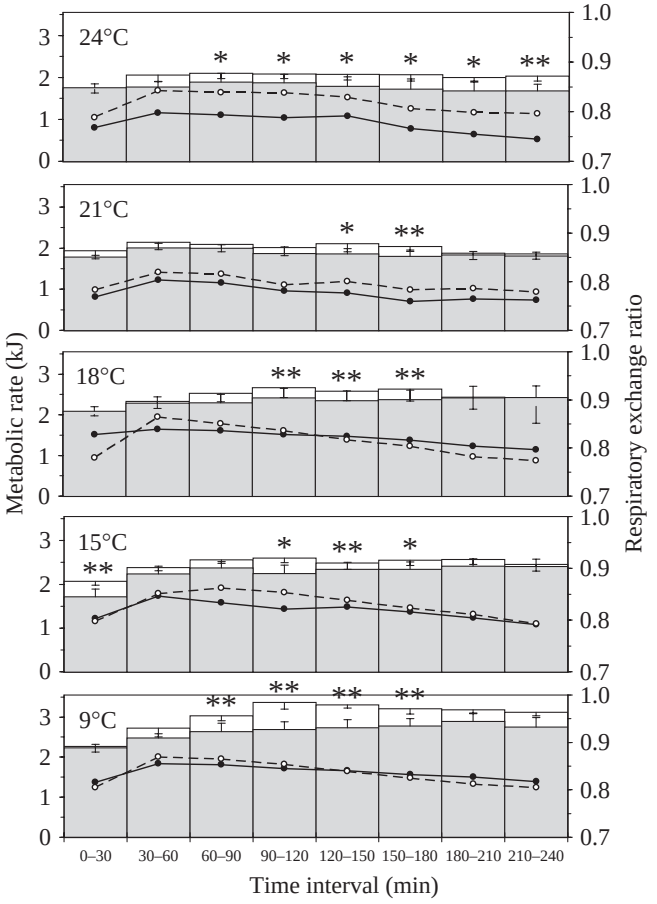

Fig. 4. Resting metabolic rates (columns) and respiratory exchange ratios (circles) of five star-nosed moles held at air temperatures of 9–24 °C following either an 8 h fast (shaded columns and filled circles, respectively) or a single 10 min session of feeding on earthworms (open columns and open circles, respectively). For each 30 min interval, metabolic rate is presented as the mean of the lowest 3 min recordings. Asterisks indicate periods when metabolic rates of fasted and postprandial star-nosed moles were significantly different (paired *t*-test; $^{**}P<0.05$, $^{*}P<0.075$). Vertical bars denote 1 S.E.M.

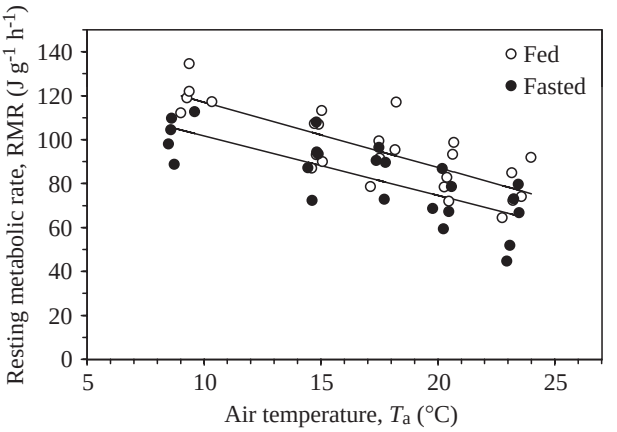

Fig. 5. The relationship between resting metabolic rate (RMR) and air temperature (T_a) of fasted and fed star-nosed moles (N=5 animals). Regression lines were fitted by the method of least squares (RMR_{fasted}=128.86–2.71T_a, N=25, r^2=0.60; RMR_{fed}=146.74–2.97T_a, N=25, r^2=0.63).

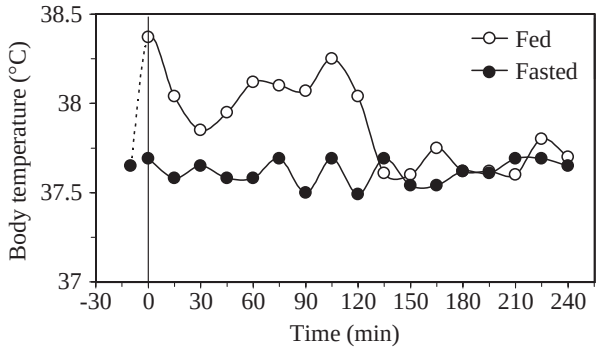

Fig. 6. Telemetered abdominal temperature of a radio-implanted star-nosed mole held at an air temperature of 9 °C. Filled circles indicate the body temperature following an 8 h fast; open circles denote the body temperature of the same animal following a 10 min session of feeding on earthworms (period indicated by dashed line).

(Fig. 4). In most 30 min sampling periods, fed moles exhibited a higher rate of heat production than their fasted counterparts. Maximal differences in metabolic rate between fasted and postprandial moles typically occurred between 60 and 180 min post-feeding (Fig. 4). Contrary to our expectations, we found no evidence that biochemical HIF substitutes for facultative thermogenesis in star-nosed moles exposed to low air temperatures (Figs 4, 5).

On the basis of the cumulative difference in metabolic rate between fed and fasted moles for all T_a values combined, the calculated HIF averaged 2.08±0.29 kJ (Fig. 4). Assuming an average metabolizable energy digestibility of 68.0 % (Table 2), this HIF estimate represents 12.8 % (range 7.7–20.1 %) of the mole's intake of metabolizable energy and 8.7 % (range 5.2–13.7 %) and of the mole's intake of gross energy.

For both fed and fasted moles, RMR varied inversely with air temperature (Fig. 5): RMR_{fed}=146.74–2.97T_a (N=25; r^2=0.63); RMR_{fasted}=128.86–2.71T_a (N=25, r^2=0.60). The calculated slopes for these two regressions were not significantly different (F=0.15, d.f.=46, P=0.70). However, the regression intercept was higher for fed than for fasted moles (F=17.52, d.f.=47, P<0.0001). Over the entire range of test temperatures, the mean resting $\dot{V}_{O_2}$ (data not shown) of postprandial moles was 1.27 times the level established for postabsorptive animals, and the mean resting RMR (Fig. 5) was 1.17 times the level for postabsorptive animals.

Body temperature

Following the 8 h fast, the telemetered abdominal T_b of the radio-implanted mole held at 9 °C varied little over the 4 h metabolic trial and averaged 37.6 °C (Fig. 6). A biphasic T_b response was observed following the single session of feeding on earthworms. Soon after the mole had ingested the meal, T_b increased by 0.8 °C, an effect that persisted for only a few

minutes before T_b gradually declined towards fasted levels. At 30 min post-feeding, T_b again increased and remained elevated (above 38.0 °C) for approximately 90 min (Fig. 6). Thereafter, postprandial T_b declined and remained similar to fasting T_b for the remainder of the trial.

Discussion

Heat released secondarily to the consumption, absorption, storage and excretion of ingested nutrients is an unavoidable consequence of feeding (Carefoot, 1990). Because this energy is unavailable to support other physiological processes (Chappell et al., 1997; Rosen and Trites, 1997), the HIF is often deemed to be 'wasted' energy. As early as 1910, however, Rubner proposed that the HIF may offset expenses incurred by active thermogenesis, thereby reducing maintenance costs at sub-thermoneutral temperatures. Such partial or complete thermal substitution would be especially beneficial to endothermic species living in cold climates where this compensatory mechanism would favour more efficient use of metabolic heat production (Klaassen et al., 1989). Despite the potential for HIF to mitigate thermoregulatory costs, we found no evidence to support the contention that the biochemical HIF substitutes either partially or fully for thermogenic heat production in cold-challenged star-nosed moles. Instead, star-nosed moles exhibited a significant and prolonged 9–26 % (mean 17.0 %) increase in RMR following a single session of feeding at all air temperatures tested (Fig. 5).

Indirect calorimetry is commonly used to assess the metabolic rate of animals *via* the conversion of $\dot{V}_{O_2}$ and/or $\dot{V}_{CO_2}$ into corresponding units of metabolic heat production (Gessaman and Nagy, 1988). Typically, metabolic rate is calculated by multiplying $\dot{V}_{O_2}$ by a pre-determined conversion factor (e.g. $20.1\,J\,ml^{-1}\,O_2$) or by an appropriate energy equivalent deduced from the RER, with the assumption that amino acid catabolism is negligible (see equation 2). Errors arising from these techniques are usually small for postabsorptive animals or those catabolizing mainly lipids and carbohydrates (±0.6 %; Gessaman and Nagy, 1988). In addition, assuming an energy equivalent of $19.5\,J\,ml^{-1}\,O_2$ for protein catabolism, Gessaman and Nagy (1988) calculated that inaccuracies introduced by this method were typically less than +3 % following ingestion of high-protein diets. However, on the basis of a comprehensive analysis of amino acid metabolism in man, Jungas et al. (1992) concluded that the average thermal equivalent of protein catabolism ($17.71\,J\,ml^{-1}\,O_2$) was substantially lower than previously thought. Consequently, if amino acids solely are being catabolized for energy, the application of RER measurements to calculate thermal equivalents (i.e. equation 2) will overestimate true metabolic heat production by 10.4–18.2 % (assuming RERs ranging from 0.71 to 1.0, respectively). Previous studies examining the HIF response of animals fed diets rich in protein have failed to account for this potential source of error when converting $\dot{V}_{O_2}$ into units of heat production and, hence, may have overestimated the true postprandial metabolic rate. To avoid this error, we assumed that substrate oxidation by postprandial moles was in direct proportion to the amount of substrate consumed.

The RER of fed moles (mean 0.823; range 0.795–0.847) was similar at all T_a values (Figs 3, 4) and close to the value calculated for the catabolism of amino acids (0.838; Jungas et al., 1992). In addition, as the RER typically remained near this level for more than 3 h before declining towards fasting levels (Figs 3, 4), we believe our technique for determining heat equivalents closely reflects both the actual proportion of substrates being catabolized and the true rate of heat production by star-nosed moles. Thus, with the assumption that amino acid oxidation/gluconeogenesis accounted for 82 % of the postprandial metabolic rate of *C. cristata* (Table 1), the average HIF across the entire range of test temperatures was 8.7 % of the GE intake (12.8 % of ME intake). This value is nearly 50 % lower than that (16.5 % of GE intake) derived from the multiplication of $\dot{V}_{O_2}$ by the RER energy equivalent, assuming negligible protein oxidation (equation 2). These HIF estimates (8.7 and 16.5 % of GE intake) encompass the range (4.7–16.8 %) reported for other aquatic endotherms fed high-protein diets of animal tissue (see Janes and Chappell, 1995; Rosen and Trites, 1997; and references therein).

As noted above, star-nosed moles exhibited a discernible HIF at all temperatures tested, with a significant elevation in metabolic rate persisting for approximately 1–3 h post-feeding in most cases (Fig. 4). Surprisingly, the largest postprandial rise in resting metabolic rate occurred at the lowest test temperature, 9 °C (Figs 4, 5). The intensity of the HIF response at this T_a was sufficient to elevate the internal temperature of the metabolic chamber 0.7 °C above that maintained by fasted moles, despite the continuous inflow (approximately $900\,ml\,min^{-1}$) of cool outside air into the chamber.

This finding suggests the possibility that the HIF could contribute to warming the microclimate of star-nosed moles in nature. It is noteworthy that free-ranging, radio-tagged European moles (*Talpa europaea*) exhibit regular bouts of foraging activity lasting 3–4 h, separated by periods of similar duration spent in the nest sleeping and digesting meals (Godfrey, 1955; Gorman and Stone, 1990). Captive star-nosed moles share a remarkably similar cycle of activity and rest to the European mole (Campbell et al., 1999). Excavated nest chambers of both species are invariably lined with dried grasses and leaves and are presumably well insulated (Hamilton, 1931; Schmidt, 1931; Gorman and Stone, 1990). We suggest that the supplementary heat liberated from the prolonged biochemical HIF contributes to maintaining an elevated T_a within the nesting chamber, thereby minimizing the gradient for sensible heat loss. This indirect form of thermal substitution could reduce facultative thermogenesis by *Condylura cristata* for up to 12 h per day and may prove to be an important component of the daily energy budget of free-living star-nosed moles. Similar benefits may accrue to other species that routinely spend significant periods within their nests following bouts of foraging.

The basal rate of metabolism of *C. cristata* is double that expected on the basis of body mass (Campbell et al., 1999). Not

surprisingly, star-nosed moles have a ravenous appetite and presumably spend much of their waking hours in search of food. On the basis of their mean GE intake of 108.9 kJ over the 12 h digestibility trials, and assuming that the dry mass of individual earthworms averages 0.10±0.01 g (*N*=40; this study) with an energy equivalent of 18.4 kJ g^{-1} (Table 1), star-nosed moles would need to consume approximately 120 worms per day just to meet their minimum energy requirements. This estimate is consistent with the observation of Schmidt (1931) that a single star-nosed mole consumed 1850 earthworms, two mice, a frog and three large grubs over a 2 week period. It is noteworthy that both these values were obtained from captive specimens. Undoubtedly, prey ingestion by free-ranging moles is much greater, particularly in cold weather. Given the enormous appetite of *C. cristata*, it is not surprising that the calculated mean retention time (67 min, Fig. 1) and the time to first appearance of faecal marker (15 min) were remarkably short. In fact, the rate of passage of digesta through the alimentary tract of the relatively large *C. cristata* (60 g) is similar to those observed in small (3–18 g) shrews (Warner, 1981). Despite the extremely rapid throughput time of star-nosed moles, the apparent digestibility coefficients for ash-free dry matter and digestible energy on the earthworm diet were still close to 80 % (Table 2).

The mean retention time of digesta was of similar duration to the mechanical HIF (Fig. 2), suggesting that this component of the HIF was mainly associated with events occurring in the alimentary tract. At thermoneutral temperatures, the mechanical HIF accounted for 1.4–2.9 % of GE intake by star-nosed moles (Fig. 2). However, as metabolic costs associated with prey handling, mastication and ingestion were not accounted for, this value is probably a conservative estimate. In many instances, these voracious feeders tear prey items into smaller, more manageable segments before consuming them. The celerity of this behaviour suggests that it could generate considerable metabolic heat. In fact, a single session of feeding on earthworms increased the T_b of star-nosed moles by 0.8 °C over pre-feeding values in a matter of minutes (Fig. 6).

It is interesting to note that, during the initial 30 min following feeding, the mechanical HIF response that was clearly evident in *C. cristata* tested at thermoneutrality (Fig. 2) was often absent at sub-thermoneutral temperatures (Fig. 4). This observation implies that the incidental storage of heat resulting from the muscular work of feeding may offset the thermoregulatory costs of star-nosed moles exposed to cold. Muskrats (*Ondatra zibethicus*) allowed to feed in water exhibited reduced body cooling compared with non-fed controls, presumably because of the muscular work associated with the handling and ingestion of aquatic vegetation (MacArthur and Campbell, 1994). A similar substitution of heat generated during activity and grooming for facultative thermogenesis has been proposed for short-tailed shrews *Blarina brevicauda* (Platt, 1974) and sea otters *Enhydra lutris* (Costa and Kooyman, 1984).

It should be noted that, even following an extended fast, star-nosed moles exposed to air temperatures ranging from 0 to 33 °C are able to regulate T_b close to 37.7 °C (Campbell et al., 1999; Fig. 6). However, even brief immersion in cold water may severely tax the thermogenic abilities of small semiaquatic mammals, and these animals often cool quickly in the aquatic medium (MacArthur, 1984). Earlier studies have underscored the value of stored body heat to aquatic thermoregulation in small-bodied amphibious mammals (Costa and Kooyman, 1984; MacArthur, 1984). Still unanswered, however, is the question of whether heat produced *via* the biochemical HIF may be of thermoregulatory benefit to star-nosed moles during periods of aquatic activity. Our results indicate that heat liberated *via* biochemical HIF elevates T_b by approximately 0.5 °C over fasting levels for at least 2 h following feeding (Fig. 6). It is conceivable that this supplemental source of heat may augment active thermogenesis by *C. cristata* while swimming and diving and thus attenuate body cooling. A similar benefit has been suggested for the HIF response of sea otters and Steller sea lions (*Eumetopias jubatus*) feeding at sub-thermoneutral water temperatures (Costa and Kooyman, 1984; Rosen and Trites, 1997). Alternatively, it is possible that star-nosed moles passively cool in the aquatic medium and, like muskrats (MacArthur, 1984), defer recovery of T_b until after emergence from water. It is possible that heat generated *via* HIF may expedite rewarming and perhaps diminish the metabolic cost of rewarming by *C. cristata* following excursions in cold water. Studies addressing the potential benefits of HIF to the aquatic temperature regulation of these diminutive divers are currently in progress.

We thank J. E. Dubois, Manitoba Museum of Man and Nature, for supplying mole distribution records and providing lodging quarters while trapping and R. Pearce for permitting us to live-trap moles on his land. We thank G. A. Fines and B. D. Jeske for assisting in the live-trapping and husbandry of moles, and J. W. Reynolds for identifying earthworm specimens. The helpful comments and critical review of the manuscript by D. A. S. Rosen are appreciated. Funding for this study was provided by an NSERC Postdoctoral Fellowship to K.L.C. and an NSERC operating grant to R.A.M.

References

Biebach, H. (1984). Effect of clutch size and time of day on the energy expenditure of incubating starlings (*Sturnus vulgaris*). *Physiol. Zool.* **57**, 26–31.

Campbell, K. L., McIntyre, I. W. and MacArthur, R. A. (1999). Fasting metabolism and thermoregulatory competence of the star-nosed mole, *Condylura cristata* (Talpidae: Condylurinae). *Comp. Biochem. Physiol.* **123**A, 293–298.

Carefoot, T. H. (1990). Specific dynamic action (SDA) in the supralittoral isopod, *Ligia pallasii*: identification of components of apparent SDA and effects of dietary amino acid quality and content on SDA. *Comp. Biochem. Physiol.* **95**A, 309–316.

Chappell, M. A., Bachman, G. C. and Hammond, K. A. (1997). The heat increment of feeding in house wren chicks: magnitude, duration and substitution for thermostatic costs. *J. Comp. Physiol.* B **167**, 313–318.

Costa, D. P. and Kooyman, G. L. (1984). Contribution of specific

dynamic action to heat balance and thermoregulation in the sea otter *Enhydra lutris*. *Physiol. Zool.* **66**, 45–52.

French, C. E., Liscinsky, S. A. and Miller, D. R. (1957). Nutrient composition of earthworms. *J. Wildl. Mgmnt* **21**, 348.

Gessaman, J. A. and Nagy, K. A. (1988). Energy metabolism: errors in gas-exchange conversion factors. *Physiol. Zool.* **61**, 507–513.

Godfrey, G. K. (1955). A field study of the activity of the mole (*T. europaea*). *Ecology* **36**, 678–685.

Gorman, M. L. and Stone, R. D. (1990). *The Natural History of Moles*. Ithaca, NY: Cornell University Press.

Hamilton, W. J., Jr (1931). Habits of the star-nosed mole, *Condylura cristata*. *J. Mammal.* **12**, 345–355.

Hawkins, P. A. J., Butler, P. J., Woakes, A. J. and Gabrielsen, G. W. (1997). Heat increment of feeding in Brunnich's guillemot *Uria lomvia*. *J. Exp. Biol.* **200**, 1757–1763.

Janes, D. N. and Chappell, M. A. (1995). The effect of ration size and body size on specific dynamic action in Adelie penguin chicks, *Pygoscelis adeliae*. *Physiol. Zool.* **68**, 1029–1044.

Jungas, R. L., Halperin, M. L. and Brosnan, J. T. (1992). Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiol. Rev.* **72**, 419–448.

Klaassen, M., Bech, C. and Slagsvold, G. (1989). Basal metabolic rate in arctic tern chicks and the effect of heat increment of feeding on thermoregulatory expenses. *Ardea* **77**, 193–200.

MacArthur, R. A. (1984). Aquatic thermoregulation in the muskrat (*Ondatra zibethicus*): energy demands of swimming and diving. *Can. J. Zool.* **62**, 241–248.

MacArthur, R. A. and Campbell, K. L. (1994). Heat increment of feeding and its thermoregulatory benefit in the muskrat (*Ondatra zibethicus*). *J. Comp. Physiol.* B **164**, 141–146.

Masman, D., Daan, S. and Dietz, M. (1988). Heat increment of feeding in the kestrel, *Falco tinnunculus* and its natural seasonal variation. In *Physiology of Cold Adaptation in Birds* (ed. C. Bech and R. E. Reinertsen), pp. 123–135. New York: Plenum Press.

Merriam, C. H. (1884a). The vertebrates of the Adirondack Region, Northeastern New York. *Trans. Linn. Soc. NY* **2**, 48–55.

Merriam, C. H. (1884b). The star-nosed mole amphibious. *Science* **4**, 429.

Peterson, K. E. and Yates, T. L. (1980). *Condylura cristata*. *Mammal. Species* **129**, 1–4.

Platt, W. J. (1974). Metabolic rates of short-tailed shrews. *Physiol. Zool.* **47**, 75–90.

Robbins, C. T. (1993). *Wildlife Feeding and Nutrition*. Second edition. New York: Academic Press.

Rosen, D. A. S. and Trites, A. W. (1997). Heat increment of feeding in Steller sea lions, *Eumetopias jubatus*. *Comp. Biochem. Physiol.* **118**A, 877–881.

Rubner, M. (1910). Über Kompensation und Summation von funktionellen Leistungen des Körpers. *Sitzungeber. Koniglich Preussichen Akad. Wiss*., 316–324.

Schmidt, F. J. W. (1931). Mammals of western Clark County. *J. Mammal.* **12**, 99–117.

Seton, E. T. (1909). *Life-histories of Northern Animals – An Account of the Mammals of Manitoba*, vol. II. New York: Charles Schribner's Sons. pp. 1136–1144.

Šimek, V. (1976). Influence of a single administration of different diets on the energy metabolism at temperature of 10, 20 and 30 °C in the golden hamster. *Physiol. Bohemoslov.* **25**, 251–253.

Tenny, S. (1871). On the appearance of the star-nosed mole on the snow at Niles, Michigan. *Am. Nat.* **5**, 314.

Warner, A. C. I. (1981). Rate of passage of digesta through the gut of mammals and birds. *Nutr. Abstr. Rev.* B **51**, 789–820.