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ORIGINAL PAPER

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Seasonal changes in morphology and function of the gastrointestinal tract of free-living alpine marmots (*Marmota marmota*)

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Abstract The gastrointestinal tracts of 76 free-living alpine marmots (*Marmota marmota*) shot during a population control program in Switzerland were collected and analysed for patterns of change in morphology and function over the period from emergence from hibernation in April to just before re-entry into hibernation in September. Between first emergence and mid-summer (July) the fresh tissue mass of the stomach increased by 105%, the small intestine by 259% (among the largest recorded for a mammal), caecum by 185%, proximal colon by 138%, and distal colon by 144%. Mitotic activity was greatest in the small intestine; the mitotic index was high (40%) compared with indexes in the stomach and hindgut (approximately 4%) even at emergence, and increased to approximately 60% by mid-summer. Microbial activity in the caecum was also significant at emergence. The stomach (length) and caecum (length and fresh mass) increased in response to ingested food earlier than did the small intestine. Between mid-summer and September there were decreases in small intestinal tissue mass and mitotic activity. It is concluded that the gastrointestinal tract of alpine marmots probably continues to function throughout hibernation at a low level, with a mid-winter trough as part of an endogenous circannual rhythm. However, after emergence in spring, increases in size and activity of the tract appear to be a response to ingested food rather than to an endogenous signal. The early signs of down-regulation of the small intestine before re-entry into hibernation, together with its delayed up-regulation in response to food in spring, are consistent with the high costs of maintaining this section of the digestive system.

Keywords Marmot · Gastrointestinal tract · Hibernation · Mitotic activity · Microbial activity

Abbreviations *BrdU* bromodeoxyuridine · *PCNA* perichromonucleolin antigen · *PUFA* poly-unsaturated fatty acid · *SCFA* short-chain fatty acid

Introduction

The gastrointestinal tract is one of the most metabolically intense organs of the vertebrate body in terms of energy utilisation and protein synthesis, so it is expensive to maintain (Stevens and Hume 1995). Although Speakman and Johnson (2000) concluded that no particular tissue of lactating mice could be singled out as costly to maintain on the basis of mass, estimates of the gastrointestinal tract's contribution to the total oxygen consumption of the animal range from 12% in rats to 25% in pigs (McBride and Kelly 1990). Thus it might be expected that, if the organ is not being used, it should be reduced in size and/or activity. Many studies have shown that the gastrointestinal tract is remarkably flexible in size (Piersma and Lindström 1997). Some of the largest changes in gastrointestinal tract mass have been demonstrated in sit-and-wait foraging Burmese pythons (*Python molurus*), which consume large meals at long and unpredictable intervals (Secor and Diamond 1997), and in long-distance migratory garden warblers (*Sylvia borin*), which fatten prior to migration but may not feed during migratory flights of several thousand kilometres (Hume and Biebach 1996). When not feeding, gut mass in both species is substantially reduced, and the energy not used in maintaining expensive intestinal tissue maximises the intervals that pythons can survive between meals and the distance that garden warblers can

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fly across inhospitable deserts. An extreme illustration of gut mass change is provided by an invertebrate, the sea cucumber *Parastichopus californicus*, which sheds its gut in autumn and regains it in the spring. Evidently the cost of maintaining the gut over the winter, when only poor quality food is available, exceeds the cost of its entire replacement (Self et al. 1995).

Arctic and alpine mammals face strongly seasonal environments, with extreme changes in ambient temperature and food availability (Körtner and Heldmaier 1995; Buck and Barnes 1999; Barnes and Buck 2000); herbivores also face large changes in food quality. Many small mammals hibernate to survive the harsh winter conditions. During hibernation, rates of oxygen consumption are reduced at low body temperatures to a small fraction of normothermic values (Geiser 1988; Ortmann and Heldmaier 2000). Additional mechanisms used by alpine marmots (*Marmota marmota*) for minimising energy consumption during hibernation include nest building, group hibernation and synchrony in timing of arousals (Arnold 1988, 1990b; Ortmann and Heldmaier 2000; Ruf and Arnold 2000).

Another mechanism might be to selectively reduce the tissue mass of organs that are not in use during hibernation and are especially expensive to maintain. A prime example is the gastrointestinal tract. Several studies have demonstrated down-regulation of the small intestine of hibernators. For example, Kruman et al. (1986, 1988) found that rates of DNA synthesis in jejunal crypt cells of Arctic ground squirrels (*Spermophilus undulatus*) decreased during autumn at the time of preparation for hibernation and rose again during late hibernation at the time of preparation for terminal arousal, suggestive of an endogenous rhythm. Carey (1990) and Carey and Cooke (1991) reported decreases in mucosal wet mass per centimetre, villus height and mucosal surface area in the small intestine of the omnivorous thirteen-lined ground squirrel (*S. tridecemlineatus*) during hibernation. In hibernating red-cheeked ground squirrels (*S. erythrogenys*), there is thinning of the gastric mucosa and duodenal villi become progressively shorter (Vinogradova and Shesstopalova 1996).

We could find no information on changes in the large intestine (hindgut) during hibernation. The caecum and/or proximal colon is the main site of digesta retention and microbial fermentation in many small to medium-sized herbivorous mammals, including marmots (Hume et al. 1993; Stevens and Hume 1995). The marmots (*Marmota* spp.) are the largest herbivores that hibernate. This paper describes the changes that take place in the fore-, mid- and hindgut of free-living alpine marmots (*Marmota marmota*) following emergence from hibernation and over the active season in the Grisons district of Switzerland. We placed considerable emphasis on samples collected within the 1st week after emergence, in order to examine the question of whether gastrointestinal tract morphology and activity change only in response to ingested food, or are already up-regulated in response to some endogenous signal in preparation for terminal arousal.

Materials and methods

The project utilised a field site at 1600–2300 m above sea level in Switzerland in which marmots are shot by professional hunters in a population control program conducted under the supervision of the chief hunting inspector of the Grisons District. At our request, nearly all animals were shot between 11.00 h and 14.00 h, over the period from first emergence from hibernation (in April) to May, then in summer (July) and just before re-entry into hibernation (in mid-late September). Samples from a total of 76 animals (44 male, 32 female) were collected over the active seasons of 1999 and 2000. The animals were grouped into age classes on the basis of body mass (Arnold 1990a). Accordingly, 13 of the animals were classified as yearlings (born in the previous year), 15 were presumed to be 2-year-olds, and the remainder were clearly 3 years or older.

All animals were killed instantly by a single neck or head shot, brought immediately to a field laboratory, weighed, and the lengths of the head, body, and left hind foot recorded. Body length (from tip of snout to base of tail) was measured with the animal lying flat on its back in a frame used for standardising this measurement as part of the marmot population control program. The abdominal cavity was quickly opened through a ventral midline incision, and the gastrointestinal tract from the end of the oesophagus to anus removed and dissected free from mesenteric attachments and adherent fat. The tract was divided into stomach, small intestine, caecum and colon. The colon was further divided into proximal colon and distal colon (including the rectum) at a point based on separation of blood drainage patterns; the small intestine, caecum and proximal colon are drained by the vena mesenterica cranialis, while the distal colon and rectum are drained by the vena mesenterica caudalis (Nickel et al. 1984). Lengths, and full and empty masses of each tract section were recorded. The mass of contents was the difference between full and empty mass. Stomach length was measured along the greater curvature from cardia to pylorus, and is thus an index of stomach size rather than a strictly linear measure. Caecum length was measured from the ileo-caecal valve, along the centre line of the organ following its natural curvature, to its blind apex.

Prior to these gross morphometric measurements, small (3 cm×3 cm) sections of the wall of the cardiac and fundic regions of the stomach and of the central region of the caecum, and 3-cm lengths of duodenum, ileum, proximal colon and distal colon were resected, taking care not to damage the mucosa. Each section was rinsed with physiological saline to remove adherent digesta, preserved in 8% formalin at room temperature for no more than 24 h, then stored in 70% ethanol for later analysis of microscopic morphometrics and mitotic activity. Immediately after the gross morphometric measurements, representative samples of the contents of the stomach, small intestine, caecum, proximal colon and distal colon were stored under N_2 at –20 °C for later analysis of the total concentrations and molar proportions of short-chain fatty acids (SCFA) as a measure of microbial fermentative activity.

All samples were transported to Vienna by road. In Vienna, preserved tissues were embedded in paraffin, and 3-μm sections stained with eosin-haematoxylin. In each stained section, ten measurements were made of the thickness of the mucosa, including the lamina propria mucosae and lamina muscularis mucosae and villi in the small intestine, and the submucosa and mucosal folds in the stomach and hindgut, taking care to avoid pillars and any damaged sections. Further 3-μm sections of paraffin-imbedded tissues were stained with MIB-5, a clone of the monoclonal antibody Ki-67, which detects a nuclear antigen that is present only in proliferating cells (Sawhney and Hall 1992; Yu et al. 1992). Ki-67 and MIB-5 stain nuclei in all phases of cell division (including actual mitosis), whereas other proliferation markers stain only (bromodeoxyuridine; BrdU) or primarily (perichromonucleolin antigen; PCNA) nuclei in the S-phase (synthetic phase). Proliferating cells in the lower sections (the mitotic region) of gastric glands, crypts between villi in the duodenum and ileum, and crypts of mucus-producing glands in the caecum and colon were identified under a light microscope following the criteria of van Dierendonck

et al. (1989). Twenty nuclei were counted on both sides of the crypt from its base, and ten crypts were counted in each region of the gastrointestinal tract.

Samples of the contents of the stomach, small intestine, caecum, proximal colon and distal colon were analysed for total concentrations of SCFA, and the molar proportions of acetic, propionic, iso-butyric, n-butyric, iso-valeric and n-valeric acids, by gas-liquid chromatography (Perkin Elmer Auto System XL with Hewlett Packard High Performance Capillary Column 30 m×0.25 mm ID packed with cross-linked FFAP). Further samples were dried at 90 °C for 24 h to determine dry matter content, which allowed us to calculate the pool sizes of SCFA in the caecum, proximal colon and distal colon.

All data except total SCFA concentrations and individual SCFA proportions were analysed by analysis of covariance (ANCOVA), using body length as the covariate in order to correct for any influence of body size. We found that body length explained more of the residual variance than did head length or hindfoot length. SCFA concentrations ($mmol.l^{-1}$) and individual SCFA proportions (%) were analysed by ANOVA because of the absence of any body size effect. Individual SCFA proportions were arcsine-transformed, and SCFA pool data (mmol) were log-transformed. All statistical analyses were conducted using S-PLUS 2000 (Venables and Ripley 1999). Because it was not possible to ascertain time of emergence from hibernation more precisely than ± 1 day, data were grouped into six time periods: up to 3 days after emergence, 4–7 days after emergence, 8–19 days after emergence (all in April), 20–30 days after emergence (May), mid-summer (July) and late summer (September). The three age classes all contributed to all sample times. Initial inspection of the data revealed considerable heteroscedasticity of variances for most parameters. Therefore, ANCOVA models were computed using generalised least squares analysis which allows the incorporation of non-standard variance structures. We used a variance model with different variables at each time period (function varIdent in S-PLUS 2000, Pinheiro and Bates 2000). Specific comparisons between mean values at different times are based on the ANCOVA model contrasts.

Results

No pathological condition was observed in any of the 76 marmots dissected. Large numbers of helminths, mainly the cestode *Ctenotaenia marmotae* (Callait and Gauthier 2000), were present in the small intestine in July and September. These parasites no doubt contributed significantly to the mass of contents of the small intestine at these times, but the mucosa showed no thickening or any other change either macroscopically or microscopically that could be attributed to damage by the tape worms.

Gross morphometrics

Lengths of stomach, small intestine, caecum and proximal and distal colon are shown in Fig. 1, fresh tissue masses in Fig. 2, and the fresh mass of contents of each section in Fig. 3. The first significant increase in lengths of the stomach and caecum occurred 4–7 days following emergence. Small intestine and distal colon length increased later, with the first significant increases 8–19 days after emergence. Although proximal colon length followed the same general pattern, it did not increase significantly until July. Distal colon length decreased ($P < 0.001$) between July and September.

Fresh tissue mass of the stomach (Fig. 2) first increased significantly later than length, 20–30 days after emergence. A similar pattern of increase was seen in the small intestine, but in contrast to the stomach, the small intestine decreased ($P < 0.001$) between July and September. Fresh tissue mass of the hindgut followed the same pattern as in the stomach, except that the first significant increases occurred earlier in both the caecum (4–7 days after emergence) and distal colon (8–19 days), though not in the proximal colon.

The fresh mass of contents (Fig. 3) increased significantly 4–7 days following emergence in all sections of the gastrointestinal tract except the proximal colon (8–19 days). Maximal gut fill was reached in July in all sections of the tract except the proximal colon (September). There was a significant ($P = 0.05$) decrease in mass of contents in the small intestine between July and September, and a trend ($P = 0.075$) for a decrease in the distal colon.

Microscopic morphometrics

Mucosal thickness in the cardiac region of the stomach initially decreased ($P < 0.01$) 4–7 days after emergence, but then was not significantly different from initial values throughout the rest of the active season (Fig. 4). There was no decrease in mucosal thickness in the fundic region, but it increased significantly only between May and July. An initial decrease ($P < 0.05$) in mucosal thickness was also recorded in the proximal colon, but then both regions of the colon increased significantly 20–30 days after emergence. In the small intestine and caecum, mucosal thickness remained unchanged until significant increases 8–19 days after emergence (duodenum), 20–30 days after emergence (ileum) and between May and July (caecum).

Mitotic activity

Mitotic indexes in the mucosa of the stomach and hindgut were generally low, between 4% and 8%, showed no significant changes over the active season, and are not shown in Fig. 5. In contrast, indexes in the duodenum and ileum were approximately 40% even within 3 days after emergence, and increased to approximately 60% in July (Fig. 5). The initial significant increase in the duodenum was recorded 20–30 days after emergence, but in the ileum not until between May and July. This was followed in the ileum by a significant ($P < 0.05$) decrease between July and September. It was not possible to compare our results based on the monoclonal antibody MIB-5 with other published data based on BrdU or PCNA because of the different parts of the cell cycle stained by the different proliferation markers.

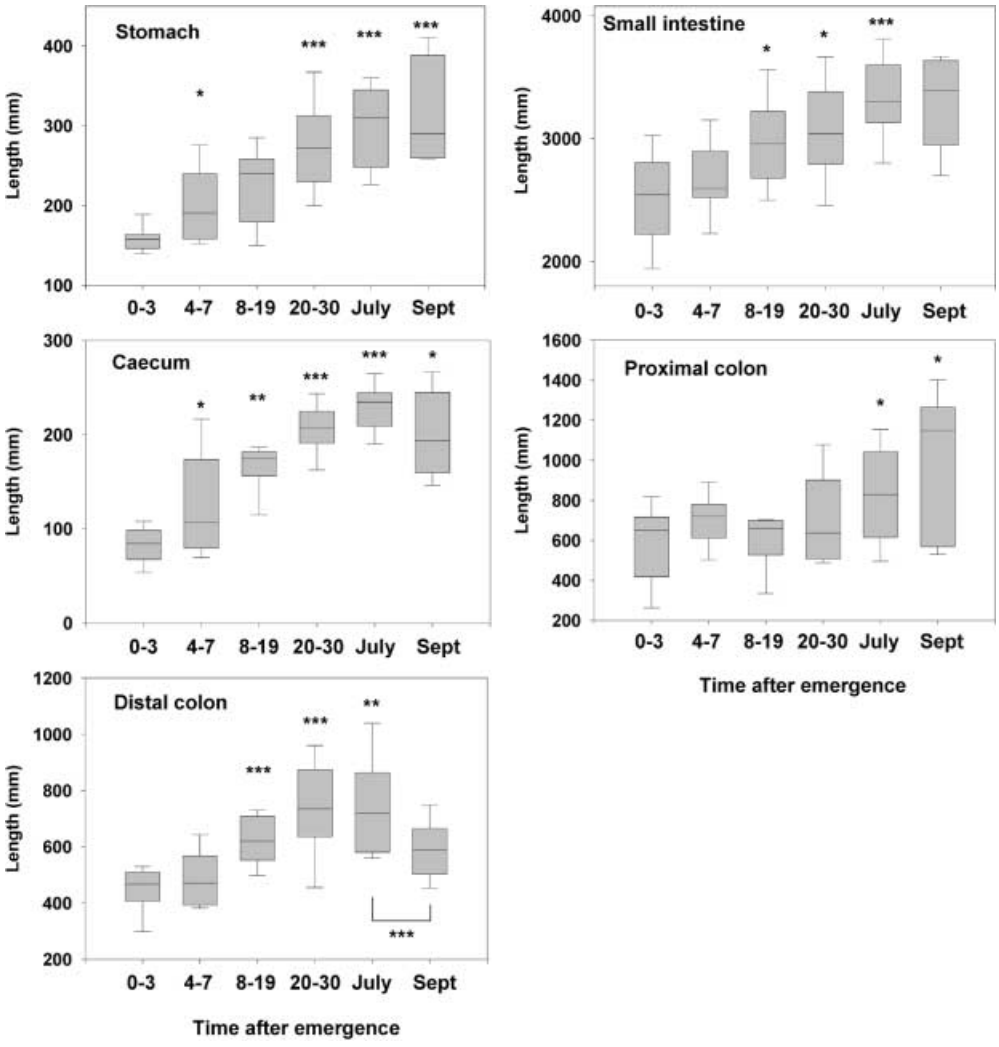

Fig. 1 Box plots of the length (mm) of the stomach, small intestine, caecum, proximal colon and distal colon of free-living alpine marmots over the active season. Stomach length measured along the greater curvature. Time after emergence in April and May (in days), July and September. Data in all box plots presented as medians (*horizontal line*) with the 25th to the 75th percentiles (*box*) and the 10th and 90th percentiles (*bar*). Significant differences from 0–3 days after emergence indicated by *asterisks* above the boxes. Significant decreases between July and September indicated by a *bar* and *asterisk*(*s*) below these two boxes (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

Microbial activity

Total SCFA concentrations were uniformly low in stomach and small intestinal contents (1–6 mmol.l^{-1} and 1–21 mmol.l^{-1}, respectively). The caecum of 6 of the 11 marmots shot within 3 days and 1 of 10 shot 4–7 days after emergence was empty. The proximal colon of three and the distal colon of two of the animals shot within 3 days of emergence were also empty. Caecal SCFA concentrations in the other early samples ranged from 29 mmol.l^{-1} to 168 mmol.l^{-1}. The highest individual concentration in the caecum was 248 mmol.l^{-1}, recorded in July. Mean SCFA concentrations in the caecum increased significantly 8–19 days after emergence (Table 1), following significant increases in the mass of stomach and caecal contents in the previous period (4–7 days) (Fig. 3). Similar patterns were observed in the proximal colon and distal colon (Table 1). There were significant decreases in total SCFA concentrations between July and September in all three hindgut regions.

The molar proportions of the individual SCFA are also shown in Table 1. Acetic acid constituted 70–76% of total SCFA throughout the active season in all three regions of the hindgut, with lowest values in May; this was significant in the caecum ($P < 0.001$), approached significance in the proximal colon ($P = 0.060$), but was not significant in the distal colon. In the caecum, propionic acid proportions were highest 0–3 days after emergence, then declined rapidly by 8–19 days, and remained low until July. The same pattern was evident for the branched-chain acids iso-butyric and iso-valeric. Conversely, the proportion of n-butyric acid increased from low levels 0–3 days after emergence to reach a peak in May–July. Patterns in the proximal colon were similar but the changes were not as marked. In the distal colon, the same pattern in n-butyric acid proportions was evident, but there were only minor changes in the proportions of propionic, iso-butyric and the two isomers of valeric acid. For all SCFA, there was a consistent trend, not always significant, for their molar proportions to return to emergence values between July and September.

The quantitative importance of SCFA to the animal is best illustrated by calculating pool sizes, using the mass of contents of each hindgut region, the dry matter content and the total SCFA concentration in each region. Pool sizes are shown in Fig. 6. They show that,

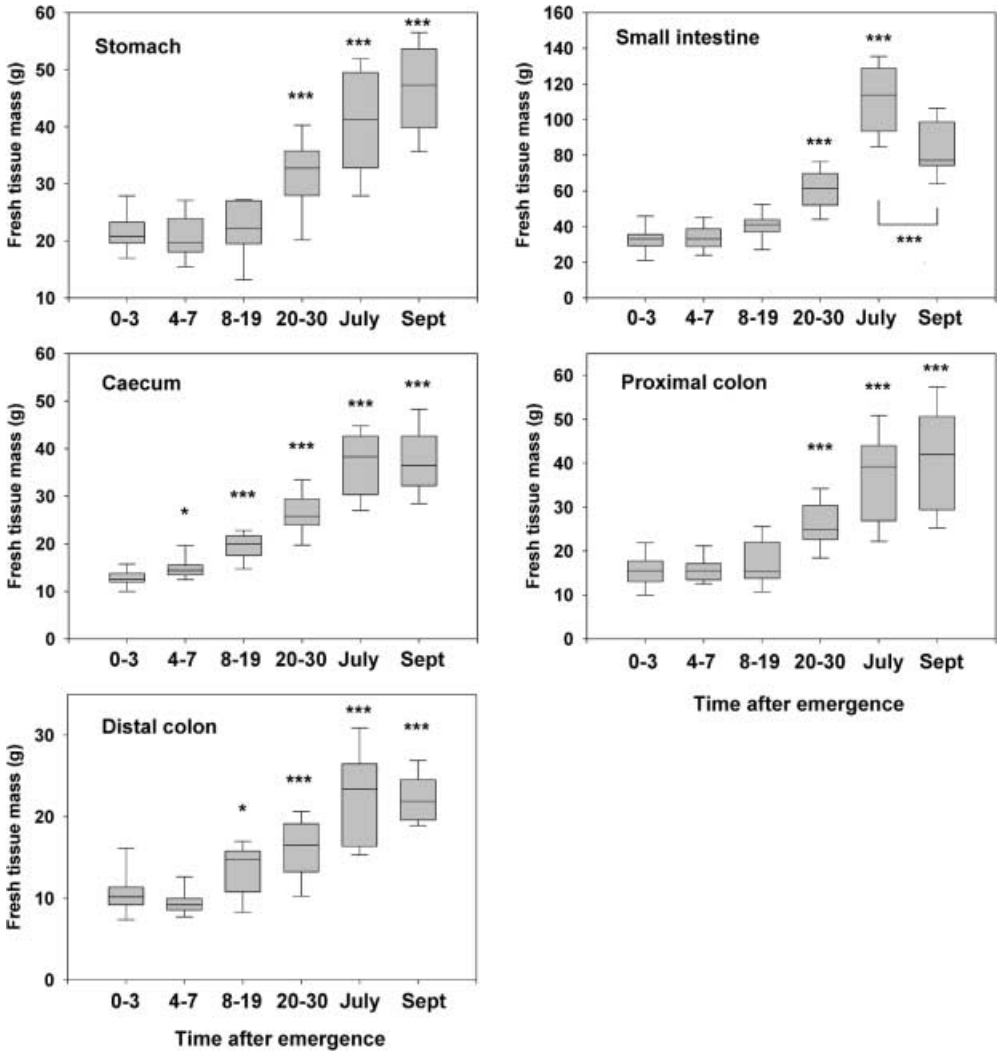

Fig. 2 Box plots of the fresh tissue mass (g) of the stomach, small intestine, caecum, proximal colon and distal colon of free-living alpine marmots over the active season. For details see legend to Fig. 1

although concentrations of SCFA are considerable even in early samples, the mass of contents at this time is small, and so pool sizes are tiny. Only after contents increase (Fig. 3) do SCFA pool sizes indicate an important contribution by SFCAs to the animal's energy supply. Maximum pool sizes were reached by July, but then they decreased significantly in all three regions of the hindgut between July and September.

Discussion

The results from the four sections of the study (gross morphometrics, microscopic morphometrics, mitotic activity and microbial activity) are consistent in showing that activity of the gastrointestinal tract of alpine marmots is lowest immediately after emergence from hibernation, and does not increase until ingested food is present in the tract. They also show that some of the earliest responses to ingested food occur in the stomach (increase in length) and caecum (increases in both length and fresh tissue mass; all 4–7 days) rather than the small intestine (length at 8–19 days, fresh tissue mass at 20–30 days) (Figs. 1, 2), even though the mass of contents increased significantly in the 1st 7 days after emergence in all three sections (Fig. 3). In the colon, increases in both length and fresh tissue mass of the distal segment preceded those in the proximal segment.

Between emergence from hibernation and mid-summer (a period of 3 months), the fresh tissue mass of the stomach increased by 105%, that of the small intestine by 259%, the caecum by 185%, the proximal colon by 138% and the distal colon by 144%. The increase in the small intestine is similar to the increase in mucosal mass per centimetre of small intestine recorded in thirteen-lined ground squirrels by Carey (1990) over a similar time period. It is also similar to the increase in tissue mass in pythons reported by Secor and Diamond (1998). Only the time scale differs; in the python the small intestine doubled in wet and dry tissue mass within 1 day of feeding after a prolonged fast. The 50% increase in the python's stomach tissue mass was more modest than the 105% increase in the alpine marmot, but was achieved in the python in 3 days after feeding. Similarly

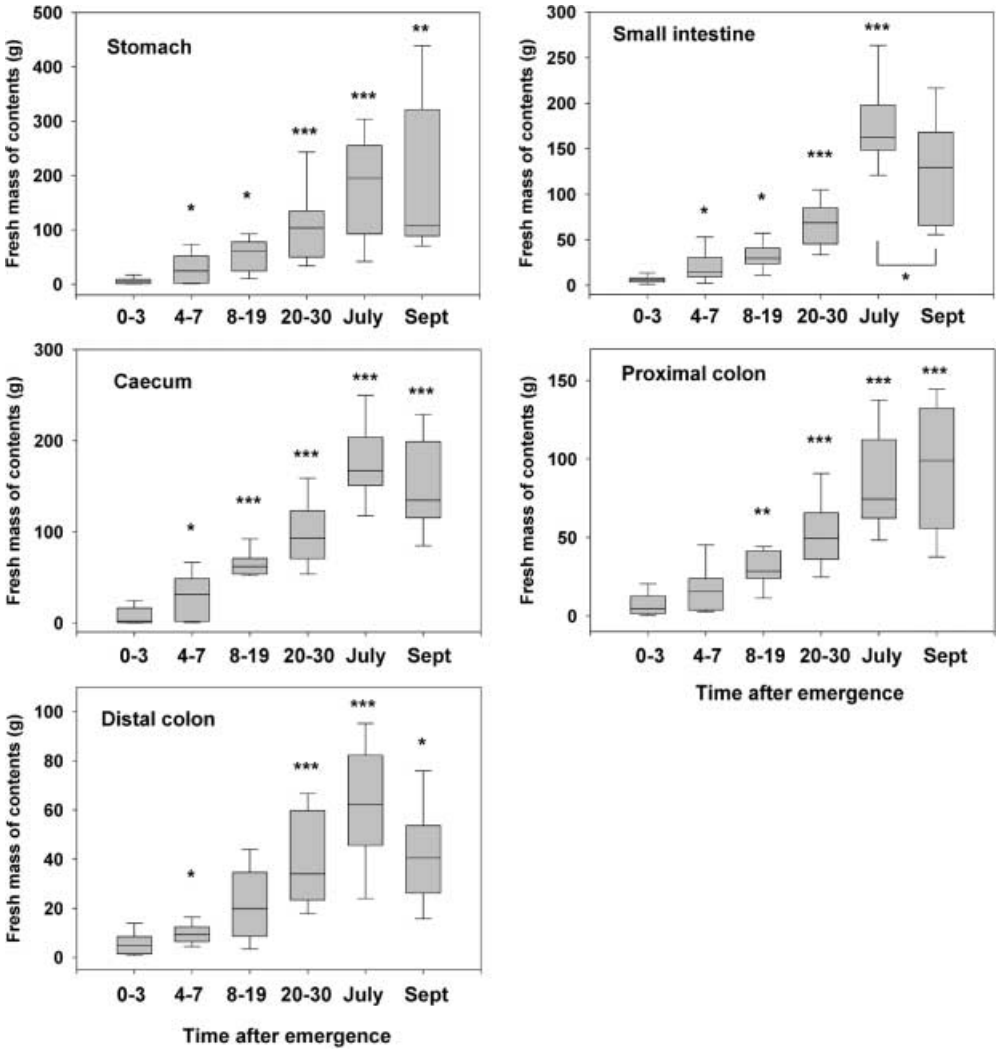

Fig. 3 Box plots of the fresh mass of contents (g) of the stomach, small intestine, caecum, proximal colon and distal colon of free-living alpine marmots over the active season. For details see legend to Fig. 1

rapid changes to those seen in the python, but in the opposite direction, were reported by Hume and Biebach (1996) in the small intestine of garden warblers, in response to a 48-h fast designed to partially simulate a migratory flight, during which the birds normally do not feed. In this case the dry tissue mass of the small intestine before the fast was 167% greater than that afterwards.

Like the gross morphological changes, post-hibernation changes at the microscopic level in the gastrointestinal tract of the alpine marmot were also delayed relative to time of emergence. Gastric mucosal thickness did not increase significantly until mid-summer, and actually decreased in the cardiac region during the 1st 7 days, possibly as this region underwent receptive relaxation (Stevens and Hume 1995) to accommodate the first ingested food. Mucosal thickness in the duodenum and ileum nearly doubled between 20 days after emergence and mid-summer (Fig. 4). Significant, but smaller, increases in mucosal thickness also occurred in the caecum and proximal colon, but not until 20–30 days post-emergence in the colon and July in the caecum. In the 1st 7 days the proximal colon mucosa thinned, as occurred in the cardiac region of the stomach, but there is no clear explanation for this finding.

Mitotically, the small intestine was by far the most active section of the gastrointestinal tract, with minimal mitotic indexes of 40%, compared with 4% in the stomach and hindgut. Mitotic indexes increased by at least a half in both the duodenum and ileum, but only after 20 days post-emergence in the duodenum and after 30 days in the ileum. Whether this minimal level of mitotic activity in the small intestine is maintained throughout hibernation is unclear. Kruman et al. (1988) found that the proportion of enterocytes in the S-phase of the cell cycle in the jejunum of Arctic ground squirrels declined in early hibernation (November) to a low in mid-hibernation (January) but increased again in late hibernation (March). The same pattern was present in normothermic animals prevented from hibernating, suggestive of an endogenous circannual rhythm. If these endogenous rhythms are a common feature of hibernators, the mitotic indexes we measured in April were probably already above mid-winter values.

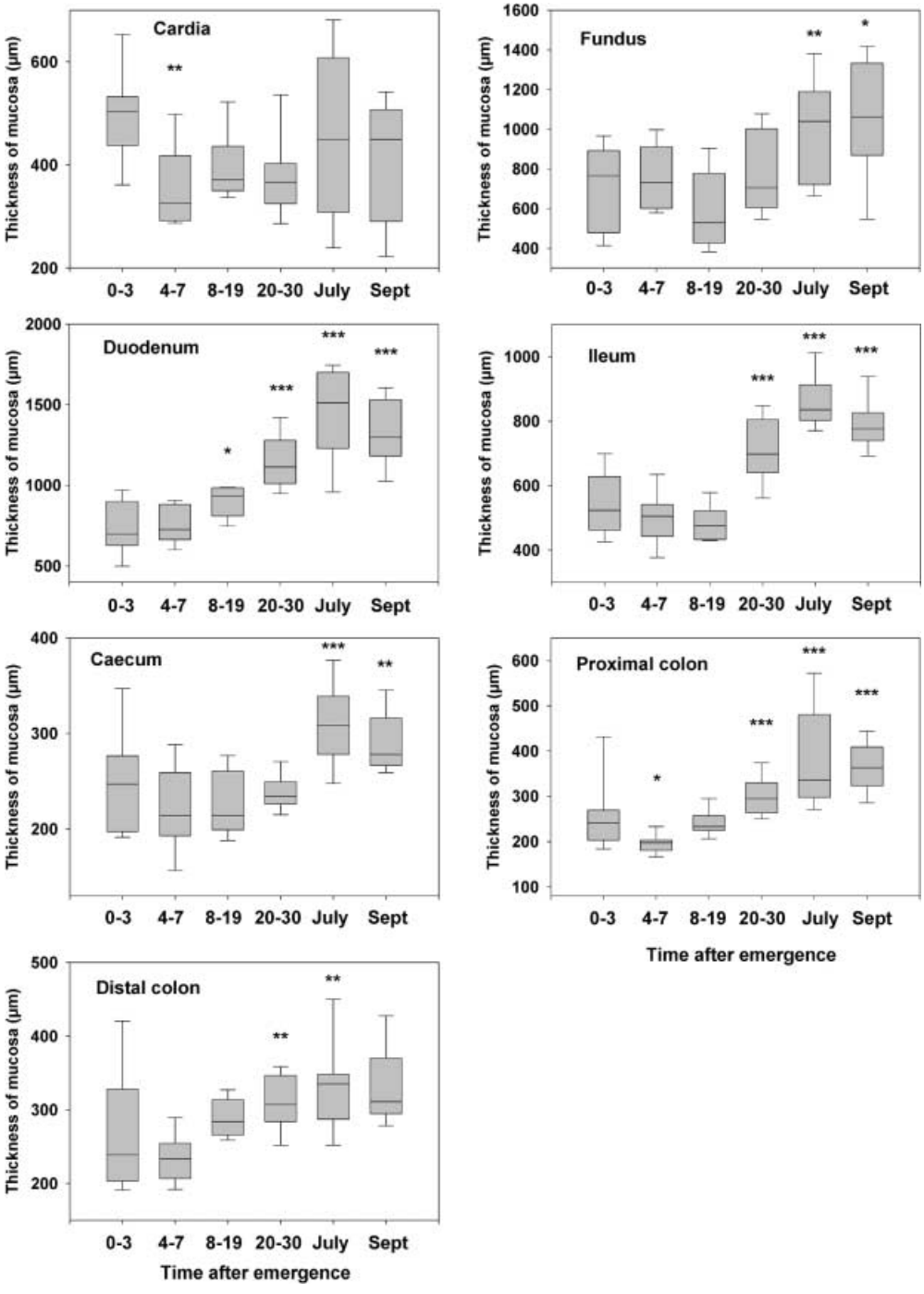

Fig. 4 Box plots of the thickness (µm) of the mucosa of the cardiac and fundic regions of the stomach, duodenum, ileum, caecum, proximal colon and distal colon of free-living alpine marmots over the active season. For details see legend to Fig. 1

Carey (1995) reviewed studies that showed that, although mitotic activities are greatly depressed during deep torpor, they resume rapidly as body temperature rises during periodic arousals and then are depressed again as the animals re-enter torpor. It is not clear how the rates of proliferation during inter-bout arousals compare with rates after arousal in spring, but clearly food ingestion per se is not needed to increase mitotic activities above torpor values, at least in the small intestine. Similarly, the capacity for digestive activity, nutrient uptake and electrolyte secretion is maintained in the jejunum of hibernating thirteen-lined ground squirrels (Carey and Martin 1996), but it is only expressed at normothermic body temperatures during inter-bout arousals.

Even if the animals do not feed during their periodic arousals in winter, the ability to absorb amino acids against a steep concentration gradient may allow them to scavenge any amino acids present in the intestinal lumen from endogenous sources at these times. Carey (1995) concluded that a basal level of hydrolytic enzyme and transporter abundance may be maintained

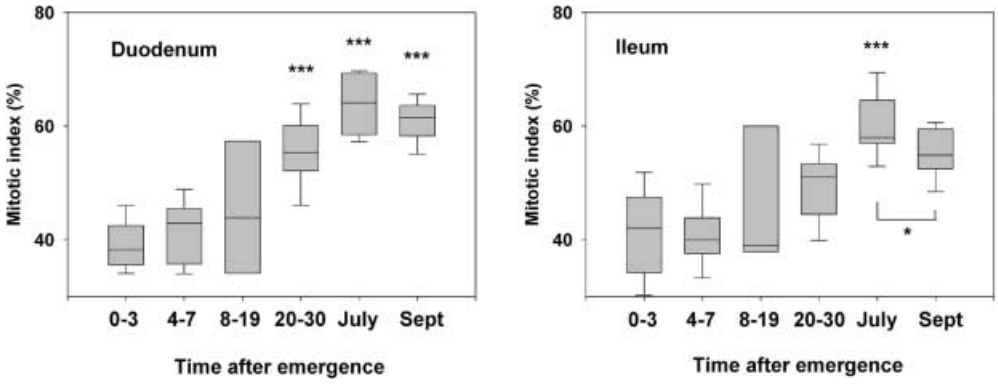

Fig. 5 Box plots of the mitotic index (percentage proliferating cells) in the mucosa of the duodenum and ileum of free-living alpine marmots over the active season. For details see legend to Fig. 1

Table 1 Total concentrations of Short-chain fatty acids (SCFA; mmol.l^{-1}) and molar proportions (%) of the individual acids in the hindgut of free-living alpine marmots; means + SD

	0–3 days	4–7 days	8–9 days	20–30 days	July	September
Caecum	($n=4$)	($n=9$)	($n=5$)	($n=19$)	($n=14$)	($n=13$)
Total SCFA (mmol.l^{-1})	75.2 ± 41.3	97.4 ± 44.8	129.5 ± 12.4^{a}	150.9 ± 39.6^{b}	178.6 ± 26.3^{c}	143.5 ± 40.5^{z}
Acetic acid	75.4 ± 1.6	73.9 ± 5.3	75.2 ± 5.1	69.8 ± 4.3^{c}	71.8 ± 4.0^{b}	75.3 ± 2.1
Propionic acid	15.8 ± 3.1	13.0 ± 4.6	9.4 ± 0.7^{c}	7.7 ± 1.2^{c}	6.6 ± 0.4^{c}	7.7 ± 2.3^{z}
Iso-butyric acid	1.0 ± 0.8	0.5 ± 0.5	0.1 ± 0.3^{a}	0.4 ± 0.3	0.3 ± 0.0	0.7 ± 0.8^{y}
n-Butyric acid	6.1 ± 3.7	11.1 ± 6.8	14.5 ± 5.4^{b}	20.7 ± 4.7^{c}	20.4 ± 3.6^{c}	14.7 ± 2.9^{z}
Iso-valeric acid	1.0 ± 1.8	0.5 ± 0.7	0.1 ± 0.3	0.6 ± 0.4	0.4 ± 0.1	0.9 ± 1.0
n-Valeric acid	0.6 ± 0.8	0.8 ± 0.8	0.6 ± 0.5	0.8 ± 0.2	0.5 ± 0.1	0.7 ± 0.4
Proximal colon	($n=7$)	($n=11$)	($n=5$)	($n=19$)	($n=15$)	($n=12$)
Total SCFA (mmol.l^{-1})	68.8 ± 17.1	103.4 ± 65.7	92.8 ± 15.0^{a}	114.4 ± 18.0^{c}	136.5 ± 21.3^{c}	94.0 ± 31.6^{x}
Acetic acid	71.3 ± 6.0	72.2 ± 5.8	75.1 ± 3.5	71.5 ± 3.6	76.2 ± 4.6	73.7 ± 7.3
Propionic acid	18.9 ± 3.4	13.2 ± 4.7^{a}	10.7 ± 1.3^{c}	9.8 ± 2.1^{c}	8.7 ± 2.8^{c}	11.7 ± 3.7^{y}
Iso-butyric acid	1.5 ± 0.8	1.0 ± 0.9	0.5 ± 0.6^{a}	0.8 ± 0.5^{a}	0.5 ± 0.3^{c}	1.0 ± 0.8^{y}
n-Butyric acid	6.0 ± 2.2	11.0 ± 3.9^{b}	11.9 ± 2.5^{c}	15.8 ± 3.2^{c}	13.3 ± 2.2^{c}	11.2 ± 4.2^{z}
Iso-valeric acid	1.9 ± 1.4	1.5 ± 1.7	0.9 ± 0.4	1.1 ± 0.6	0.7 ± 0.4^{a}	1.5 ± 1.3^{y}
n-Valeric acid	1.2 ± 1.2	1.1 ± 0.7	0.8 ± 0.6	0.9 ± 0.3	0.7 ± 0.3	0.9 ± 0.5
Distal colon	($n=8$)	($n=11$)	($n=5$)	($n=19$)	($n=15$)	($n=13$)
Total SCFA (mmol.l^{-1})	48.0 ± 26.7	72.9 ± 43.0	106.8 ± 24.8^{c}	129.1 ± 36.4^{c}	135.8 ± 48.0^{c}	96.8 ± 22.3^{z}
Acetic acid	71.2 ± 9.6	74.5 ± 5.4	73.9 ± 3.0	69.8 ± 4.8	74.2 ± 6.4	71.4 ± 10.1
Propionic acid	15.7 ± 5.3	12.1 ± 4.6	11.7 ± 1.4	11.3 ± 2.8^{a}	11.2 ± 3.9^{a}	13.3 ± 3.8
Iso-butyric acid	1.5 ± 1.0	1.3 ± 1.1	1.6 ± 0.9	1.1 ± 0.6	0.8 ± 0.6	1.6 ± 1.2^{x}
n-butyric acid	8.4 ± 3.6	8.6 ± 3.6	10.7 ± 3.2	15.1 ± 6.5^{b}	11.8 ± 2.2^{a}	10.2 ± 3.8^{z}
Iso-valeric acid	2.5 ± 1.6	2.1 ± 2.1	1.4 ± 0.7	1.7 ± 1.0	1.2 ± 0.7^{a}	2.2 ± 1.7^{y}
n-Valeric acid	0.8 ± 0.9	1.3 ± 1.0	0.9 ± 0.6	1.0 ± 0.4	0.8 ± 0.4	1.3 ± 0.8

^{a,b,c} Significantly different from 0–3 days value
^{x,y,z} September value significantly different from July value
^{a,x} $P<0.05$ ^{b,y} $P<0.01$ ^{c,z} $P<0.001$

throughout hibernation through synthesis de novo during periodic arousals. More recently, Toole et al. (1999) suggested that, in hibernating golden hamsters (*Mesocricetus auratus*), there were significant increases in numbers of myenteric neurones that helped to maintain the integrity of the muscular and mucosal layers of the small intestine and hindgut in the absence of luminal contents. These findings help to explain the relatively high mitotic indexes in our alpine marmots immediately post-emergence. Nevertheless, there is clearly a delay before biosynthetic activities increase above this basal level in response to ingested food.

Interestingly, microbial activity in the hindgut increased more rapidly than small intestinal function. The concentrations of SCFA recorded in the caecum within 3 days of emergence, and before any evidence of food in the stomach, also suggest that a basal level of fermentation continues throughout hibernation in the alpine marmot. Alternatively, ingestion of bedding material just prior to emergence may initiate microbial activity, but this is unlikely given that marmots emerge in spring with almost no delay after terminal arousal (Arnold 1993, 1995). In relatively large herbivores such as marmots, there may be survival value in maintaining a certain minimal level of microbial activity in the hindgut during hibernation if it means a greater ability to maximise intake of new grass as soon as it becomes available in spring. Also, the only grass available for the first few weeks after emergence from hibernation may be dead material from the previous growing season. Utilisation of this material, with its high cell wall content, can only proceed via microbial fermentation (Hume et al. 1993; Stevens and Hume 1995). There is no information available on numbers of bacteria in the hindgut of

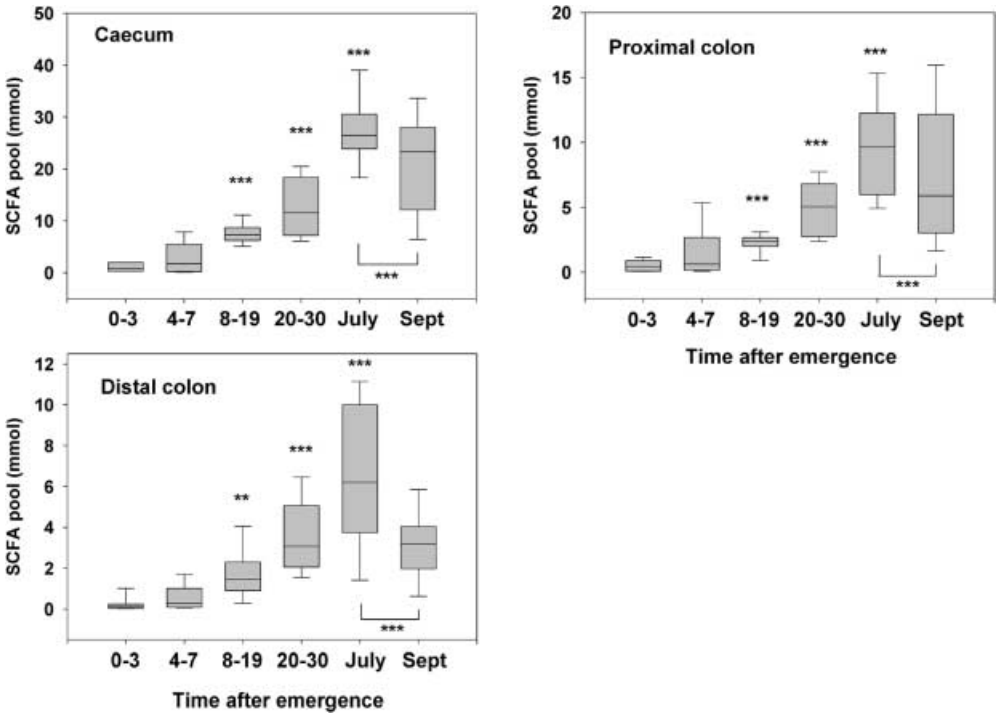

Fig. 6 Box plots of the short-chain fatty acid pool (mmol) in the caecum, proximal colon and distal colon of free-living alpine marmots over the active season. For details see legend to Fig. 1

hibernating marmots. In thirteen-lined ground squirrels, Barnes and Burton (1970) found that, after 6 weeks of hibernation, only 32% of the total caecal microflora could be recovered by the roll tube technique, compared with 84% in active animals. Thus many dead organisms were present in the hibernating squirrels, suggesting that certain elements of the microflora disappear, or are reduced to very low numbers during hibernation. However, clearly some bacteria survived during hibernation, indicating a remarkably stable association between these organisms and their host. Most were probably in a resting state, as they were unable to grow in culture at the hibernation temperature of 7 °C. Presumably this is also the case in marmots, the largest herbivores known to hibernate, and in which deep body temperatures reach 4 °C during hibernation (Arnold 1993).

The nature of any substrate fermented in the hindgut of alpine marmots during hibernation is unknown. The relatively high molar proportions of the branched-chain SCFA iso-butyric and iso-valeric acids in the 1st 3 days after emergence suggest fermentation of protein, probably derived from endogenous materials such as the mucous layer lining the hindgut and secretions entering from the small intestine. The only other potential substrate is the bedding material (grass) collected immediately before entry into hibernation in late summer/autumn. Hibernation burrows of the Asian species *Marmota camtschatica* may be lined with up to 15 kg of grass (Bibikow 1996). It is unlikely that alpine marmots eat much of their bedding material during arousals, but the ingestion of even small amounts would supply additional substrate to the hindgut microflora. Körtner and Heldmaier (1995) concluded that, at least in captivity, feeding and hibernation are not mutually exclusive in alpine marmots.

Taken together with those of other workers, our results suggest that the gastrointestinal tract of alpine marmots is already functioning, albeit at a low level, when the animals emerge from hibernation in early spring. However, increases in tract capacity and activity after emergence are clearly a response to ingested food, and take some time. Such a strategy would seem to be suitable for alpine marmots, which do not cache food (Arnold 1993), and therefore probably do not feed between terminal arousal and emergence. At the high altitudes that characterise their habitat, food is not always immediately available, and may not appear until almost 2 months after emergence (Arnold 1990b). In this case, it would be prudent to have ingested food act as the signal for reactivation of the gastrointestinal tract above pre-emergence levels. The relatively high levels of depot fat carried by emerging alpine marmots (up to 16% of body mass; I.D. Hume, personal observation) fuel the reproductive activities of the animals until food does become available. Peak concentrations of plasma non-esterified fatty acids in *M. flaviventris* in Colorado in April (Hill and Florant 1999) provide additional evidence that marmots are in a fasting rather than a fed state in early spring.

Once food is available, ingestion rates increase slowly at first (Davis 1976), then rapidly (Körtner and Heldmaier 1995) and the gastrointestinal tract responds in capacity (Figs. 1, 2, 3) and activity (Figs. 4, 5, 6). Peak levels in most parameters were recorded in mid-summer (July) . The high quality of the diet at this time is reflected in peak concentrations of SCFA in the caecum, in the high molar proportion of n-butyric acid, and the relatively low molar proportion of acetic acid. Butyric acid is produced during the fermentation of soluble carbohydrates, particularly sugars, but acetic acid pro-

duction is directly related to the amount of structural carbohydrate (fibre) in the diet (Stevens and Hume 1995). Thus the ratio of acetate to n-butyrate in the caecal SCFA was highest (8.9–16.7) in the 1st week after emergence from hibernation when only old grass of low quality from the previous growing season was available, and fell to a low of 2.9 in July.

The relatively low molar proportions of propionic acid, particularly in summer even though the nutritive value of the forage was high, suggest the possibility of reductive acetogenesis in the caecum of the alpine marmot. Acetate production from H_2 and CO_2 may be a significant alternative to methanogenesis and propionate production as a sink for reducing equivalents in the hindgut (Demeyer 1991; Jensen 1996). In theory, this should increase the yield of SCFAs per unit of substrate fermented in hindgut fermenters such as marmots, but the quantitative significance of this possibility has not been investigated.

Several parameters decreased between July and September, including fresh mass of the small intestinal tissue (Fig. 2) and contents (Fig. 3), mitotic index in the ileum (Fig. 5) and length of the distal colon (Fig. 1). Some of these pre-hibernation changes in autumn have also been reported in the small intestine of Arctic ground squirrels (Kruman et al. 1986, 1988) and thirteen-lined ground squirrels (Carey 1990). These changes coincide with decreasing day length and ambient temperature, reductions in activity levels of the animals (Bibikow 1996), and decreased rates of feeding (Körtner and Heldmaier 1995). Levels of depot fat approach an asymptote, and proportions of polyunsaturated long-chain fatty acids (PUFA) in the depot fat increase as the marmots switch their diet from forage to more flowers and seeds, many of which are rich sources of PUFA (Hill and Florant 1999). It has been demonstrated that increases in the levels of certain PUFA in the depot fat of marmots reduce energy expenditure and increase depth of torpor during hibernation, at least in captivity (Geiser and Kenagy 1987; Frank 1992; Florant et al. 1993; Florant 1998). Any one or more of these changes could be a signal for the gastrointestinal tract to prepare for hibernation by down-regulating activities and capacities. The early signs of down-regulation of the small intestine, together with its delayed up-regulation in response to food in spring, are consistent with the high costs of maintaining this part of the digestive system.

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References

Arnold W (1988) Social thermoregulation during hibernation in alpine marmots (*Marmota marmota*). J Comp Physiol B 158:151–156

Arnold W (1990a) The evolution of marmot sociality. I. Why disperse late? Behav Ecol Sociobiol 27:229–237

Arnold W (1990b) The evolution of marmot sociality. II. Costs and benefits of joint hibernation. Behav Ecol Sociobiol 27:239–246

Arnold W (1993) Energetics of social hibernation. In: Carey C, Florant GL, Wunder BA, Horwitz B (eds) Life in the cold: ecological, physiological, and molecular mechanisms. Westview, Boulder, pp 65–80

Arnold W (1995) Social behaviour and telemetric assessment of thermoregulation in hibernating marmots. In: Alleva E, Fasolo A, Lipp HP, Nadel L, Ricceri L (eds) Behavioural brain research in naturalistic and semi-naturalistic settings. Kluwer, Amsterdam, pp 395–411

Barnes BM, Buck CL (2000) Hibernation in the extreme: burrow and body temperatures, metabolism, and limits to torpor bout length in Arctic ground squirrels. In: Heldmaier G, Klingenspor M (eds) Life in the cold. Eleventh International Hibernation Symposium. Springer, Berlin Heidelberg New York, pp 65–72

Barnes EM, Burton GC (1970) The effect of hibernation on the caecal flora of the thirteen-lined ground squirrel (*Citellus tridecemlineatus*). J Appl Bact 33:505–514

Bibikow DI (1996) Die Murmeltiere der Welt. Westarp Wissenschaften, Magdeburg

Buck CL, Barnes BM (1999) Annual cycle of body composition and hibernation in free-living Arctic ground squirrels. J Mammal 80:430–442

Callait M-P, Gauthier D (2000) Parasite adaptations to hibernation in alpine marmots (*Marmota marmota*). In: Heldmaier G, Klingenspor M (eds) Life in the cold. Eleventh International Hibernation Symposium. Springer, Berlin Heidelberg New York, pp 139–146

Carey HV (1990) Seasonal changes in mucosal structure and function in ground squirrel intestine. Am J Physiol 259:R385–R392

Carey HV (1995) Gut feelings about hibernation. News Physiol Sci 10:55–61

Carey HV, Cooke HJ (1991) Effect of hibernation and jejunal bypass on mucosal structure and function. Am J Physiol 261:G37–G44

Carey HV, Martin SL (1996) Preservation of intestinal gene expression during hibernation. Am J Physiol 271:G805–G813

Davis DE (1976) Hibernation and circannual rhythms of food consumption in marmots and ground squirrels. Quart Rev Biol 51:477–514

Demeyer DI (1991) Quantitative aspects of microbial metabolism in the rumen and hindgut. In: Jouany JP (ed) Rumen microbial metabolism and ruminant digestion. INRA, Paris, pp 217–237

Dierendonck van JH, Keijer R, Van de Velde CJH, Cornelisse CJ (1989) Nuclear distribution of the Ki-67 antigen during the cell cycle: comparison with growth fraction in human breast cancer cells. Cancer Res 49:2999–3006

Florant GL (1998) Lipid metabolism in hibernators: the importance of essential fatty acids. Am Zool 38:331–340

Florant GL, Hester L, Ameenuddin S, Rintoul DA (1993) The effect of a low essential fatty acid diet on hibernation in marmots. Am J Physiol 264:R747–R753

Frank CL (1992) The influence of dietary fatty acids on hibernation by golden-mantled ground squirrels (*Spermophilus lateralis*). Physiol Zool 65:906–920

Geiser F (1988) Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? J Comp Physiol B 158:25–37

Geiser F, Kenagy GJ (1987) Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. Am J Physiol 252:R897–R901

Hill VL, Florant GL (1999) Patterns of fatty acid composition in free-ranging yellow-bellied marmots (*Marmota flaviventris*). Can J Zool 77:1494–1503

Hume ID, Biebach H (1996) Digestive tract function in the long-distance migratory garden warbler, *Sylvia borin*. J Comp Physiol B 166:388–395

Hume ID, Morgan KR, Kenagy GJ (1993) Digesta retention and digestive performance in sciurid and microtine rodents: effects of hindgut morphology and body size. Physiol Zool 66:396–411

Jensen BB (1996) Methanogenesis in monogastric animals. Environ Monit Assess 42:99–112

Körtner G, Heldmaier G (1995) Body weight cycles and energy balance in the alpine marmot (*Marmota marmota*). Physiol Zool 68:149–163

Kruman II, Kolaeva SG, Iliasova EN, Zubrikhina GN, Khachko VN, Petrova AS (1986) Seasonal variations of DNA synthesis in intestinal epithelial cells of hibernating animals. I. DNA synthesis in intestinal epithelial cells of ground squirrel (*Citellus undulatus*) during deep hibernation. Comp Biochem Physiol B 83:173–177

Kruman II, Kolaeva SG, Rudchenko SA, Khurkhulu ZS (1988) Seasonal variations of DNA-synthesis in intestinal epithelial cells of hibernating animals. 2. DNA-synthesis in intestinal epithelial cells of ground squirrel (*Citellus undulatus*) during autumn and late hibernation season. Comp Biochem Physiol B 89:271–273

McBride BW, Kelly JM (1990) Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. J Anim Sci 68:2997–3010

Nickel R, Schummer A, Seiferle E (1984) Lehrbuch der Anatomie der Haustiere. Paul Parey, Berlin

Ortmann S, Heldmaier G (2000) Regulation of body temperature and energy requirements of hibernating Alpine marmots (*Marmota marmota*). Am J Physiol 278:R698–R704

Piersma T, Lindström A (1997) Rapid reversible changes in organ size as a component of adaptive behaviour. TREE 12:134–138

Pinheiro JC, Bates DM (2000) Mixed effects models in S and S-PLUS. Springer, Berlin Heidelberg New York

Ruf T, Arnold W (2000) Mechanisms of social thermoregulation in hibernating alpine marmots (*Marmota marmota*). In: Heldmaier G, Klingenspor M (eds) Life in the cold. Eleventh International Hibernation Symposium. Springer, Berlin Heidelberg New York pp 81–94

Sawhney N, Hall PA (1992) Ki67 – structure, function, and new antibodies. J Path 168:161–162

Secor SM, Diamond J (1997) Effects of meal size on postprandial responses in juvenile Burmese pythons (*Python molurus*). Am J Physiol 272:R902–R912

Secor SM, Diamond J (1998) A vertebrate model of extreme physiological regulation. Nature 395:659–662

Self RFL, Jumars PA, Mayer LM (1995) In vitro amino acid and glucose uptake rates across the wall of a surface deposit feeder. J Exp Mar Biol Ecol 192:289–318

Speakman JR, Johnson MS (2000) Relationships between resting metabolic rate and morphology in lactating mice: what tissues are the major contributors to resting metabolism? In: Heldmaier G, Klingenspor M (eds) Life in the cold. Eleventh International Hibernation Symposium. Springer, Berlin Heidelberg New York, pp 479–486

Stevens CE, Hume ID (1995) Comparative physiology of the vertebrate digestive system, 2nd edn. Cambridge University Press, Cambridge

Toole L, Belai A, Shochina M, Burstock G (1999) The effects of hibernation on the myenteric plexus of the golden hamster small and large intestine. Cell Tissue Res 296:479–487

Venables WN, Ripley BD (1999) Modern applied statistics with S-PLUS. Springer, Berlin Heidelberg New York

Vinogradova MS, Shestopalova LV (1996) Seasonal changes in the morphology and function of digestive tract epitheliocytes in the ground squirrel *Spermophilus erythrogenys* inhabiting west Siberia. In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptations to the cold. Tenth International Hibernation Symposium. University of New England Press, Armidale, pp 389–392

Yu CC-W, Woods AL, Levison DA (1992) The assessment of cellular proliferation by immunohistochemistry: a review of currently available methods and their applications. Histochem J 24:121–131