

Metabolism of normothermic woodchucks during prolonged fasting

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Summary

The energy metabolism of hibernators has not been characterized for normothermic fasting, and our goal was to quantify oxidative fuel selection of non-hibernating woodchucks *Marmota monax* during prolonged food deprivation. Indirect calorimetry and nitrogen excretion measurements were used to assess changes in metabolic rate (\dot{V}_{O_2}), fuel selection and composition of nitrogen wastes, as well as seasonal differences. For reference, matching experiments were also performed on rabbits. The results show that woodchucks have a higher metabolic rate in summer ($271 \mu\text{mol O}_2 \text{ kg}^{-1} \text{ min}^{-1}$) than in spring ($200 \mu\text{mol O}_2 \text{ kg}^{-1} \text{ min}^{-1}$) and that fasting-induced metabolic depression is only possible in summer (–25% in 14 days). The metabolic rate of rabbits is high at all times ($383 \mu\text{mol O}_2 \text{ kg}^{-1} \text{ min}^{-1}$), but they show a more rapid depression in response to fasting (–32% in 7 days). Woodchucks have a naturally low reliance on proteins in the fed state (accounting for 8% \dot{V}_{O_2} in spring; 17% \dot{V}_{O_2} in summer; vs 28% \dot{V}_{O_2} in rabbits) and are able to decrease

it even further during fasting (spring, 5% \dot{V}_{O_2} ; summer, 6% \dot{V}_{O_2} ; vs 20% \dot{V}_{O_2} in rabbits). This study shows that, apart from their notorious capacity for hibernation, woodchucks are particularly well adapted for normothermic fasting. Their ability to cope with prolonged food deprivation is based on a series of integrated responses eliciting deep metabolic depression and a rapid change in fuel selection to spare limited protein reserves. Information presently available on prolonged fasting suggests that such an ability for metabolic depression, possibly down to minimal levels still compatible with normothermic life, may be common among mammals. In contrast, the extreme protein sparing demonstrated in woodchucks is a unique metabolic feature of fasting champions.

Key words: metabolic depression, fuel selection, lipids, protein sparing, metabolic rate, oxygen consumption, energy expenditure, food deprivation, woodchuck, *Marmota monax*, rabbit.

Introduction

Coping successfully with prolonged periods of fasting depends on two key physiological processes: metabolic depression (Craven, 1951; Markussen and Oritsland, 1986; Merkt and Taylor, 1994) and regulated changes in fuel selection (Cahill, 1970). During fasting, birds and mammals go through distinct metabolic stages, starting with the use of limited carbohydrate stores (phase I), and followed by the mobilization of large lipid reserves to spare body proteins (phase II). Eventually, energy metabolism becomes primarily dependent on protein oxidation (phase III), and muscle function starts being compromised, particularly in the heart (Garnett et al., 1969; Van Itallie and Yang, 1984). The prolonged fasting period characterized by phase II shows two important features: (1) the overall energy budget is dominated by lipid oxidation regardless of the organism's natural history, but (2) the relative importance of proteins varies widely among animals, depending on how often and for how long food deprivation normally occurs in each species. Protein oxidation supports 1–10% of total ATP production in species adapted for fasting (Cherel et al., 1995; Galster and Morrison, 1966;

Lundberg et al., 1976; Nørdoy et al., 1990), but this value can exceed 25% in species that are not (Cahill, 1970; Cherel et al., 1992; Goodman et al., 1980; Henry et al., 1988). One of the important roles of protein catabolism is to provide amino acids as a gluconeogenic substrate to sustain hepatic glucose production (Owen et al., 1998; Peroni et al., 1997). Nitrogenous waste products are subsequently excreted as ammonia, urea, uric acid and creatinine. In mammals, prolonged fasting is typically associated with progressively lower rates of urea excretion, partly compensated by a gradual increase in ammonia production (Nørdoy et al., 1990; Owen et al., 1969, 1998). However, the composition of nitrogenous end products has never been monitored in a hibernator fasting under normothermic conditions.

Hibernators are among the mammalian champions of long-term fasting and a lot of information is available on their metabolism during hypothermia (Wang and Lee, 1996). Much less is known about oxidative fuel selection during normothermia, even though many hibernators continue to rely on their endogenous energy reserves long after coming out of

hibernation. For example, woodchucks *Marmota monax* (Davis, 1967; Hamilton, 1934; Snyder et al., 1961) and American black bears *Ursus americanus* (Nelson, 1980) are known to continue fasting for several weeks after spring arousal, even though snow has melted and food is readily available. Numerous studies have investigated the biology of hibernators (for a review, see Humphries et al., 2003), but no information is available on their response to fasting under normothermic conditions. They show seasonal changes in energy expenditure, with lower normothermic metabolic rates during the fall, and as much as a 96% decrease during actual hibernation (Körtner and Heldmaier, 1995). However, it is not known whether they use metabolic depression during prolonged normothermic fasting, a strategy commonly observed in non-hibernators (Choshniak et al., 1995; Keys et al., 1950; Ma and Foster, 1986; Merkt and Taylor, 1994). Depressing metabolism under normothermic conditions could be a very valuable compromise to delay the depletion of energy reserves without the loss of alertness and mobility imposed by hibernation. In this study, our goal was to quantify the metabolic response of normothermic woodchucks to prolonged fasting. We used indirect calorimetry and nitrogen excretion analysis to determine: (1) the presence and/or extent of metabolic depression, (2) the pattern of changes in oxidative fuel selection and nitrogenous waste production, and (3) whether these metabolic responses show seasonal differences by comparing animals in early spring and in summer. As a reference, we also carried out parallel experiments under identical conditions, but on New Zealand white rabbits, a species of similar size and diet as the woodchuck, but not adapted for prolonged fasting.

Materials and methods

Animals

Woodchucks *Marmota monax* L. and New Zealand white rabbits *Oryctolagus cuniculus* L. of both sexes were used. They were obtained from a captive colony (Cornell University, Ithaca, NY, USA) and from Charles River (St. Hyacinthe, Quebec, Canada), respectively. The woodchucks ate food pellets (54% carbohydrate, 2% fat, 15% protein, 18% crude fibre and 11% water) and the rabbits were fed rabbit chow (52% carbohydrate, 3% fat, 16% protein, 14% crude fibre and 15% water). All the measurements were carried out at two different times of the year: (i) in early spring following hibernation (February–March, $N=4$; spring woodchucks) and (ii) during the summer following a winter without hibernation (July–August, $N=4$; summer woodchucks). For rabbits, we found no seasonal differences and, therefore, all results were pooled in this species (March–October, $N=9$ rabbits).

Fasting protocols

Food was provided for the first 3 days of each experiment while food consumption rate was monitored to obtain baseline values. Then, the animals were fasted for the remainder of the measurements. Water was available *ad libitum* at all times. All

experiments were approved by the Animal Care Committee of the University of Ottawa that imposed maximal values for fasting duration and weight loss in each species. For the rabbits, the experiments had to be terminated after a maximum of 7 days without food, or when they had lost 15% of initial body mass. For the woodchucks, fasting was allowed for up to 14 days, or until they had lost 30% of initial body mass.

Measurements of gas exchange and nitrogen excretion

Animals were measured individually in a closed Plexiglas respirometer (54 cm×38 cm×67 cm) supplied with room air at 3–8 l min⁻¹. Rates of O₂ consumption and CO₂ production were measured in a respirometer (Oxymax system, Columbus Instruments, Columbus, Ohio, USA) as described previously (Fournier and Weber, 1994). Gas exchange measurements were interrupted for ~30 min once a day to weigh the animals, measure rectal temperature, clean the respirometer, and calibrate the analyzers. The respirometer floor was modified to allow the collection of urine in a vessel containing a thymol crystal and placed on ice to prevent bacterial growth. The volume of urine produced was measured every 24 h and a daily subsample was frozen to measure total nitrogen and the main nitrogenous waste products: ammonia, urea, uric acid and creatinine.

Body composition

Additional animals of the same batch (2 spring woodchucks, 2 summer woodchucks and 3 rabbits) were euthanized by overdose of pentobarbital in the post-absorptive state (12 h after their last meal) to determine body composition before fasting. Skeletal muscle, adipose tissue, liver and heart mass were measured after careful dissection.

Analyses and calculations

Total urinary nitrogen was measured using the Kjeldahl method (Tecator analyzers, 1007 Digester and Kjeltac System 1002 Distilling Unit, Hoganas, Sweden). The concentrations of individual nitrogenous compounds of urine were quantified spectrophotometrically. Urea was determined using the urease/glutamate dehydrogenase method and uric acid was measured using the uricase method (Bergmeyer, 1985). Creatinine was measured using a commercial kit (Sigma diagnostics kit, St Louis, MO, USA). Total ammonia (NH₃+NH₄⁺) was measured according to the method of Verdouw (1978). Protein oxidation (in g) was calculated by multiplying total urinary nitrogen excretion (in g) by 6.25, assuming that the proteins oxidized contained an average of 16% N by weight. Rates of carbohydrate and lipid oxidation were calculated according to the equations of Frayn (1983) as follows:

Carbohydrate oxidation =

$$4.55\dot{V}_{\text{CO}_2} - 3.21\dot{V}_{\text{O}_2} - 2.87(\text{urinary N excretion})$$

and

Lipid oxidation =

$$1.67\dot{V}_{\text{O}_2} - 1.67\dot{V}_{\text{CO}_2} - 1.92(\text{urinary N excretion}),$$

Table 1. Body mass, oxygen consumption, food consumption and body temperature of fed rabbits and woodchucks after 3 days in the respirometer

	Woodchuck		Rabbit
	Spring	Summer	
<i>N</i>	4	4	9
Body mass (kg)	3.130±0.31	4.195±0.28*	3.052±0.08*
Mean 24 h oxygen consumption (μmol O ₂ kg ⁻¹ min ⁻¹)	200.2±17.4	270.7±20.9*	383.1±11.4*
Food consumption (g kg ⁻¹ day ⁻¹)	18.77±4.43	28.23±9.29	28.30±3.36
Body temperature (°C)	36.75±0.32	36.45±0.61	39.12±0.22*

Values are means ± S.E.M. *Significant difference between the marked value and the value to its left.

where the rates of carbohydrate and lipid oxidation and urinary N excretion are in g min⁻¹ and \dot{V}_{O_2} and \dot{V}_{CO_2} in l min⁻¹. The rates of lipid oxidation in g min⁻¹ were converted to molar equivalents, using a molecular mass of 861 g mol⁻¹ for an average triacylglycerol (Frayn, 1983). After 1 complete day of fasting, lipid and protein oxidation were assumed to account for 100% of total oxygen consumption; the oxygen consumption linked to lipid oxidation was therefore calculated by subtracting the oxygen consumption of protein oxidation from total oxygen consumption. This was done because long-term fasting causes a significant stimulation of gluconeogenesis and ketone metabolism (Baba et al., 1995; Krilowicz, 1985), thereby invalidating the usual post-absorptive calculations of indirect calorimetry for partitioning lipid and carbohydrate oxidation (Frayn, 1983; Owen et al., 1998).

Statistics

The effects of fasting were assessed by repeated-measures analysis of variance (ANOVA) with time, individual animal and season or species as main factors. When significant temporal changes were detected, the Dunnett's *post-hoc* test was used to determine which fasting means were different from control values from fed animals. Percentages were transformed to the arcsine of their square root before statistical analyses. All values are presented as means ± S.E.M. and significant differences are indicated when $P < 0.05$.

Results

Seasonal effects in woodchucks

In the fed state, the woodchucks had a higher body mass and a higher metabolic rate in summer than in spring ($P < 0.05$), but their food consumption rate and body temperature were not significantly different between seasons (Table 1). Muscle, heart and liver mass were the same between seasons in the fed woodchucks, but adipose tissue mass was significantly higher in the summer than in the spring (Table 2). During prolonged fasting, the rate of weight loss was identical between spring and summer (Fig. 1) and it decreased progressively from 17.3±1.7 g kg⁻¹ day⁻¹ on the first day to 5.2±0.6 g kg⁻¹ day⁻¹ after 14 days without food ($P < 0.05$; Fig. 1B). Therefore, after 2 weeks of fasting the woodchucks had only lost 13% of initial body mass (Fig. 1A). The oxygen consumption of the fed animals was 35% higher in summer than in spring ($P < 0.05$; Table 1). The summer woodchucks reduced metabolic rate by 25% during fasting ($P < 0.05$), whereas the spring woodchucks did not (Fig. 2). By the end of the fasting period, the initial difference in oxygen consumption observed between seasons had therefore disappeared (Fig. 2A). In the summer woodchucks, the metabolic depression observed during fasting was accompanied by a decrease in body temperature ($P < 0.05$; Fig. 3), and a significant correlation between O₂ consumption and body temperature was observed in this group (Pearson correlation coefficient=0.595; $P < 0.05$).

The relative contributions of the different metabolic fuels to total energy expenditure are presented in Fig. 4. Before fasting,

Table 2. Body composition of a subsample of post-absorptive rabbits and woodchucks determined by carcass dissection

	Woodchuck		Rabbit
	Spring	Summer	
<i>N</i>	2	2	3
Body mass (kg)	2.828±0.372	3.955±0.200	3.633±0.09
Skeletal muscle (% LBM)	52.41±1.19	54.32±1.59	48.23±0.70*
Adipose tissue (% body mass)	40.31±0.53	56.10±0.48*	17.85±1.80*
Heart (% LBM)	1.10±0.09	1.17±0.01	0.42±0.02*
Liver (% LBM)	6.08±0.11	6.16±0.44	3.10±0.37*

Values are means ± S.E.M. *Significant difference between the marked value and the value to its left. LBM, lean body mass.

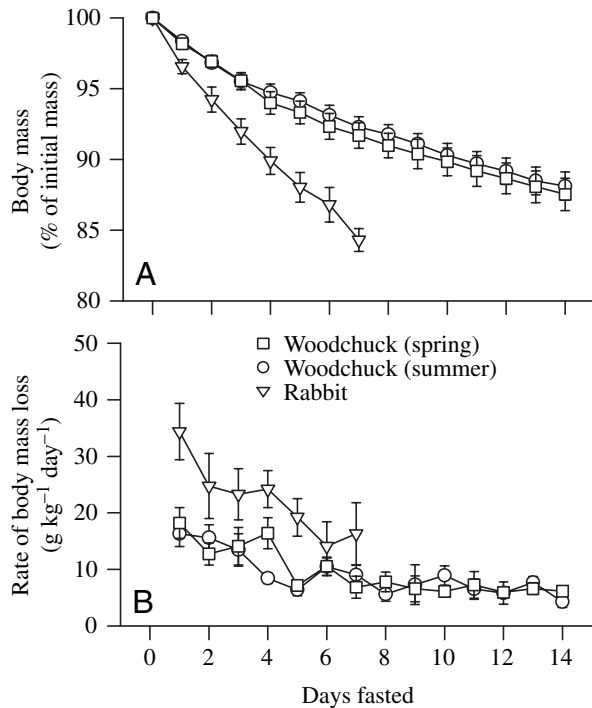


Fig. 1. Changes in body mass of adult woodchucks and rabbits during prolonged fasting. (A) Body mass expressed as % of initial mass measured in fed animals (the value for day 0), and (B) rate of absolute mass loss in $\text{g kg}^{-1} \text{day}^{-1}$. Values are means \pm S.E.M. for summer woodchucks ($N=4$), spring woodchucks ($N=4$) and rabbits ($N=9$).

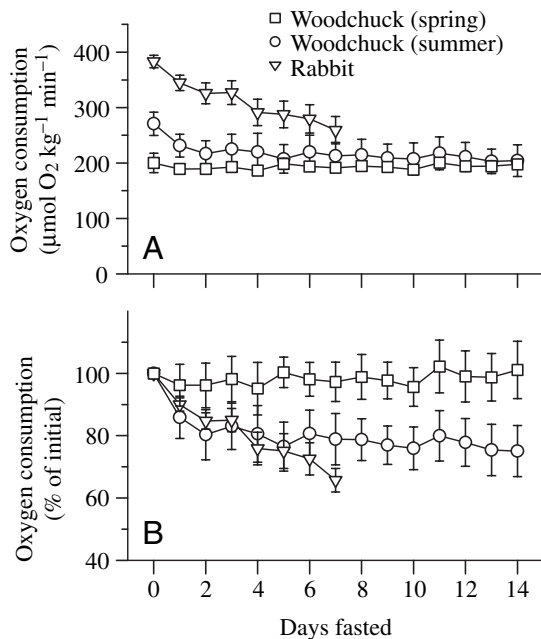


Fig. 2. Mean rate of oxygen consumption over a 24 h period of adult woodchucks and rabbits during prolonged fasting. Values are means \pm S.E.M. (A) Absolute rates in $\mu\text{mol O}_2 \text{kg}^{-1} \text{min}^{-1}$ and (B) values expressed as % of initial metabolic rate measured on day 0 in fed animals. Summer woodchucks ($N=4$), spring woodchucks ($N=4$) and rabbits ($N=9$).

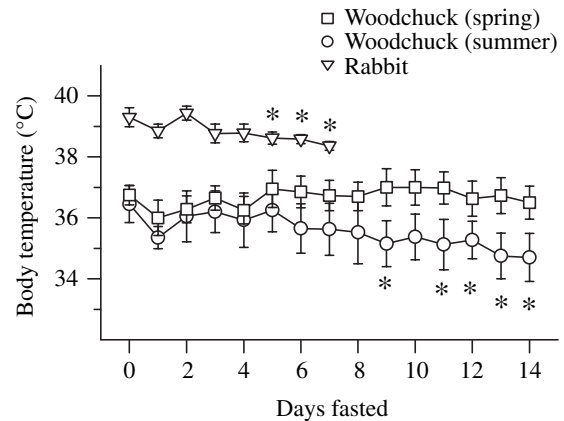


Fig. 3. Body temperature of woodchucks and rabbits before and during a prolonged fast. Values are means \pm S.E.M. for summer woodchucks ($N=4$), spring woodchucks ($N=4$) and rabbits ($N=9$). * indicates significant differences from the fed values (day 0) within each group.

the percentage contribution of lipid and carbohydrate oxidation was the same between spring and summer woodchucks ($P>0.05$), whereas protein oxidation was higher in the summer than in the spring ($P<0.05$; Fig. 4A). Lipids rapidly became the dominant fuel when fasting was initiated (Fig. 4B) and remained so until the end of the experiments (Fig. 4C). The seasonal difference in protein metabolism observed in the fed woodchucks disappeared during fasting because food deprivation decreased protein oxidation in the summer animals.

Differences between woodchucks and rabbits

No seasonal differences in body mass, metabolic rate, food consumption or body temperature were observed in the rabbits ($P>0.05$) and, therefore, spring and summer results were pooled for this species. A large difference in oxygen consumption was observed between species. Both summer and spring woodchucks had lower mass-specific metabolic rates than the rabbits ($P<0.05$; Table 1). The summer woodchucks consumed as much food as the rabbits ($P>0.05$), but their average body temperature was lower ($P<0.05$) (Table 1).

During fasting, the rabbits lost weight more rapidly than the woodchucks ($P<0.05$; Fig. 1A). On the first day of fasting, the rabbits lost $34.4 \pm 5.0 \text{ g kg}^{-1} \text{day}^{-1}$ compared to $17.3 \pm 1.7 \text{ g kg}^{-1} \text{day}^{-1}$ for the woodchucks. However, the rate of weight loss of the rabbits decreased over time to reach $16.3 \pm 5.5 \text{ g kg}^{-1} \text{day}^{-1}$ by the end of the fasting period ($P<0.05$; Fig. 1B). Regardless of this decrease, the rabbit experiments had to be interrupted after 7 days of fasting because they had already lost 15% of their body mass, compared to only 8% for the woodchucks (Fig. 1A). There was a very large difference in adipose tissue size between the 2 species. Carcass analysis of fed animals showed that the woodchucks had 2–4 times more fat than the rabbits relative to total body mass ($P<0.05$; Table 2).

The rate of oxygen consumption of the rabbits decreased sharply during fasting ($P<0.05$; Fig. 2). It showed a 32%

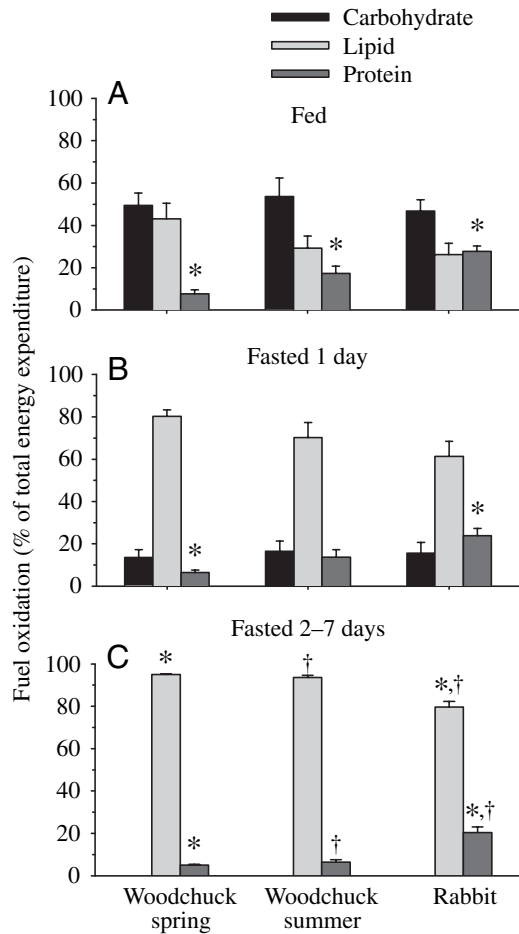


Fig. 4. Fuel selection in woodchucks and rabbits before and during a prolonged fast. The % contributions of carbohydrates, lipids and proteins to total oxygen consumption are given for fed animals (A), for animals fasted for 1 day (B) and for animals fasted for 2–7 days (C). Values are means \pm S.E.M. for summer woodchucks ($N=4$), spring woodchucks ($N=4$) and rabbits ($N=9$). Values of the same fuel type marked with the same symbol are significantly different from each other.

reduction over 1 week without food, decreasing from 383 ± 11 to $259 \pm 25 \mu\text{mol O}_2 \text{ kg}^{-1} \text{ min}^{-1}$. This change in metabolic rate was correlated with a small decrease in body temperature (Pearson correlation coefficient=0.786; $P<0.05$). Mean body temperature was reduced from $39.3 \pm 0.3^\circ\text{C}$ in the fed state to $38.4 \pm 0.1^\circ\text{C}$ after 7 days of fasting ($P<0.05$; Fig. 3).

In fed animals, the contributions of lipid and carbohydrate oxidation to total oxygen consumption did not differ between woodchucks and rabbits, but protein oxidation was higher in the rabbits ($P<0.05$; Fig. 4A). This species difference in fuel utilization persisted during fasting ($P<0.05$; Fig. 4), even though food deprivation caused the rabbits to reduce protein oxidation ($P<0.05$; Fig. 4) and urinary nitrogen excretion ($P<0.05$; Fig. 5A).

Urinary nitrogen excretion

Total urinary nitrogen excretion of fed rabbits was

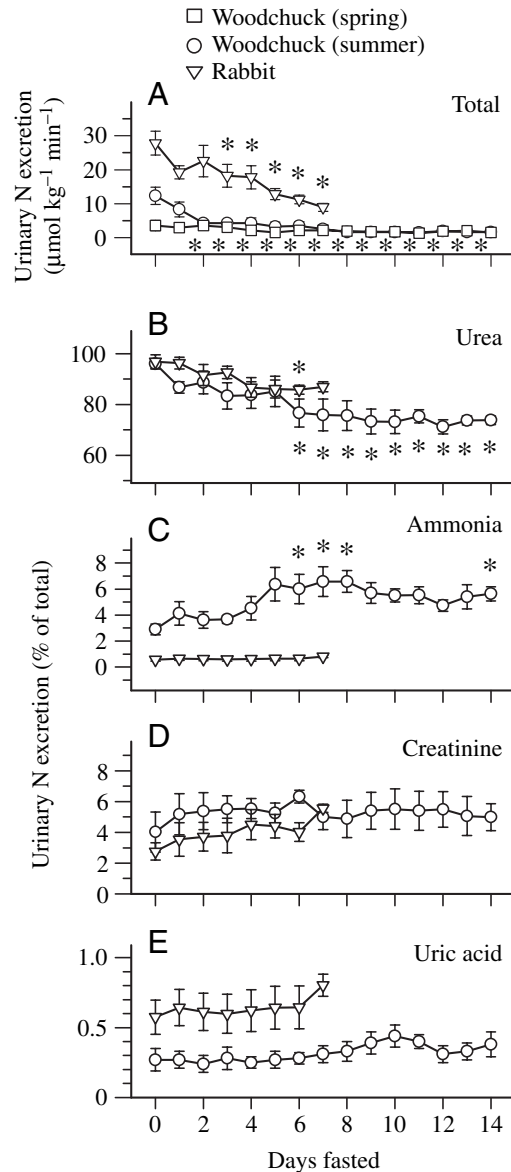


Fig. 5. Urinary excretion rates of woodchucks and rabbits before and during prolonged fasting. (A) Absolute rates of N excretion ($N=4$ for spring and summer woodchucks, $N=9$ for rabbits). (B–E) The relative contributions of urea (B), ammonia (C), creatinine (D) and uric acid (E) to total N excretion. For relative contributions (% of total N excretion), the values for spring and summer woodchucks were pooled ($N=8$ for woodchucks and 9 for rabbits). Values are means \pm S.E.M. *Significant differences from fed values measured on day 0 within each group.

$27.37 \pm 2.69 \mu\text{mol N kg}^{-1} \text{ min}^{-1}$ compared to $12.26 \pm 2.13 \mu\text{mol N kg}^{-1} \text{ min}^{-1}$ for the summer woodchucks and $3.61 \pm 1.54 \mu\text{mol N kg}^{-1} \text{ min}^{-1}$ for the spring woodchucks ($P<0.05$; Fig. 5A). Rabbit nitrogen excretion decreased to $8.93 \pm 1.15 \mu\text{mol N kg}^{-1} \text{ min}^{-1}$ ($P<0.05$; Fig. 5A) during the 7-day fast (a 67% change). Total urinary N excretion of the summer woodchucks decreased during the first 2 days of fasting to reach the low levels found in spring woodchucks (Fig. 5A). The % composition of individual nitrogenous compounds

relative to total N excretion did not differ between seasons in the woodchucks and, therefore, spring and summer data were pooled (Fig. 5B–E). However, differences did exist between species. The contribution of uric acid to total urinary N content was higher in rabbits than in woodchucks, both before and during the fast ($P < 0.05$; Fig. 5E). Conversely, the contribution of ammonia to total N content was higher in woodchucks than in rabbits, before and during the fast ($P < 0.05$; Fig. 5C). The % contributions of urea and creatinine to total N excretion were not different between species (Fig. 5B,D). As total nitrogen excretion decreased with fasting (Fig. 5A), so did the percentage contribution of urea after seven days of fasting ($P < 0.05$; Fig. 5B) (-22% for the woodchucks and -10% for the rabbits). The contribution of ammonia to total N excretion of the woodchucks doubled during the fasting period ($P < 0.05$; Fig. 5C). A similar increase did not occur in the rabbits, where the contribution of ammonia to total urinary nitrogen content remained around 1% throughout the fasting period.

Discussion

Our goal was to characterize the major metabolic adjustments of a hibernator during prolonged fasting under normothermic conditions, and to assess possible seasonal effects. This study shows that woodchucks use a dual strategy to cope with normothermic fasting: (1) they rapidly depress their metabolic rate (unless they already function at very low rates as in the spring), possibly reducing energy expenditure to the lowest level still compatible with normothermic life, and (2) they reorganize their fuel selection pattern to spare limited reserves of proteins.

Fasting-induced metabolic depression

In the fed state, metabolic rate is much higher in the summer than in the spring ($+35\%$; see Table 1), and this observation is consistent with published measurements on post-absorptive woodchucks and marmots (Bailey, 1965; Körtner and Heldmaier, 1995; Rawson et al., 1998). After spring arousal, a low basal metabolic rate appears critical for surviving the normothermic fasting period that typically follows 5 months of hibernation (Davis, 1967; Hamilton, 1934; Snyder et al., 1961). During fasting, metabolic depression was only used in the summer (25% reduction in \dot{V}_{O_2} over 2 weeks; Fig. 2), and it did not occur in the spring. This observation suggests the interesting possibility that normothermic, basal metabolism has a lower limit. The potential existence of a minimum basal metabolic rate for normothermic mammals is supported by the fact that summer and spring woodchucks reach the same rate of energy expenditure after 14 days of fasting, even though their pre-fasting rates are very different. Measurements on rabbits provide additional support for the same concept because this species starts with a much higher post-absorptive metabolic rate than spring or summer woodchucks, but it shows stronger metabolic depression during fasting (-32% in only 7 days). The rapid decrease in energy expenditure shown by rabbits is insufficient to completely offset their high post-

absorptive metabolic rate (Table 1, Fig. 2A), and, therefore, loss of body mass is much more rapid in rabbits (-15% in 7 days) than in woodchucks (-13% in 14 days) (Fig. 1). For this reason, the rabbit experiments had to be terminated after 1 week (according to the limit set by our animal care committee), and we could not determine whether a longer period of fasting would eventually decrease the metabolic rate of rabbits to the lower levels observed in woodchucks. In both species, metabolic depression was accompanied by a small, but significant decrease in body temperature (Fig. 3), as previously observed in other species including the rat (Ma and Foster, 1986). Like spring woodchucks, other mammals with naturally low basal metabolic rates seem to lack the capacity for metabolic depression during fasting. Experiments on the Virginia opossum *Didelphis virginiana*, a marsupial of similar body size (3–4 kg), revealed that this nocturnal species reaches its lowest metabolic rate of $\sim 200 \mu\text{mol O}_2 \text{ kg}^{-1} \text{ min}^{-1}$ (or $4.5 \text{ ml O}_2 \text{ kg}^{-1} \text{ min}^{-1}$) during daylight sleep (see fig. 1 in Weber and O'Connor, 2000). This minimum post-absorptive rate is identical to the lowest value observed here in woodchucks (Fig. 2A) and the Virginia opossum is not able to decrease its metabolic rate further in response to fasting (Weber and O'Connor, 2000).

The two main ATP-consuming processes accounting for basal metabolic rate are trans-membrane ion pumping and protein synthesis (Rolfe and Brown, 1997). It is conceivable that mammals can only downregulate these essential processes to a minimal level, below which normothermic life is compromised. For example, decreasing the cost of ion pumping can be achieved by lowering ion leakiness of membranes through changes in the degree of saturation of phospholipids (Hulbert and Else, 2000). However, modifying phospholipid saturation will also affect many other important membrane functions through changes in overall fluidity (e.g. insulin sensitivity), and such widespread disruption may not be compatible with mammalian life at $\sim 37^\circ\text{C}$. Another way to decrease energy expenditure during fasting would be to lower mitochondrial proton leak, a process that uncouples oxygen consumption from ADP phosphorylation. Two recent studies show that fasting and calorie restriction do not affect proton leak (Bézaire et al., 2001; Ramsay et al., 2004), whereas another suggests that proton leak is decreased *via* complex mechanisms that vary with the duration of calorie restriction (Bevilacqua et al., 2004). Clearly, the potential existence of a minimum normothermic metabolic rate in endotherms, and the mechanistic limitations for its specific set point, warrant further investigation.

Changes in fuel selection: protein sparing

In addition to metabolic depression, prolonged fasting has important effects on fuel selection. In our experiments, the major changes elicited by food deprivation took place within 2 days, and, therefore, all the values measured after this time were pooled for each group of animals (Fig. 4). In both species, the dominant use of carbohydrates that normally support energy metabolism in the post-absorptive state (phase I) was

rapidly replaced by high lipid use (phase II) as fasting was continued (Fig. 4). However, the most striking differences in fuel selection were observed in relation to protein sparing. In the spring, woodchucks had the lowest rate of net protein oxidation, presumably because their fuel selection pattern reflected the hibernation state more closely than in the summer. In the fed state, proteins only accounted for 8% of metabolic rate in spring woodchucks, whereas it reached 17% \dot{V}_{O_2} in summer woodchucks, and a high value of 28% \dot{V}_{O_2} in rabbits (Fig. 4A). All groups had the ability to decrease absolute and relative rates of net protein oxidation in response to fasting. After more than 3 days without food, the contribution of proteins was reduced to 5% \dot{V}_{O_2} in spring woodchucks, 6% \dot{V}_{O_2} in summer woodchucks and 20% \dot{V}_{O_2} in rabbits. Therefore, woodchucks show a superior ability for protein sparing (also reflected by much lower rates of water consumption and urine production than the rabbits; see Fig. 6), particularly during the summer. At this time of year, they can not only decrease their overall rate of energy expenditure to cope with fasting, but also dramatically reduce their reliance on proteins. This metabolic strategy helps to conserve muscle mass and it has been observed in other species adapted for prolonged fasting. Breeding adult seals and post-weaning seal pups commonly cope for several weeks without food by reducing their metabolic rate to ~45% of normal values in fed animals and their reliance on proteins down to 6% of \dot{V}_{O_2} (Nørdoy et al., 1993, 1990; Worthy and Lavigne, 1987). However, the most extreme capacity for protein sparing may be found in bears. During the summer, pack ice recession prevents polar bears *U. maritimus* from hunting seals and they appear to lower protein

oxidation to 1% of \dot{V}_{O_2} in response to fasting (Atkinson et al., 1996; Nelson, 1987). Black bears *Ursus americanus* and grizzly bears *U. arctos* decrease body temperature by a few degrees and metabolic rate by ~30% during their 'pseudo-hibernation', which can last for up to 6 months (Watts et al., 1981). During this time, no nitrogen wastes are excreted (Barboza et al., 1997; Nelson, 1973, 1980; Nelson et al., 1973) and two possible mechanisms have been invoked to explain this observation: (1) a complete inhibition of protein oxidation and/or (2) the recycling of nitrogen waste products. More recent experiments where muscle biopsies have been sampled at the beginning and at the end of winter show that protein breakdown of hibernating bears is actually significant but low (Tinker et al., 1998). Therefore, they do have the ability to recycle nitrogen because no waste products are accumulated during hibernation.

The exact pathways for nitrogen recycling have not been investigated in detail, but urea hydrolysis may be the most important mechanism used by woodchucks. To some extent, all mammals seem to be able to reabsorb urea through the bladder, and to bring it to their digestive system where it can undergo bacterial hydrolysis. The ammonia produced can then be recycled, and this process has been demonstrated in several mammals (Campbell and MacArthur, 1997; Harlow, 1987; Singer, 2002). The large difference in nitrogen excretion between the two species examined here may be explained by the fact that urea hydrolysis is particularly active in woodchucks compared to rabbits. This hypothesis is consistent with the observations that woodchucks produce very small volumes of urine (Fig. 6B) (thus favoring urea reabsorption through the bladder), and have a very large caecum (where extensive bacterial fermentation can take place). Surprisingly, however, metabolic tracer measurements have revealed that a closely related species (the marmot: *Marmota flaviventris*) strongly reduces its rate of urea hydrolysis during fasting (Harlow, 1987). Identifying the exact pathways and quantifying their flux in bears and true hibernators like woodchucks during normothermic fasting or hibernation are exciting avenues for future research.

Apart from fueling energy metabolism, protein breakdown can also play an important role in providing amino acids as a substrate for gluconeogenesis (Peroni et al., 1997). Liver glycogen is essentially depleted during phase I of fasting and glucose production becomes dependent on gluconeogenesis during phase II (Goodman et al., 1990; Nilsson and Hultman, 1973). In rats and humans for example, amino acids account for ~25% of total gluconeogenic flux during phase II (Owen et al., 1998), a contribution matching that of glycerol, an end-product of lipolysis.

Nitrogen excretion

The nitrogenous waste products of amino acid oxidation are excreted in urine as ammonia, urea and uric acid. Our analysis of changes in the relative composition of nitrogen wastes reveals another energy-saving mechanism associated with normothermic fasting in the woodchuck: a gradual shift away

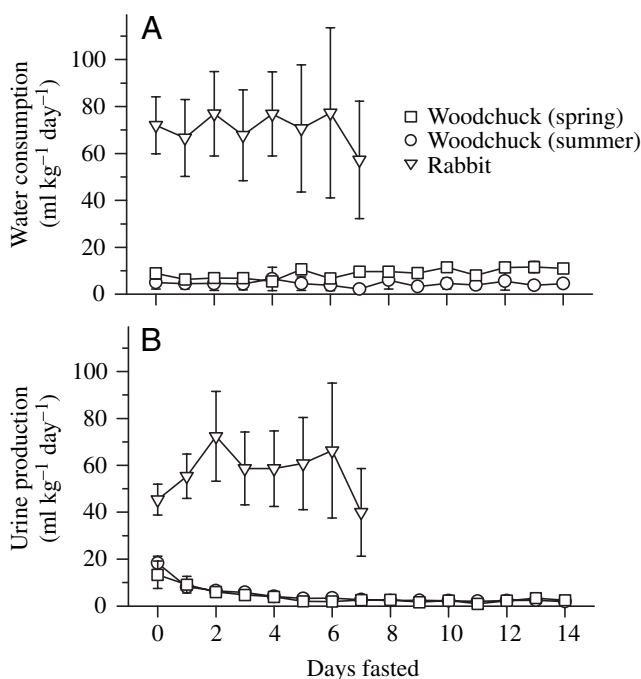


Fig. 6. Rates of water consumption and urine production in woodchucks and rabbits before and during prolonged fasting ($N=4$ for spring and summer woodchucks, $N=9$ for rabbits).

from urea excretion accompanied by an increase in ammonia excretion (Fig. 5). This change in ammonia excretion may be related to an increase in urea hydrolysis. Alternately, it could reflect a metabolic strategy aiming at decreasing the energy cost of nitrogen waste disposal in woodchucks. Interestingly, rabbits did not show this strategy, and increased their relative excretion of uric acid instead of ammonia. It is possible that such costly nitrogen waste disposal is only found in species that are not adapted for long-term fasting. As observed previously by others (e.g. see Hannaford et al., 1982), rabbits showed a particularly high rate of nitrogen excretion and this may also be related to the very high extraction of dietary protein afforded by coprophagy (Thacker and Brandt, 1955).

Conclusions

This study shows that, apart from their notorious capacity for hibernation, woodchucks are particularly well adapted for normothermic fasting. Their unusual ability to cope with prolonged food deprivation is based on a series of integrated mechanisms eliciting deep metabolic depression and a rapid change in fuel selection to spare limited protein reserves. The few studies available on prolonged fasting suggest that such an ability for depressing metabolism – to what could be minimal levels still compatible with normothermic life – may be common among mammals. In contrast, extreme protein sparing as demonstrated here in woodchucks, appears to be a unique metabolic feature of the fasting champions.

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