The heat increment of feeding and its thermoregulatory implications in the short-tailed shrew (*Blarina brevicauda*)

Allyson G. Hindle, Ian W. McIntyre, Kevin L. Campbell, and Robert A. MacArthur

Abstract: The nature and potential thermoregulatory benefits of the heat increment of feeding (HIF) were investigated in short-tailed shrews (*Blarina brevicauda*). At thermoneutrality, the postprandial rate of oxygen consumption ($\dot{V}_{O_2}$) of shrews increased by an average of 18% beyond fasting levels for ca. 2 h following the consumption of 3.5 g of earthworms. Over the same period, body temperature increased by an average of 0.6 °C. The digesta-retention time calculated from nickel alloy tracer excretion rates (168.1 ± 11.4 min (mean ± SE); $n = 7$) exceeded the duration of HIF (117.5 ± 10.4 min; $n = 6$) by 43%. This finding suggests that the mechanical costs of feeding may be a relatively minor component of HIF in this species. Regression of resting $\dot{V}_{O_2}$ on ambient temperature (T_a) below thermoneutrality yielded similar slopes ($P = 0.71$) and intercepts ($P = 0.33$) for fed and fasted animals, suggesting that HIF substitutes, at least partially, for facultative thermogenesis at low T_a. We found no evidence that HIF enhanced microclimate warming of an insulated, open-flow metabolic chamber occupied by recently fed shrews. Occupancy of this chamber by shrews increased microclimate T_a from 5 to 9.0–9.5 °C regardless of their nutritional status.

Résumé : Nous avons étudié la nature et les bénéfices thermorégulateurs potentiels de l'accroissement de température dû à l'alimentation (HIF) chez la grande musaraigne (*Blarina brevicauda*). Dans des conditions de neutralité thermique, le taux de consommation d'oxygène ($\dot{V}_{O_2}$) des musaraignes après un repas augmente en moyenne de 18 % au-dessus du niveau de jeûne pendant ca. 2 h après la consommation de 3,5 g de vers de terre. Durant cette même période, la température du corps croît en moyenne de 0,6 °C. La durée de rétention du bol alimentaire calculée d'après les taux d'excrétion d'un traceur d'alliage de nickel (168,1 ± 11,4 min (moyenne ± erreur type); $n = 7$) dépasse la durée de l'HIF (117,5 ± 10,4 min; $n = 6$) de 43 %. Cette constatation laisse croire que les coûts mécaniques de l'alimentation sont une composante relativement mineure de l'HIF chez cette espèce. Des régressions de $\dot{V}_{O_2}$ au repos sur la température ambiente (T_a) sous la neutralité thermique génèrent des pentes ($P = 0,71$) et des distances à l'origine ($P = 0,33$) semblables chez les animaux nourris et à jeun, ce qui indique que HIF se substitue peut-être, au moins en partie, à la thermogenèse facultative aux T_a basses. Il n'y a pas d'indications que HIF améliore le réchauffement du microclimat dans une enceinte métabolique isolée à circuit ouvert occupée par des musaraignes qui viennent de manger. La présence des musaraignes dans l'enceinte fait augmenter la température du microclimat T_a de 5 à 9,0–9,5 °C, quel que soit leur statut alimentaire.

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Introduction

The elevation of metabolic rate in response to consumption of a meal has been termed the heat increment of feeding (HIF). HIF can be partitioned into two major postprandial energy-requiring processes. The first involves the muscular work of mastication and peristalsis (Carefoot 1990). The second, biochemical component results from the postabsorptive metabolism of carbohydrates, lipids, and especially proteins (Carefoot 1990). HIF is an obligate response that potentially can be exploited by cold-stressed animals to

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offset costs of thermogenesis at low temperatures (Kleiber 1975). In theory, the metabolic rate of fed animals should always exceed that of fasted animals in the thermoneutral zone (TNZ). However, if HIF substitutes for cold-induced thermogenesis, the metabolic rates of fed and fasted animals should converge below the TNZ (Robbins 1993). To date, the largest body of data suggesting that HIF substitutes, at least partially, for active thermogenesis in cold has been gathered for aquatic birds (Janes and Chappell 1995; Hawkins et al. 1997) and mammals (Costa and Kooyman 1984; MacArthur and Campbell 1994; but see Campbell et al. 2000). Thermoregulatory substitution by HIF has also been noted in terrestrial endotherms, including white-tailed deer (*Odocoileus virginianus*) fawns (Jensen et al. 1999) and house wren (*Troglodytes aedon*) chicks (Chappell et al. 1997).

Campbell et al. (2000) recently proposed that the HIF response of small endotherms may also elevate the ambient temperature (T_a) within insulated nests, thus providing a thermoregulatory benefit to the nest occupants. If so, then

the potential exists for indirect substitution of HIF for facultative thermogenesis in nest-building endotherms (Campbell et al. 2000). The energetic benefits of nest building, irrespective of nutritional status, have been amply demonstrated in small mammals (Glaser and Lustick 1975; Pauls 1981; Vogt and Lynch 1982; McDevitt and Andrews 1994). For example, studies of the red squirrel (*Tamiasciurus hudsonicus*) and white-footed mouse (*Peromyscus leucopus*) have shown that the construction and occupation of nests reduces winter energy expenditure by 18%–28% (Pauls 1981; Vogt and Lynch 1982). We suggest that any additional metabolic heat released secondarily to feeding should only serve to augment this energy saving.

Estimated basal metabolic rates (BMR) of shrews tend to exceed allometric predictions (Pearson 1947; McNab 1991) and it is generally recognized that shrews consume more food per unit body mass than most other mammals (Buckner 1964). Consequently, shrews should experience a relatively large HIF response, especially given that their diet consists almost entirely of protein-rich animal tissue (Morrison et al. 1957; Musharaf and Latshaw 1999). Furthermore, shrews exhibit a nearly continuous activity cycle consisting of frequent foraging bouts interspersed with brief periods of rest. Therefore, shrews may exhibit a chronic HIF response that persists over the course of the day.

A species ideally suited for investigating the potential thermoregulatory benefits of feeding is the short-tailed shrew (*Blarina brevicauda*). A northern distribution (George et al. 1986), a lack of a definitive circadian activity pattern (Antipas et al. 1990), and an apparent absence of energy-saving strategies such as huddling or torpor (Merritt 1986; McNab 1991) all imply that this shrew is faced with daily as well as seasonal thermoregulatory challenges. Given these constraints, the short-tailed shrew is likely to adopt strategies that optimize the use of the obligate HIF response. Furthermore, the tendency for individuals of this species to cache food in or close to the nest implies that they do not always leave their nests to feed (Robinson and Brodie 1983). This observation, combined with the potential for a large HIF response, supports the hypothesis that HIF may substitute indirectly for facultative thermogenesis in short-tailed shrews occupying insulated nests by increasing microclimate T_a.

The objectives of this study were twofold. First, we wished to quantify the magnitude and time course of the HIF response of short-tailed shrews. The elevation in rate of oxygen consumption ($\dot{V}_{O_2}$) in response to feeding and the duration of this $\dot{V}_{O_2}$ increment were determined. The passage rate of digesta through the alimentary tract of captive short-tailed shrews was also measured to determine the maximum period over which the mechanical events associated with feeding could be expected to contribute to the observed metabolic response. Body-temperature (T_b) dynamics of selected test animals were also measured in conjunction with the HIF response.

Our second goal was to test two hypotheses regarding the potential thermoregulatory benefits of the HIF response in short-tailed shrews. The possibility that HIF substitutes for facultative thermogenesis was tested by comparing resting metabolic rates (RMR) of fasted and fed shrews over a T_a range of 0–29 °C. We specifically tested for convergence of

thermal-conductance slopes derived by regressing metabolic rate on T_a below thermoneutrality, and for a decrease in the lower limit of the TNZ (lower critical temperature; LCT) of fed animals that would imply a direct substitution effect (Kleiber 1975). We also considered the alternative hypothesis that HIF substitutes indirectly for facultative thermogenesis, by raising the microclimate T_a of an insulated nest in a cold environment.

Materials and methods

Animals

Adult short-tailed shrews (20.4–32.2 g) were captured in Nopoming Provincial Park (50°45′N, 95°20′W) and at the Fort Whyte Centre, Winnipeg, Manitoba (49°50′N, 97°10′W), during the fall of 1999. Though the sex ratio of subject animals is unknown, trials were conducted during fall and winter when this species is not reproductively active. Animals were transported to the Animal Holding Facility, University of Manitoba, where they were housed separately in 72-L plastic containers fitted with screen lids in a controlled-environment room held at 20 ± 1 °C with a 12 h light : 12 h dark photoperiod. A 10 cm deep mixture of sterilized peat moss and potting soil in the bottom of each container provided a substrate for burrowing. Each shrew was provided with a wooden nest box (11 cm × 12 cm × 10 cm), nesting materials (leaves, grasses, and cotton wool), and sections of plastic pipe for additional cover. Constructed nests were collected periodically and used for conductance determinations (see below). Animals were maintained on a mixture of beef heart, beef liver, canned dog food, and ground beef enriched with calcium and multivitamin supplements. This diet was supplemented daily with sunflower seeds, live earthworms (*Lumbricus* sp.), and mealworms (*Tenebrio molitor*). Water was provided ad libitum. Animals were acclimated to holding conditions for a period of 3 weeks prior to commencing experiments. All study animals were captured and cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Digesta-passage rate

The rate of food passage through the alimentary tract of short-tailed shrews ($n = 7$) held at a T_a of 25 °C was estimated using an inorganic tracer (Campbell et al. 2000). Shrews were fasted for 4 h then placed in a clean 72-L plastic container for a 15-min period during which time they were provided earthworms injected with a small quantity (ca. 500–1000) of Microtracer™ iron–nickel alloy particles (Micro Tracers Inc., San Francisco, Calif.). At the end of this feeding session, all uneaten tracer-labeled food was removed and the bottom of the container cleaned to remove any alloy contaminants. Shrews were then monitored continuously for 8 h, during which time they were provided with unlabeled maintenance rations (see above) and water ad libitum. During this period, all feces were collected and defecation times recorded to the nearest minute. Fecal samples were stored at –20 °C until analyzed. Dim lighting and the absence of a lid on the container minimized disturbance to the subject when feces were collected. Fecal samples were removed when shrews were either preoccupied with feeding

or resting in a darkened shelter (open-bottomed nest box). Fecal samples were stored at –20 °C until analyzed.

Fecal samples were ashed individually for 20 min at 550 °C. The ash was dissolved in water and filtered through Whatman No. 1 filter paper (12.5 cm diameter) to trap alloy particles. Filter papers were dried and placed on clean glass plates. They were then treated with 2–3 mL of acid reagent (25 g tartaric acid dissolved in 100 mL water and 100 mL 0.1 M hydrochloric acid), dried, sprinkled with a 1:1 mixture by volume of dimethylglyoxime (1% alcohol solution) and ammonium hydroxide, and redried. This treatment converts nickel to nickel dimethylglyoxime, causing tracer particles to appear on the filter paper as discrete red specks that are easily viewed and quantified using a dissecting microscope (Campbell et al. 2000).

Treated tracer particles were counted to determine the cumulative excretion of particles over time. Mean retention time (t_m) was calculated from the equation (Warner 1981)

$$[1] \qquad t_m = \sum m_i t_i / \sum m_i$$

where m_i represents the amount of marker excreted at a given time (t_i). The times required for initial excretion of the marker (t_i), 50% marker excretion (t_{50}), and 90% marker excretion (t_{90}) were also calculated.

Metabolic-rate determinations

$\dot{V}_{O_2}$ was measured using negative-pressure open-circuit respirometry. The metabolic chamber consisted of a modified 0.95-L paint can fitted with inlet and outlet air ports (Campbell et al. 1999) and provided with 3–4 mm of sterile soil as substrate. During testing, the metabolic chamber was placed in a controlled-environment cabinet set to the desired temperature. Room air was drawn through the chamber at a rate of 560 mL·min^{-1} using a calibrated rotameter. Exhaust gas from the chamber was dried and made CO_2-free by passing it sequentially through columns of Drierite and soda lime, and a sample of this gas (250 mL·min^{-1}) was then drawn through the M-22 sensor of an Applied Electrochemistry S3-A oxygen analyzer. Relative motor activity of the shrew was monitored independently of $\dot{V}_{O_2}$ using a motion-activity detector (Sable Systems Inc., Henderson, Nev.) situated beneath the metabolic chamber. The temperature inside the metabolic chamber was recorded by a temperature probe placed in the exhaust port. During each trial, the fractional oxygen content of the exhaust gas, cabinet T_a, metabolic chamber T_a, and relative activity were recorded at 5-s intervals by computer using Datacan V data-acquisition software (Sable Systems Inc.). $\dot{V}_{O_2}$ was calculated using eq. 4a of Withers (1977). To familiarize shrews with the metabolic chamber, each animal participated in two mock trials prior to starting experiments.

Time course and magnitude of HIF response

Shrews were fasted for 6 h prior to all feeding trials. In the feeding trials, shrews were weighed and placed for 15 min in an empty 72-L plastic container furnished with a shelter, drinking water, and a weighed ration of live earthworms. Following feeding, ingested meal mass was recorded and animals were placed in the metabolic chamber. An average of 3 min elapsed between the end of the feeding session

and the start of $\dot{V}_{O_2}$ recording. Mean body mass was calculated from values obtained prior to feeding and at the end of each trial. Sham-feeding control trials were also performed on each subject. These were identical with feeding trials, except that shrews were provided with only fresh water during the 15-min "feeding" session. $\dot{V}_{O_2}$ of the subject was measured continuously for a 3-h period following either a single-feeding or a sham-feeding treatment. Both treatments were conducted on each animal ($n = 6$ shrews) at test temperatures of 5 and 28 °C. In all experiments, temperature and feeding treatments were randomized and a minimum of 24 h separated consecutive trials on the same individual.

For purposes of analysis, each $\dot{V}_{O_2}$ recording was divided into consecutive 5-min blocks and mean $\dot{V}_{O_2}$ calculated for each of these intervals. In ca. 19% of these data blocks, $\dot{V}_{O_2}$ measurements were clearly affected by movement of shrews in the chamber. Peaks in animal activity identified by the motion-activity detector corresponded with obvious spikes in $\dot{V}_{O_2}$. These spikes were deleted and replaced by linearly interpolated data calculated from rates of metabolism immediately preceding and following each period of activity (Chappell et al. 1997; Campbell et al. 2000). Duration of the HIF response was defined as the time required for $\dot{V}_{O_2}$ of a fed shrew to return to within 5% of the mean $\dot{V}_{O_2}$ of the sham-fed control. Time to achieve maximum HIF was defined as the 5-min interval during which the difference between postprandial $\dot{V}_{O_2}$ and baseline $\dot{V}_{O_2}$ (sham-fed controls) was the greatest.

Test for direct thermal substitution by HIF

We compared the RMRs of fed and fasted animals. Food was withheld for 4 h prior to determining the fasting $\dot{V}_{O_2}$ of shrews. According to Platt (1974), the HIF response of the short-tailed shrew is maximal at 1 h following feeding and dissipates after ca. 2 h. Therefore, to ensure that measurements of $\dot{V}_{O_2}$ of fed shrews incorporated a significant portion of the HIF, animals were provided with food ad libitum at 1.5 and 0.5 h prior to testing. On average, 3.5 g (range = 1.7–5.6 g) of food were consumed in total. $\dot{V}_{O_2}$ of fed and fasted shrews was recorded at each of the following chamber T_a (±1 °C): 0, 5, 10, 15, 25, and 28 °C ($n = 7–9$ shrews in all cases). Animals were weighed to the nearest 0.01 g before feeding and after each trial, and mean body mass was used in all calculations.

Data collected during the first 20 min of each 75-min trial were excluded from analyses, accounting for the time required by the shrew to adjust to the metabolic chamber and for the system to reach equilibrium. To ensure that $\dot{V}_{O_2}$ measurements from fed animals strongly reflected the HIF response, trial length was set at a maximum of 75 min. RMR was thus calculated as the average of the three lowest 2-min $\dot{V}_{O_2}$ recordings (determined by the Datacan "nadir" function) over the final 55 min of each trial.

To derive metabolic relationships for fed and fasted shrews, data were grouped according to whether they fell within or below the TNZ previously established for this species by Neal and Lustick (1973). RMR values that were below thermoneutrality were assumed to vary linearly and inversely with T_a (Feist and White 1989). Linear regressions were performed on both "fasted" and "fed" shrews. To estimate the LCT, the regression line in each case was extended

to intercept the line denoting mean RMR at thermoneutrality (i.e., range of air temperatures over which resting $\dot{V}_{O_2}$ is minimal and independent of T_a).

Test for indirect thermal substitution by HIF

A metabolic chamber (0.95-L paint can) was wrapped in an insulative layer of fabric so as to achieve an overall thermal conductance approximating that of nests constructed by captive short-tailed shrews when provided with leaves, grasses, and wool. Conductance cooling constants derived by regressing $\ln(T_b - T_a)$ against time (Morrison and Tietz 1957) for prewarmed empty nests constructed by captive shrews were compared with those for the prewarmed metabolic chamber surrounded by varying thicknesses of insulative fabric. When the cooling constants of nest and chamber were similar, it was assumed they had comparable insulative values (Morrison and Tietz 1957). Activity, $\dot{V}_{O_2}$, and air temperature inside and immediately adjacent to the chamber were measured for eight short-tailed shrews, using methods described above. For this experiment, air flow to the chamber was maintained at 560 mL·min^{-1} using a mass flowmeter (model TR-SS1, Sable Systems Inc.). Measurements were obtained from fasted and fed individuals following procedures identical with those described above for the test of direct thermal substitution. Metabolic rates of fed and fasted shrews resting in the insulated chamber were compared with those of shrews held at a similar cabinet (outside) air temperature, 5 °C, in a non-insulated metabolic chamber. Heat production of fed and fasted animals was also assessed indirectly from measurements of chamber T_a. Body mass and meal mass were recorded to the nearest 0.01 g before and after each trial.
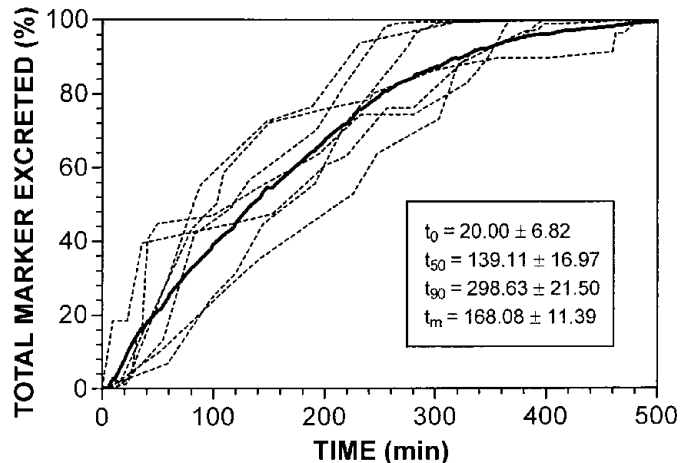
T_b measurements

To compare the T_b responses of fed and fasted shrews, abdominal T_b recordings were obtained telemetrically from three shrews implanted with 1.5-g model X-M temperature transmitters (Mini-Mitter Inc., Sunriver, Oreg.). Shrews were anesthetized with an intraperitoneal injection of ketamine hydrochloride (40 mg·kg^{-1}, Bimeda-MTC, Cambridge, Ont.) and Rompun (8 mg·kg^{-1} Bayer Inc., Etobicoke, Ont.) supplemented by an inhalant anesthetic (Halothane, MTC Pharmaceuticals, Cambridge, Ont.) as required. Transmitter implantation and calibration procedures followed those described by Dyck and MacArthur (1992) and Campbell et al. (1999). Transmitter signals were detected with a Sony AM receiver. All surgery was completed at least 7 days prior to starting experiments.

T_b's of resting fasted shrews were recorded over a T_a range of 0–29 °C. Following a 4-h fast, radio-implanted shrews were placed in the metabolic chamber installed in a controlled-environment cabinet. Each shrew was allowed 30 min to adjust to the chamber prior to recording resting T_b.

The time course of the T_b response to feeding was determined for each of the radio-implanted shrews. Following a 4-h fast, shrews were placed in a 38-L plastic container furnished with 1–2 cm of sterilized soil, a resting chamber, and a water dish. The container was placed on the motion-activity detector in a controlled-environment cabinet set at 25 °C. Activity and chamber T_a were recorded at 5-s intervals using Datacan V data-acquisition software (Sable Sys-

Fig. 1. Rate of passage of digesta in seven short-tailed shrews (*Blarina brevicauda*) held at 25 °C. The solid line represents mean cumulative percent excretion of the particulate tracer; the dotted lines denote excretion patterns of individual animals. t_m is mean retention time (min), t_0 is time to the first appearance of the marker, t_{50} is time to 50% excretion of the marker, and t_{90} is time to 90% excretion of the marker.

tems Inc.). After allowing shrews 30 min to adjust to the container, they were provided with either live earthworms (shrews 3 and 4) or a frozen-meat ration (shrew 11) for exactly 15 min, during which time T_b was recorded at 1-min intervals. Food was then removed and T_b monitored at 5-min intervals for the remainder of the 2-h trial. A matching control trial was also performed on each fasted animal, during which time no food was provided and T_b was recorded at 5-min intervals throughout the 2-h trial.

Statistical analyses

Comparisons of internal chamber T_a and $\dot{V}_{O_2}$ between fed and fasted shrews were made with paired t tests. Regression analyses were performed on linear data sets by the method of least squares. To test for direct thermal substitution of the HIF response, $\dot{V}_{O_2}$ regressions for fed and fasted shrews were compared using t tests for comparisons of slopes and intercepts of linear functions (Zar 1984). Mean values are presented ±1 SEM.
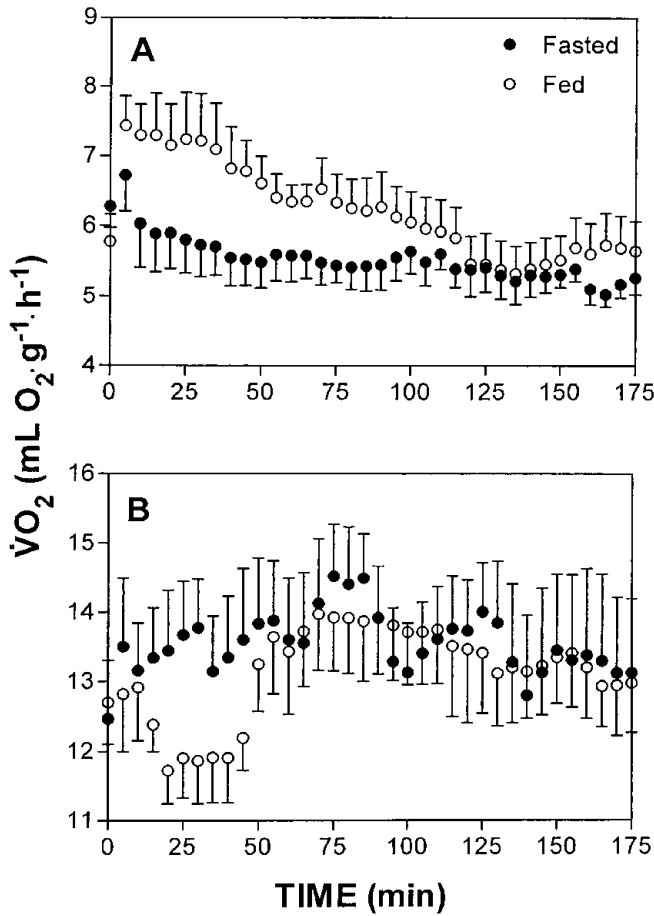
Results

Digesta passage rate

Excretion patterns were similar in all seven shrews tested (Fig. 1). Inorganic alloy tracer consumed by shrews was first detected in feces at 20.0 ± 6.8 min post ingestion. The retention time calculated for these animals (t_m) was 168.1 ± 11.4 min. Half of the marker excretion occurred within 139.1 ± 17.0 min, while 90% of total excretion occurred within 298.6 ± 21.5 min.

Time course of HIF response

Metabolic rate

In general, $\dot{V}_{O_2}$ of fed shrews tested at 28 °C exceeded that of sham-fed control animals (Fig. 2A). Analyses of

Fig. 2. Temporal changes in $\dot{V}_{O_2}$ (mean ± SE) of fed ($n = 6$) and sham-fed (fasted; $n = 6$) short-tailed shrews held at 28 °C (A) and 5 °C (B). Metabolic measurements were initiated 3 min following completion of the 15-min feeding or sham-feeding (control) treatment (time = 0).
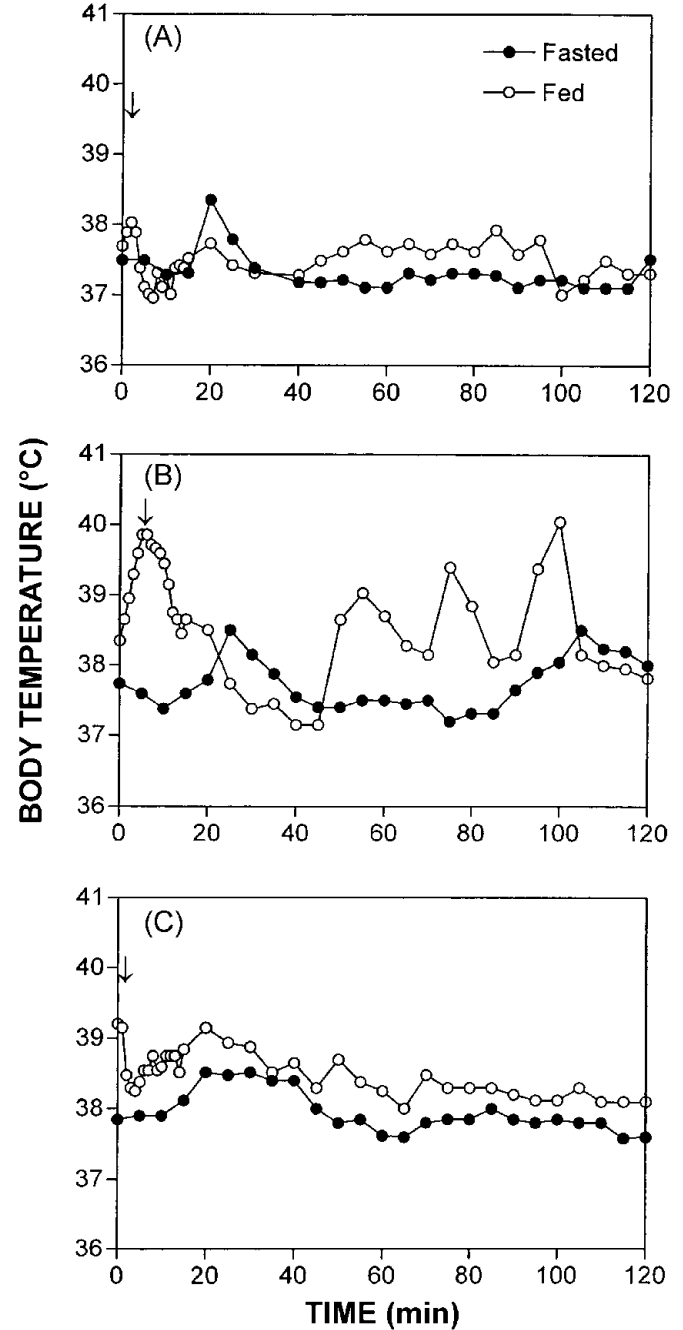

Fig. 3. Temporal changes in body temperature of three fed and sham-fed radio-implanted short-tailed shrews, Nos. 3 (A), 4 (B), and 11 (C), held at 25 °C. In feeding trials, food was provided at time = 1 min and removed at time = 15 min. In each case, the arrow indicates the precise time food was consumed.

interpolated data revealed that the increase in $\dot{V}_{O_2}$ of fed animals peaked at 25 ± 12 min (range = 5–75 min) post feeding, attaining a maximum value that averaged 36.7% (range = 22–58.5%) higher than the maximum $\dot{V}_{O_2}$ of the sham-fed controls. The $\dot{V}_{O_2}$ of fed animals returned to within 5% of control values at 117.5 ± 10.4 min (range = 95–155 min) following food removal (Fig. 2A). Over this period, $\dot{V}_{O_2}$ of fed animals was elevated an average of 18% over that of sham-fed controls. In sharp contrast to results at 28 °C, $\dot{V}_{O_2}$ of sham-fed animals tested at 5 °C exceeded that of fed animals for ca. 50 min post feeding, following which $\dot{V}_{O_2}$ values for both groups were indistinguishable (Fig. 2B). Despite a roughly threefold variation in meal mass (1.7–5.6 g), neither the magnitude nor the duration of the HIF response varied with the amount of food consumed by shrews ($P > 0.05$).

T_b values

The T_b of three radio-implanted shrews exhibited an overall increase of 0.6 ± 0.2 °C for ca. 2 h following feeding. However, within the first 30 min of each trial, T_b's of fed and fasted shrews occasionally overlapped (Fig. 3). In two of the fed animals (Nos. 3 and 4), a transient increase in T_b was observed immediately following the presentation of live

earthworms. This response did not occur in shrew 11, which was fed a frozen-meat ration in lieu of live earthworms. The T_b of shrews 3 and 4 declined immediately after the live prey had been immobilized (i.e., earthworms were no longer observed moving). Shrew 11 exhibited a sharp decrease in T_b during the first few minutes after consuming the frozen meal, following which T_b gradually recovered (Fig. 3). In all cases, fasted animals displayed a transient increase in T_b

ca. 20 min following sham-feeding that appeared to be associated with increased locomotor activity (Fig. 3).

Metabolic and T_b responses to varying air temperature

Over a T_a range of 0–29 °C, RMR of short-tailed shrews appeared to conform to the pattern expected for a typical normothermic mammal (Fig. 4B). However, abdominal T_b data recorded for three fasted resting shrews revealed a positive correspondence between T_b and T_a over the T_a range 0.5–29.5 °C (Fig. 4A; $T_b = 0.044T_a + 37.006$; $r^2 = 0.42$, df = 25, $P = 0.0003$). In all cases, T_b was recorded following 30 min exposure to a given T_a. RMR of fasted animals at thermoneutrality was 4.59 mL $O_2 \cdot g^{-1} \cdot h^{-1}$. Data presented in Fig. 4B suggest that the lower limit of the thermoneutral zone (LCT) occurs at ca. 24 °C. RMR of fasted shrews varied linearly with declining air temperature below thermoneutrality (RMR = $-0.39T_a + 13.64$; $r^2 = 0.49$, df = 18, $P = 0.0006$). Extrapolation of this line to the x axis yields a predicted T_b (Feist and White 1989) of 35 °C, which is reasonably close to the overall T_b (37.7 ± 0.1 °C) measured in the three radio-implanted shrews. In fed animals, RMR also varied linearly with declining T_a below thermoneutrality (RMR = $-0.44T_a + 14.82$; $r^2 = 0.565$, df = 23, $P < 0.0001$). There was no tendency for RMR of either fed or fasted shrews to increase at the higher ambient temperatures tested (27–29 °C; Fig. 4B), which suggests that none of these temperatures exceed the TNZ of short-tailed shrews.

Evidence for direct thermogenic substitution by HIF

The regression of RMR on T_a below thermoneutrality yielded similar slopes ($t = 0.064$, df = 39, $P = 0.71$) and intercepts ($t = 0.294$, df = 40, $P = 0.33$) for fasted and fed animals (Fig. 4B). However, within the TNZ, RMR averaged 28% higher for fed (5.89 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) than for fasted (4.59 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) animals (Fig. 4B; $t = 4.184$, df = 22, $P = 0.0002$). The increase in RMR of fed shrews in the thermoneutral zone resulted in a reduction of the calculated LCT by ca. 3.5 °C, from 24.0 °C in fasted shrews to 20.5 °C in fed animals (Fig. 4B).

### Evidence for indirect thermogenic substitution by HIF

For fasted animals, the air temperature within the insulated metabolic chamber increased by 4.5 ± 0.3 °C over the course of the 75-min trial. However, contrary to predictions, the internal chamber T_a was 9.5 ± 0.3 °C for control (fasted) animals, nearly 6% higher than that for fed shrews (9.0 ± 0.2 °C; $t = 2.50$, df = 7, $P = 0.041$). Similarly, $\dot{V}_{O_2}$ of fasted shrews in the insulated chamber (6.17 ± 0.26 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) was 8% higher than that (5.71 ± 0.25 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) recorded for fed animals ($t = 2.31$, df = 7, $P = 0.054$).

Discussion

RMR and T_b of short-tailed shrews

The metabolic responses of short-tailed shrews to air temperatures varying from 0 to 29 °C (Fig. 4B) conformed to the conventional model for homeothermic terrestrial mammals (Feist and White 1989). The lower limit of the TNZ was 24 °C, close to the LCT (25 °C) previously reported for this species by Neal and Lustick (1973). The mean estimated

Fig. 4. Relationship of body temperature (T_b) (A) and resting metabolic rate (RMR) (B) to chamber air temperature (T_a) in fed ($n = 6$) and fasted ($n = 7$) short-tailed shrews. T_b was recorded in three fasted radio-implanted animals. Least-squares regression analysis of T_b data yielded the equation $T_b = 0.044T_a + 37.006$ ($r^2 = 0.415$, df = 25, $P = 0.0003$). For each group, a linear regression model was fitted to RMR data below thermoneutrality (RMR = $-0.39T_a + 13.64$; $r^2 = 0.488$, df = 18, $P = 0.0006$ for fasted animals; RMR = $-0.44T_a + 14.82$; $r^2 = 0.565$, df = 23, $P < 0.0001$ for fed animals). In each case, the point where the extrapolated regression intercepts the mean RMR at thermoneutrality is defined as the lower critical temperature. The broken line is an extrapolation of the RMR regression derived for fasted animals below thermoneutrality (see the text for details).
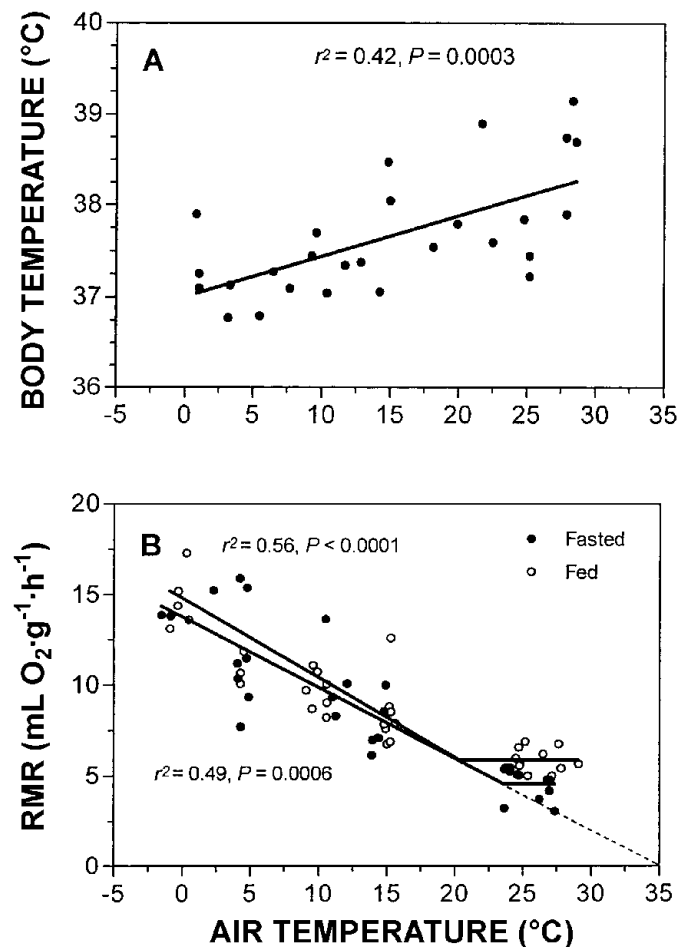
BMR (4.59 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) was 2.7 times the value for eutherians predicted from allometry (1.70 mL $O_2 \cdot g^{-1} \cdot h^{-1}$; Wunder 1975), a finding consistent with the prevailing view that shrews exhibit an exceptionally high BMR (see McNab 1983, 1991). Our estimate of BMR is comparable to values reported for this species by Buckner (1964; 4.6 mL $O_2 \cdot g^{-1} \cdot h^{-1}$), Pearson (1947; 3.4 mL $O_2 \cdot g^{-1} \cdot h^{-1}$), Randolph (1980; 4.85 mL $O_2 \cdot g^{-1} \cdot h^{-1}$), and Deavers and Hudson (1981; 3.22 mL $O_2 \cdot g^{-1} \cdot h^{-1}$). On the other hand, Platt (1974) reported a BMR of only 2.50 mL $O_2 \cdot g^{-1} \cdot h^{-1}$ for short-tailed shrews and suggested that activity may have confounded earlier metabolic measurements reported in the literature. However, it should be

noted that Platt (1974) recorded RMR only when shrews were sleeping, a state associated with reduced metabolism (Randolph 1973).

The mean thermoneutral T_b of resting, post-absorptive shrews in this study (38.0 °C) was in close agreement with mean values (38–38.5 °C) reported in the literature (Kendeigh 1945; Doremus 1965; Neal and Lustick 1973; Merritt and Adamerovich 1991). This species is not known to enter torpor (Merritt 1986; McNab 1991; Merritt and Adamerovich 1991), yet we observed T_b to exhibit a significant, albeit modest, linear decrease with declining T_a (Fig. 4A). However, with a maximum observed T_b drop of only 2 °C over a T_a range of 0–29 °C, it is unlikely that this T_b depression had any marked effect on shrew metabolism. This finding is interesting to the extent that it indicates that T_b of these shrews is somewhat labile, a trait also noted by Neal and Lustick (1973), and one that perhaps reflects a graded vasoconstriction of abdominal tissues as T_a falls.

Magnitude and duration of the HIF response

The mean mass of food (3.5 g, 10% of body mass) consumed by the short-tailed shrews during the HIF trials concurs with earlier estimates of natural meal mass reported by Morrison et al. (1957). The HIF response of fed shrews at 28 °C lasted 118 ± 10 min, during which time $\dot{V}_{O_2}$ averaged 18% higher than that for sham-fed controls. The duration of this HIF response is virtually identical with that (2 h) reported for this species by Platt (1974). Assuming energy and water-content values equivalent to those previously reported for earthworms (Campbell et al. 2000), gross energy intake (GEI) by shrews averaged 13.01 kJ. To estimate the percentage of GEI accounted for by HIF, we converted $\dot{V}_{O_2}$ of both fed and fasted shrews into units of heat production using eqs. 2 and 3 of Campbell et al. (2000), and assuming a respiratory quotient of 0.83 (Buckner 1964). On average, HIF represented 4.1% (eqs. 2 and 3 of Campbell et al. 2000) or 7.3% (eq. 2) of GEI. It is noteworthy that both values are near the lower limit of the range (4.7%–16.8% of GEI) reported for other mammals fed diets high in protein (see Campbell et al. 2000 and references therein).

Digesta-throughput time (t_m) of short-tailed shrews in this study was 168 ± 11 min. In theory, throughput time should reflect the period during which peristalsis contributes to the HIF response. The observation that t_m is ca. 40% longer than the duration of the HIF response suggests that the mechanical HIF contributes only a minor extent to the postprandial rise in $\dot{V}_{O_2}$. As the shrew has a relatively simple gut with minimal diverticulae or cecae (George et al. 1986), it is unlikely that tracer particles aggregated at these sites sufficiently to slow their passage through the digestive tract. The t_m of the short-tailed shrew (Fig. 1) was considerably longer than that for the larger (60 g) star-nosed mole, *Condylura cristata* ($t_m = 66.7 ± 7.8$ min, $n = 5$; Campbell et al. 2000). The cumulative excretion time for the short-tailed shrew also exceeded that (ca. 4.2 h) reported for the slightly smaller European water shrew, *Neomys fodiens* (Kostalecka-Myrcha and Myrcha 1964). However, t_{90} of our study animals was comparable to the 90% excretion times of 5.5–7.1 h (depending on diet) observed in the Cape rock elephant shrew, *Elephantulus edwardii* (Woodall and Currie 1989).

T_b responses to feeding

T_b's of fed shrews generally exceeded those of sham-fed control animals at a T_a of 25 °C. However, during the first 30 min of testing there was occasional overlap between T_b's of fed and fasted shrews (Fig. 3). This T_b overlap supports our conclusion that the mechanical HIF, which typically is manifested shortly after feeding, is a relatively minor component of the overall HIF response in short-tailed shrews. Immediately upon presentation of food to shrews, T_b increased dramatically. We attribute this response to excitement at the presentation of food combined with the metabolic costs of mastication. A parallel, but smaller, increase occurred in sham-fed controls, which may have reflected the excitement factor only. It seems, therefore, that the mechanical component of HIF in the short-tailed shrew reflects mainly the costs of food handling and mastication, while peristalsis appears to contribute so little to HIF that it is obscured by normal activity levels.

The basis for the HIF response has been questioned by Wilson and Culik (1991), who argued that the postprandial increment in $\dot{V}_{O_2}$ might, in some species at least, reflect the energy required to heat an ingested meal to core T_b. That this argument has merit is suggested by the transient T_b drop observed in all three radio-implanted shrews immediately following feeding (Fig. 3). However, the T_b drop was consistent for all three shrews despite considerable differences in the temperature of food consumed. As well, postprandial T_b recovered within 5 min of feeding, which suggests that all ingesta would also have equilibrated with T_b in this time. Beyond this point, the continued elevation in $\dot{V}_{O_2}$ (Fig. 2A) should, in theory, reflect digestion and assimilation processes.

Thermoregulatory implications of HIF at sub-thermoneutral temperatures

HIF has the potential to substitute directly for cold-induced thermogenesis at low T_a's. In this case, heat produced secondarily to digestion and assimilation of nutrients can be used to defend T_b, thereby reducing thermogenic demands on endogenous fuel resources. Without substitution, the obligate HIF response presents an additional metabolic expense to the animal and one that reduces the overall efficiency of energy assimilation (Robbins 1993). Consistent with theory (Kleiber 1975), thermoneutral RMR was elevated in fed shrews, effectively lowering the LCT of fed animals (Fig. 4B). Below the TNZ, the slope and intercept of the regression of RMR on T_a were nearly identical for fed and fasted animals. These findings suggest that HIF substitutes at least partially for facultative thermogenesis at low temperatures. It is noteworthy that the maximum RMRs recorded for fed and fasted animals (Fig. 4B) were well below the peak value (19.3 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) reported by Dawson and Olson (1987) for cold-challenged short-tailed shrews. Therefore, the similarity of the RMR responses of fed and fasted animals below thermoneutrality cannot be explained on the basis that the $\dot{V}_{O_2}$ values for these animals were approaching peak levels in the cold. The substitution argument is further supported by the results presented in Fig. 2B indicating that in a cold-challenge situation ($T_a = 5$ °C), postprandial $\dot{V}_{O_2}$ values for fed and fasted shrews were indistinguishable

after 50 min following feeding. No evidence of substitution by HIF was reported in previous studies of mammalian insectivores, including the star-nosed mole (Campbell et al. 2000) and short-tailed shrew (Platt 1974). However, Platt's findings are inconclusive, since he compared $\dot{V}_{O_2}$ measurements of awake, fed shrews with those of sleeping, fasted animals.

Potential nest-warming benefits of HIF

In captivity, short-tailed shrews constructed well-insulated nests (see above). In nature, construction of spherical nests composed of leaves, twigs, grasses, and occasionally hair has also been documented for the short-tailed shrew (George et al. 1986; Merritt 1986). Despite the open-circuit design of the respirometry system, shrews were able to maintain the temperature of the insulated metabolic chamber 4.0–4.5 °C above that of the incoming ambient air (5 °C). By comparison, in control trials with non-insulated chambers, shrews were able to maintain the chamber T_a only 1.8 °C above the external T_a. Interestingly, $\dot{V}_{O_2}$ and the internal chamber T_a were significantly higher for fasted than for fed shrews held in the insulated metabolic chamber. The basis for this anomalous finding is unclear, though we suspect that it relates to differences in the motor activity levels of fed and fasted shrews (see below).

Potential confounding effects of activity on $\dot{V}_{O_2}$

Shrews in general are predisposed to hyperactivity (McNab 1991), a characteristic observed in all of the subject animals used in this study. While every precaution was taken to minimize and correct for the effects of activity, this variable could not be discounted as a potential source of variation in our metabolic measurements. A post-absorptive state is a necessary criterion for measurement of BMR, yet interactions between nutritional status and activity level may confound metabolic measurements (McDevitt and Andrews 1995; McNab 1991). For instance, it has been noted that $\dot{V}_{O_2}$ of pygmy shrews, *Sorex minutus*, is the same whether they are fed or fasted (McDevitt and Andrews 1995). These authors interpret this apparent anomaly as reflecting an increase in the motor activity (likely food-searching behaviour) of fasted animals.

If a similar tendency occurs in short-tailed shrews, then our RMR measurements for post-absorptive animals may have been biased to some extent by activity. Thus, what appears to be a clear example of substitution of HIF for facultative thermogenesis at low temperatures (Fig. 4B) may instead reflect the additional metabolic costs of activity in fasted animals. Platt (1974) arrived at a similar conclusion in his earlier study of the same species. Rather than inferring a substitutive effect of HIF on metabolism at sub-thermoneutral temperatures, Platt concluded that heat produced from motor activity substitutes for facultative thermogenesis. Similarly, Campbell et al. (2000) suggested that activity associated with feeding, rather than the HIF response, substitutes for facultative thermogenesis in star-nosed moles. We suspect that feeding-related differences in motor activity also account for the marked disparity in the $\dot{V}_{O_2}$ values for fed and fasted cold-challenged shrews during the first 50 min following feeding (Fig. 2B). Though speculative, the initially lower

$\dot{V}_{O_2}$ of fed shrews apparent in Fig. 2B could reflect a sharp curtailment in the activity of satiated animals.

Irrespective of whether activity-induced thermogenesis or HIF substitutes for thermoregulatory heat production below the TNZ, $\dot{V}_{O_2}$ of fed shrews within this zone was clearly elevated for ca. 2 h following feeding (Fig. 4B). To the extent that increased metabolic heat production in the TNZ extends the lower limit of this zone, HIF provides a thermoregulatory energy saving to short-tailed shrews. Moreover, this thermoregulatory benefit may be promoted by the natural tendency of these shrews to cache food in or near their nest. We believe that our inability to demonstrate microclimate warming resulting from HIF in this species stems from an inherent difficulty in achieving comparable activity levels in fed and fasted animals. Though the short-tailed shrew proved to be an uncooperative model in this respect, that other small nest-building endotherms benefit from an HIF response that contributes to microclimate warming remains a viable hypothesis that is amenable to testing.

Acknowledgments

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