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Maintenance of a fully functional digestive system during hibernation in the European hamster, a food-storing hibernator

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ABSTRACT

Some small mammals limit energy expenditure during winter conditions through torpor bouts, which are characterized by a decrease in body temperature and metabolic rate. Individuals arise periodically from torpor to restore critical functions requiring euthermia. Although most of the species involved do not feed during hibernation and rely on body reserves to fulfil energy requirements (fat-storing species), others hoard food in a burrow (food-storing species) and can feed during interbout euthermy. Whereas fat-storing species undergo a marked atrophy of the digestive tract, food-storing species have to maintain a functional digestive system during hibernation. Our study aimed to evaluate the absorption capacities of a food-storing species, the European hamster, throughout the annual cycle. In vivo intestinal perfusions were conducted in different groups of hamsters ($n = 5$) during the different life periods, namely before hibernation, in torpor, during interbout euthermy, and during summer rest. The triglyceride, non-esterified free fatty acid, starch, glucose and protein composition of the perfusate was evaluated before and after the 1 h perfusion of a closed intestinal loop. Triglyceride, starch and protein hydrolysis rates were similar in hibernating (torpid and euthermic) and non-hibernating hamsters. Intestinal absorption of free fatty acid was also similar in all groups. However, glucose uptake rate was higher during hibernation than during the summer. In contrast with fat-storing species, the intestinal absorption capacities of food-storing species are fully maintained during hibernation to optimize nutrient assimilation during short interbout euthermy. In particular, glucose uptake rate is increased during hibernation to restore glycaemia and ensure glucose-dependent pathways.

1. Introduction

Seasonal decrease in food resources is a challenge for animals living under temperate latitudes. Specific adaptations such as hibernation have remained throughout evolution to enable individuals to limit energy expenditure and cope with winter conditions. Hibernation is a period of hypometabolism associated with decreased metabolic rate and body temperature. Hibernating rodents periodically arise from these hypothermic states, called *torpor bouts*, then return to eumetabolism and euthermia (reviewed in: Heldmaier et al., 2004). The majority of hibernating species do not feed during hibernation and rely solely on body reserves for their energy requirements (fat-storing species), whereas other hibernating species such as hamsters or chipmunks hoard food in a burrow (food-storing species) and feed between torpor bouts (reviewed in: Humphries et al., 2001; Geiser, 2004). In both cases, hibernation can be considered as a fasting state of variable duration [from 4 days in food-storing to several months in fat-storing species (Humphries et al., 2003)].

The absorption of food by the digestive tract depends on the intestinal surface area and the activities of digestive enzymes and intestinal transporters. The mechanisms involved in digestion and absorption when food is abundant have already been investigated in depth (Eckert et al., 1978; Kellett and Helliwell, 2000; Kellett, 2001; Hirsch and Cheeseman, 1998; Stahl et al., 1999, 2001; Bonen et al., 2007; Munck et al., 2000; Verrey et al., 2000). Carbohydrate digestion begins in the mouth, and continues in the small intestine (Eckert et al., 1978). The resulting monosaccharides are mainly glucose, and are absorbed through the apical wall of the enterocytes via energy-dependent or facilitative transporters (Kellett and Helliwell, 2000; Kellett, 2001). Triglycerides are digested by pancreatic lipase to yield monoacylglycerol and non-esterified fatty acids (NEFA), both of which can efficiently diffuse or be transported into the enterocytes (Hirsch and Cheeseman, 1998; Stahl et al., 1999, 2001; Bonen et al., 2007). Finally, proteins are hydrolysed into short peptides and amino acids by pancreatic and intestinal peptidases, respectively (Eckert et al., 1978) before being absorbed

Abbreviations: IBE, interbout euthermy; L/D, light/dark; NEFA, non-esterified fatty acid; TG, triglyceride.

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through ion cotransport by specific transporters (Munck et al., 2000; Verrey et al., 2000).

Many studies have explored morphological changes (Carey, 1989, 1990; Hume et al., 2002; Fleck and Carey, 2005) and food absorption capacities (Carey and Sills, 1992; Carey and Martin, 1996; Carey, 2001; Balslev-Clausen et al., 2003; Carey et al., 2012) in hibernating, fat-storing species. A marked atrophy of the small intestine occurs during hibernation in thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), with a decrease in jejunal villi length and density, and lower mucosal wet weight and protein content (Carey, 1990). In alpine marmots (*Marmota marmota*), a decrease was observed in the fresh tissue mass of all digestive organs (stomach, intestine, caecum and colon) during hibernation (Hume et al., 2002). The atrophy of the digestive organs is a consequence of fasting, and allows individuals to limit protein turnover, which is extremely costly in energy (Young et al., 1983; Macrae and Lobley, 1986). Contrariwise, some genes encoding for intestinal enzymes like sucrase or isomaltase are still expressed continuously throughout hibernation in thirteen-lined ground squirrels (Carey and Martin, 1996), whereas amylase gene expression is reduced by ~40% (Balslev-Clausen et al., 2003). Furthermore, studies performed on isolated ground squirrel intestine reveal a maintenance of glucose and amino acid absorption (at 37 °C) until at least the sixth week of hibernation (Carey and Sills, 1992, 1996). As suggested by Carey and Martin (1996), the maintaining of intestinal absorptive capacities throughout hibernation should enable the immediate absorption of nutrients when the individual emerges in spring.

Contrary to fat-storing species, some food-storing species preserve their intestinal morphology (jejunal villi length) throughout the hibernation period (Weitten et al., 2013). Their digestive enzyme activities also continue, as shown through in vitro measurements of sucrase, isomaltase, lactase and aminopeptidase activities in European hamsters (Galluser et al., 1988) and illustrated by a modelling study demonstrating a positive relationship between digestibility and torpor in chipmunks (Humphries et al., 2001). The maintenance of intestinal structure and function might allow the immediate absorption of food by animals refeeding during euthermic phases (Humphries et al., 2001). Indeed, the interbout euthermic phases are too short [less than 48 h (Fig. 1)] to ensure a complete restoration of small intestine morphology and function. We hypothesised that food-storing species are able to optimize food digestion and absorption during short IBE thanks to the maintenance of a fully functional digestive tract throughout the hibernation period. To test our hypothesis, we evaluated the food digestion and absorption capacities of European hamsters throughout the annual cycle through in vivo intestinal perfusion.

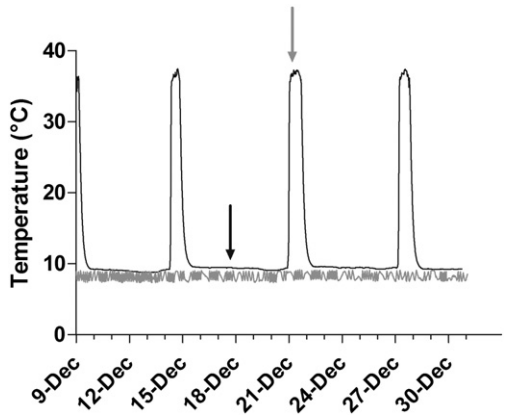

Fig. 1. Core temperature (black line) (°C) of one European hamster in midwinter, recorded over a three-week period. The grey line shows air temperature. The average time at which anaesthesis was carried out is indicated with a black arrow (Torpor group) and a grey arrow (IBE group).

2. Material and methods

The experimental protocol followed the EU Directive 2010/63/UE for animal experiments and the care and use of laboratory animals, and was validated by an Ethical Committee under agreement AL/03/32/12/12.

2.1. Animals

Twenty female European hamsters (*Cricetus cricetus*) were studied, aged 41 weeks and weighing 202.4 ± 6.3 prior to the experiment. All animals were supplied by Chronobiotron (Strasbourg, France). The hamsters were housed individually in standard cages and had ad libitum access to water and food (pellet N° 105 from Safe, Augy, France). Animals were placed in the photoperiodic and thermal conditions described in Table 1 to reproduce the environmental conditions of the annual hamster cycle. Hibernation in this species is controlled by an endogenous circannual clock (Monecke et al., 2015), and occurs from mid-October to mid-March.

2.1.1. Experimental groups

To evaluate the absorption capacity throughout the annual cycle, experimental procedures were conducted on hamsters at four different physiological states. Two of these states occur during the hibernation period, i.e. torpor (in the middle of a torpor bout, i.e. after 48 h of torpor, considering that the average duration of a torpor bout is 4.5 days/108 ± 2.5 h in European hamsters) and interbout euthermy (IBE) (Fig. 1). The other two states occur prior to hibernation (in September, Pre-hib) and one after reproduction (in June, Summer).

Before starting the perfusion experiment, all torpid animals were quickly warmed under an infrared lamp, and the experiment started when rectal temperature exceeded 35 °C.

2.1.2. Intestinal perfusion

The experiments were conducted between 10.00 and 12.00 am. After an overnight fast to ensure that the digestive tract was empty, the animals were anaesthetised (5% isoflurane in 1.2 $L \cdot min^{-1}$ oxygen for induction, followed by an intraperitoneal injection of 200 mg/kg Ketamine–0.1 mL/kg Xylasin), weighed (to the nearest 0.1 g) and placed in the supine position under an oxygen flux (1.2 $L \cdot min^{-1}$) on a warm surgery table (37 °C) to maintain body temperature.

Sublingual blood samples (100 μL) were taken (as describe by Heimann et al., 2009) with a heparinized capillary tube before and after the intestinal perfusion. All the blood samples were conserved on ice in heparinized microtubes until centrifugation (1500g, 15 min), and plasma was conserved at −80 °C until glycaemia measurements were performed.

The abdomen was shaved and a midline laparotomy was performed from below the umbilicus gland to the xyphoïd appendicle. The small intestine was isolated and the luminal content was removed by gently flushing with saline solution at 37 °C. An intestinal loop (from the cardia to the ilio-caecal junction) was cannulated to allow a continuously recirculating perfusion at 37 °C at a flow rate of 2 mL/min with a 30 mL perfusion solution. The perfusion solution was composed of 0.9% NaCl, 10% starch (88% purity, ref: 102955, MP Biomedicals, USA), 8.2% soy proteins (92% purity, ref: 905456, MP Biomedicals, USA), 5% oil (Frial, Lesieur, France, 10% saturated fatty acid, 44% mono-unsaturated acid, 46% polyunsaturated acid), 0.8% AIN-76 mineral mixture (ref: 905455, MP Biomedicals, USA) and 76% H_2O–NaCl 0.9% (in % of mass). After 1 h of perfusion, all remaining perfusate was collected and its volume was measured.

Perfusate samples were taken before and after the perfusion. They were immediately frozen in liquid nitrogen then stored at −80 °C until analysis.

The animals were killed after the absorptive period. The small intestine was excised and the mucosa was scraped free of the underlying tissue and weighed.