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RESEARCH ARTICLE

Acclimation of intestinal morphology and function in Djungarian hamsters (*Phodopus sungorus*) related to seasonal and acute energy balance

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ABSTRACT

Small mammals exhibit seasonal changes in intestinal morphology and function via increased intestine size and resorptive surface and/or nutrient transport capacity to increase energy yield from food during winter. This study investigated whether seasonal or acute acclimation to anticipated or actual energetic challenges in Djungarian hamsters also resulted in higher nutrient resorption capacities owing to changes in small intestine histology and physiology. The hamsters show numerous seasonal energy-saving adjustments in response to short photoperiod. As spontaneous daily torpor represents one of these adjustments related to food quality and quantity, it was hypothesized that the hamsters' variable torpor expression patterns are influenced by their individual nutrient uptake capacity. Hamsters under short photoperiod showed longer small intestines and higher mucosal electrogenic transport capacities for glucose relative to body mass. Similar observations were made in hamsters under long photoperiod and food restriction. However, this acute energetic challenge caused a stronger increase of glucose transport capacity. Apart from that, neither fasting-induced torpor in food-restricted hamsters nor spontaneous daily torpor in short photoperiod-exposed hamsters clearly correlated with mucosal glucose transport capacity. Both seasonally anticipated and acute energetic challenges caused adjustments in the hamsters' small intestine. Short photoperiod appeared to induce an integration of these and other acclimation processes in relation to body mass to achieve a long-term adjustment of energy balance. Food restriction seemed to result in a more flexible, short-term strategy of maximizing energy uptake possibly via mucosal glucose transport and reducing energy consumption via torpor expression as an emergency response.

KEY WORDS: Siberian hamster, Spontaneous daily torpor, Fasting-induced torpor, Mucosal electrogenic transport, Small intestine, Resorptive surface

INTRODUCTION

Temperate zone winters are accompanied by a decline in food resources, which is one of the most important energetic challenges for endothermic mammals as they need a constant energy supply to defend their high body temperature. Consequently, survival under harsh winter conditions demands considerable acclimatization of morphology and physiology to decrease energy expenditure and increase energy yield. Most of the various winter acclimatization processes of small endothermic mammals aim to reduce energy expenditure. This can be achieved by adjustments of body and fat mass, the reduction of reproductive activity or the decrease of heat loss via a better insulating winter fur, as well as by nest building or social huddling (Heldmaier, 1989). Furthermore, hibernation and daily torpor are two forms of controlled and voluntary decrease in metabolism and body temperature as important mechanisms to reduce energy expenditure (Ruf and Geiser, 2015). In contrast, acclimatizations to increase energy yield are less common. On the one hand, preventive food hoarding can increase energy availability at times of scarcity (Vander Wall, 1990), but on the other hand, it can also be beneficial to increase the amount of energy that can be obtained from food. On the level of digestion and resorption, the Andean Altiplano mouse (*Abrothrix andinus*) combines two major mechanisms that increase nutrient uptake from a given amount of food. In preparation for the winter in the Andes, characterized by low ambient temperatures as well as low food quality and quantity, these mice increase the mass and volume of their intestinal tract, resulting in a larger resorptive surface area (Karasov and Diamond, 1983). In addition, the animals show an increased mucosal transport capacity for D-glucose and L-tyrosine (Bozinovic and Iturri, 1991). A seasonality of intestinal tract size could be demonstrated for several other small rodent species, whereby all studies reported an increase in length, mass, volume or surface area with upcoming winter or in response to low ambient temperatures and low food quality (Green and Millar, 1987; Hammond, 1993; Derting and Noakes, 1995; Chi and Wang, 2011). Especially in small homeotherms that neither migrate nor become torpid, these structural and physiological changes in favor of an increased nutrient resorption serve as an essential mechanism to compensate for the decreased energy availability during times of increased energy expenditure (Bozinovic and Iturri, 1991). Many of these species also use daily torpor in response to energetic challenges (Eto et al., 2014; Tannenbaum and Pivorun, 1988; Chi et al., 2016). However, although winter acclimatization always comprises multiple, complex and interacting adjustments (Heldmaier and Lynch, 1986), it has never been reported whether and how mechanisms to decrease energy expenditure (such as torpor) work together with mechanisms to increase energy yield (nutrient resorption). Until now, information on this important relationship was available for hibernators only, which differ in many characteristics from animals expressing daily torpor.

Hibernators such as thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), European hamsters (*Cricetus cricetus*) and alpine marmots (*Marmota marmota*) show a reduction of intestinal tissue during their hibernation season (Carey, 1990, 1992; Carey and Sills,

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1992; Galluser et al., 1988; Hume et al., 2002). However, hibernation is often characterized by the complete cessation of food intake and processing, while the animals obtain energy from internal fat stores (Kenagy, 1989). During these periods of extreme fasting, the hibernators can save additional energy by reducing the intestinal tract as a metabolically expensive organ (Stevens and Hume, 1995). Interestingly, the mucosal transport mechanisms at euthermic body temperatures remain intact (Carey, 1990, 1992; Carey and Sills, 1992). But still, the absence of intestinal contents during fasting is considered to be the main reason for the observed multifaceted mucosal atrophies, as it has been shown for many non-hibernating species (Debnam and Levin, 1975; Gleeson et al., 1972; Hughes and Dowling, 1980; Karasov and Diamond, 1983; Kotler et al., 1981).

Many smaller endotherms such as the Djungarian hamster [*Phodopus sungorus* (Pallas 1773)] use a different repertoire of seasonal adjustments (Heldmaier, 1989). The hamsters reduce their body size when exposed to a winter-like short photoperiod to decrease their overall energy demand (Ruf and Heldmaier, 1992). However, they need to forage for food during their activity phase and use spontaneous daily torpor to save energy during their daily resting phase (Ruf and Heldmaier, 2000). Although low ambient temperatures or reduced food availability have been shown to facilitate torpor expression (Ruf et al., 1993), under short photoperiod, spontaneous daily torpor also occurs without acute energetic challenges (Heldmaier and Steinlechner, 1981). Given the diverse and individual acclimatization strategies that partly depend on energy availability (Scherbarth and Steinlechner, 2010), it appears necessary to examine the potential acclimation of the animals' intestinal structure and function as important factors of food processing and energy assimilation capacity. So far, only one study has investigated the Djungarian hamster's small intestine morphology during post-natal development (Wołczuk and Kobak, 2013). The present study further examined the hamsters' small intestinal morphology and function as part of seasonal as well as acute acclimation processes.

Focus is set on investigating the effect of short photoperiod acclimation on intestinal size and mucosal electrogenic nutrient transport capacity as well as a potential relationship between the incidence of spontaneous daily torpor and resorption capacity. As the hamsters face a considerable energetic challenge during the harsh winters in their Central Asian habitat, long-term acclimation to a winter-like short photoperiod was expected to result in a decrease of overall small intestine size. In contrast, small intestine nutrient resorption capacity, i.e. mucosal surface size and transport efficiency, should be increased to optimize energy yield from the available food whereby an individually lower uptake capacity would be compensated by an increased spontaneous daily torpor incidence.

As a reduced amount of food can be considered as one major part of the energetic challenge during winter, intestinal morphology and function was also measured in long photoperiod-exposed hamsters under moderate long-term food restriction, which causes another form of daily torpor, namely fasting-induced torpor (reviewed in Diedrich and Steinlechner, 2012). The acute energetic challenge due to reduced food availability was considered to increase mucosal transport capacity to maximize energy yield from reduced food, which again should negatively correlate with individual fasting-induced torpor incidence. Finally, the question was raised as to whether the applied food restriction regime would lead to changes in mucosal morphology and whether hamsters with an individually stronger reduction of resorptive surface would compensate for the lower energy assimilation capacity with a higher expression of fasting-induced torpor.

MATERIALS AND METHODS

Animals

Djungarian hamsters of experiment 1 originated from the laboratory breed in the Institute of Zoology of the University of Veterinary Medicine in Hannover, Germany (~52°N latitude). They were born and raised under natural photoperiod, ambient temperature and humidity conditions. Since weaning, the animals were kept individually in Makrolon Type II cages (22.0×16.5×14.0 cm) and received food (Altromin hamster breeding diet 7014, Lage, Germany) and tap water *ad libitum* (AL) unless otherwise stated. They were additionally supplemented with apple and approximately 5 g of sunflower seeds with oat flakes once a week. Nutritional changes during the experiments are described below. Animal husbandry and all experiments performed at the University of Veterinary Medicine in Hannover were in accordance with the German Animal Protection Law and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (11/0372).

For experiment 2, Djungarian hamsters were taken from the laboratory breed of the Institute of Neurobiology at Ulm University, Germany (~48°N latitude). Here, the animals were born and raised under an artificial long photoperiod with 16 h:8 h light:dark (16:8 LD) per day, a constant ambient temperature of 19 ±1°C and a relative humidity of 45±15%. All hamsters were kept individually in Makrolon Type III cages (26.5×42.5×18.0 cm) and received food (Altromin hamster breeding diet 7014, Lage, Germany) and tap water *ad libitum* unless otherwise stated. Supplementary feeding comprised oat flakes and cucumber once per week. Animal husbandry and all experiments performed at Ulm University were in accordance with the German Animal Protection Law and approved by the Regierungspräsidium Tübingen, Germany (1411; 1432).

Experiment 1: Effect of short photoperiod or food restriction on mucosal electrogenic transport

Experimental setup

Acclimation to short photoperiod

For experiment 1, a total of 48 female Djungarian hamsters with an age of 9±2 months were used. Thirty-six of these hamsters were transferred to artificial long photoperiod (16:8 LD), a constant ambient temperature of 19±1°C and a relative humidity of 40±10%. Animals received their *ad libitum* pellet food and water while the supplementary feeding was suspended. For the following 20 days under the new environmental and feeding conditions, body mass development and daily food intake were measured. Twelve animals stayed in artificial long photoperiod conditions (LP-AL-1), and 24 animals were transferred to artificial short photoperiod conditions (8:16 LD; SP-AL-1) to induce short photoperiod acclimation and spontaneous daily torpor expression.

Before the beginning of the experiment, all hamsters of group SP-AL-1 were implanted intraperitoneally with temperature-sensitive RFID transponders (volume 0.035 cc, mass 0.1 g) (IPTT-300, Bio Medic Data Systems, Seaford, DE, USA) under isoflurane anesthesia (®CP-Pharma, Burgdorf, Germany). These transponders allowed for the acute measurement of core body temperature from outside the cages with a hand-held reader (DAS-7009, Bio Medic Data Systems) and thus the assessment of spontaneous daily torpor.

For the next 13 weeks, the hamsters' body mass, food intake and fur index were measured in regular intervals. The fur index is a measure of the degree of winter molt in short photoperiod-exposed Djungarian hamsters, and ranges from 1 for a complete grayish-brown summer fur to 6 for a complete white winter fur (Figala et al., 1973). Three

hamsters had to be removed from the study as they showed no signs of short photoperiod acclimation such as body mass reduction or molt.

From week 6 on, all short photoperiod-exposed hamsters were checked for spontaneous daily torpor expression once per day until the end of the experiment after week 13. Four hours after the beginning of the light phase, core body temperature was measured and all hamsters with a body temperature below 32°C for at least 30 min were considered to be torpid (Ruf et al., 1989, 1991, 1993). A further six hamsters were excluded from the analysis as they did not express spontaneous daily torpor.

In week 14, all hamsters were killed via CO_2 inhalation within the first hours of their resting phase.

Acclimation to food restriction

In addition, 12 hamsters remained under artificial long photoperiod and were subjected to a special food restriction paradigm to induce fasting-induced torpor expression (LP-FR-1). Prior to the beginning of food restriction, all hamster cages were equipped with a small wooden nest box (7.0×5.0×5.0 cm inner size) for the continuous measurement of surface body temperatures (T_s) while the hamsters rested in the nest box, thereby allowing torpor assessment. A small infrared thermometer (MLX90614ESF-BAA; Melexis Microelectronic Systems, Yeper, Belgium) with a coverage angle of 90 deg was fixed to a hole in the middle of the top (distance of ∼2.5 cm between sensor and the hamsters' back) of each nest box and was connected to a microcontroller board (Leonardo, Arduino). Body temperature values with a resolution and accuracy of 0.02°C and 0.5°C, respectively, were stored every minute on a personal computer. The visual inspection of the resulting T_s curves allowed for a reliable detection of torpor bouts (Diedrich et al., 2014).

During the next 2 weeks, body mass and *ad libitum* daily food intake were determined, while the hamsters could get used to the new housing conditions. All hamsters immediately accepted the nest box and used it for their resting phases and, consequently, fasting-induced torpor. For the following 5 weeks, the hamsters' body mass was determined on a weekly basis. One hamster had to be removed from the study as it did not show fasting-induced torpor.

During week 1 of the food restriction, all hamsters were food-restricted by 70% of their individual *ad libitum* daily food intake. For a further 4 weeks, food restriction was lowered to 40% of the hamsters' initial daily food intake. Additional feeding (+10%) was provided when an animal had lost more than 25% of its initial body mass. Food rations were offered daily directly in the cages, 9 h before the beginning of the dark phase. This time point was chosen because it is known from a previous study on fasting-induced torpor that the torpor bouts usually started 15–16 h after feeding (Diedrich et al., 2015). With the feeding schedule of the present study, it was intended to evoke fasting-induced torpor with the beginning of the following light and thus resting phase to synchronize the time point of torpor expression with that of spontaneous daily torpor in the hamster under short photoperiod.

At the end of week 5, all hamsters were killed via CO_2 inhalation in the first hours of their resting phase.

Sampling

The hamsters of all three treatment groups LP-AL-1 (n=12), LP-FR-1 (n=11) and SP-AL-1 (n=15) were subjected to the same experimental and analytical protocols described below. One hamster of LP-FR-1 and six hamsters of SP-AL-1 were killed but not used for the further experiment as they had not expressed torpor.

The small intestine was removed from the peritoneal cavity, cleared from mesenterial tissue, and measured in length to the nearest 0.1 cm. For electrophysiological measurements, four small intestine samples with a length of 2 cm each were taken from distal to proximal, beginning at the ileocecal valve. Until mounting, the tissue was kept in serosal buffer solution at 4°C, and gassed with carbogen (95% O_2, 5% CO_2; for detailed composition, see 'Measurement of mucosal electrogenic transport', below). Each sample was sliced along the mesappendix and rinsed with ice-cold physiological saline, and the mucosa was stripped from the muscle layers.

Measurement of mucosal electrogenic transport

The mucosal tissue was mounted into an Ussing chamber with an aperture of 0.3 cm^2. On the mucosal side, the Ussing chamber contained 10 ml of buffer with the following composition (mmol l^{-1}): NaCl 113.6, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, Na_2HPO_4 1.5, $NaHCO_3$ 21.0, mannitol 2.0 and HEPES 20.0. The buffer on the serosal side (10 ml) consisted of (mmol l^{-1}): NaCl 113.6, KCl 5.4, $CaCl_2$ 1.2, $MgCl_2$ 1.2, Na_2HPO_4 1.5, $NaHCO_3$ 21.0, glucose 10.0, mannitol 2.0, HEPES 7.0 and Na-gluconate 6.0. Both buffer solutions had an osmolarity of 300 mosmol l^{-1}. Buffers were adjusted to a pH of 7.4 and were gassed continuously with carbogen at 37°C. Indomethacin (10 µmol l^{-1}) was added to both buffer solutions to prevent endogenous prostaglandin production in the tissue.

All measurements were performed under short-circuit current conditions using a computer-controlled voltage clamp (Mussler Scientific Instruments, Aachen, Germany). Short-circuit currents (I_{sc}, µE cm^{-2} h^{-1}) and transepithelial tissue conductance (G_t, mS cm^{-2}) were determined as previously described and continuously stored on a computer in 6-s intervals (Leonhard-Marek et al., 2009).

After an equilibration time of 30 min, 5 mmol l^{-1} of alanine, 5 mmol l^{-1} of glucose and 10 mmol l^{-1} glucose were added on the mucosal side in intervals of 15 min each. Both alanine as representative amino acid and glucose were used to induce mucosal sodium-coupled electrogenic transport mechanisms and thus a corresponding increase in I_{sc}. In addition, 0.01 mmol l^{-1} forskolin (Sigma Aldrich, Taufkirchen, Germany) was added on the serosal side of the system to evoke a maximum increase in I_{sc} via cAMP-induced Cl^- secretion on the mucosal side. This secretory response was used to evaluate the viability of the mounted mucosal tissue at the end of each experiment (Brown et al., 1990). Finally, administration of 0.5 mmol l^{-1} 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB; Sigma Aldrich) inhibited the secretory response to forskolin, thus decreasing I_{sc} back to base line values (example measurement in Fig. 1).

Experiment 2: Effect of short photoperiod or food restriction on mucosal histology

Experimental setup

Acclimation to food restriction

For experiment 2, a total of 30 Djungarian hamsters with an age of 10±5 months were used under artificial long photoperiod (16:8 LD), a constant ambient temperature of 19±1°C and a relative humidity of 40±10%. For 14 days, body mass development and daily food intake were measured, whereby supplementary feeding was suspended. Ten animals were kept under long photoperiod and *ad libitum* feeding (LP-AL-2), while another 10 animals under long photoperiod were subjected to the food restriction paradigm described in Experiment 1 in order to evoke fasting-induced torpor (LP-FR-2). For torpor assessment, the animals of group LP-FR-2 were implanted intraperitoneally with temperature-sensitive radio frequency transmitters. They have a volume of 1.1 ml, a mass of 1.6 g and

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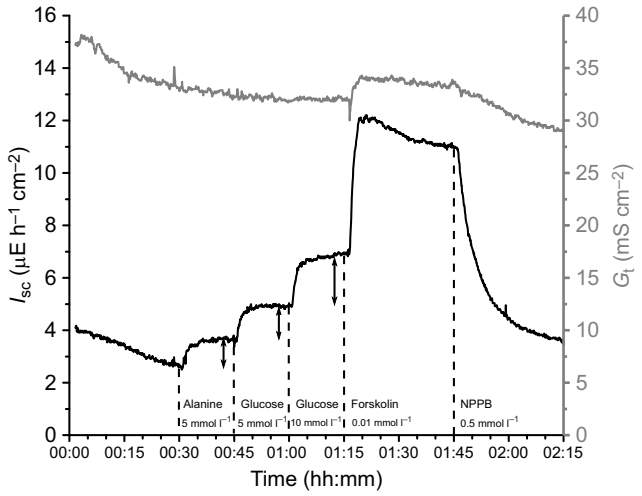

Fig. 1. Example of electrogenic transport measurement in experiment 1. Short-circuit current (I_{sc}) over the small intestine mucosa of a short photoperiod-acclimated Djungarian hamster after administration of 5 mmol l^{-1} alanine, 5 mmol l^{-1} glucose, 10 mmol l^{-1} glucose, 0.01 mmol l^{-1} forskolin and 0.5 mmol l^{-1} NPPB (marked by dashed lines). The parameter of interest is the difference between a stable baseline before the substance administration and a stable measurement after the administration (marked by arrows). The transmucosal tissue conductance (G_t) was measured in parallel as a measure of tissue integrity.

measure at an accuracy of 0.15°C. Coating in medical silicone as well as two-point calibration coefficients were provided by the manufacturer (TA11TA-F10, Data Science International, St Paul, MN, USA). Implantation was performed under isoflurane anesthesia (Forene® Abbvie, Ludwigshafen, Germany). The transmitters allowed the continuous measurement of core body temperature via a temperature-dependent radio frequency signal to receiver plates underneath the hamster cages. The mean values of the converted signal were stored every 3 min on a personal computer. Torpor was defined as a reduction of core body temperature below 32°C for more than 30 min.

During the five experimental weeks, the continuous temperature readings were used to determine the incidence of fasting-induced torpor expression for each hamster of group LP-FR-2. Body mass and food intake of group LP-AL-2 and LP-FR-2 were determined regularly. One hamster was excluded from group LP-FR-2 because it did not express torpor. At the end of week 5, the remaining animals of both groups were killed via CO_2 inhalation in the first hours of their resting phase.

Acclimation to short photoperiod

In addition, the remaining 10 hamsters were transferred to artificial short photoperiod (8:16 LD; SP-AD-2) to induce short photoperiod acclimation and spontaneous daily torpor expression. For the next 10 weeks, the hamsters' body mass and fur index (see Experiment 1) were measured at regular intervals. All hamsters showed clear signs of short photoperiod acclimation and spontaneous daily torpor bouts could be detected visually. For the further assessment of torpor expression, the hamsters were implanted intraperitoneally with the TA11TA-F10 transmitters described above (see Experiment 1). Afterwards, the hamsters were monitored for another 4 to 6 weeks regarding their body mass, fur index and torpor expression.

After approximately 16 weeks of short photoperiod acclimation, the hamsters were killed via CO_2 inhalation within the first hours of their resting phase.

Sampling

The hamsters of all three treatment groups LP-AL-2 (n=10), LP-FR-2 (n=7) and SP-AL-2 (n=10) were subjected to the same experimental and analytical protocols described below. Three hamsters of LP-FR-2 were killed but not used for the further experiment as they had not expressed fasting-induced torpor.

The complete small intestine was removed from the peritoneal cavity, divided into three segments of comparable length (proximal, medial and distal) and rinsed for approximately 5 min with ice-cold physiological saline via a peristaltic pump. The segments were measured in length to the nearest 0.1 cm before they were further divided into approximately 1 cm pieces. One piece of each segment with a defined length was dried for 2 weeks. Afterwards, the dry mass of these pieces was determined to the nearest 0.1 mg and extrapolated to the complete length of the segments. Three further pieces of each segment were fixed in 4% paraformaldehyde at 4°C for 24 h for later histological examinations. The remaining pieces were stored at −80°C for other purposes.

Histological examination of mucosal surface

The fixed small intestine pieces were dehydrated and blocked in paraffin. For the histological examination of the small intestine mucosa, 5 µm cross-sections were produced and stained with Mayer's haemalaun and 0.1% eosin Y solution (Merck, Darmstadt, Germany). In order to calculate the mucosal resorptive surface of the proximal and medial small intestine segments for the three different treatment groups, approximately 17 sections per segment (see Table S1) were analyzed via light microscopy (Leica ICC50W, 40x, 100x, Leica Camera AG, Wetzlar, Germany) and image processing (ImageJ 1.52n, National Institutes of Health, USA). According to Kisielinski and colleagues (2002) as well as Wołczuk and colleagues (2011), the following parameters were measured: segment length (L_S, mm), mucosal circumference at the villi base (C_M, mm), villus height (H_V, µm), distance to neighboring villus (D_V, µm), villus width as mean of tip, middle and base (W_V, µm), villus width at base (W_{VB}, µm) and crypt width (W_C, µm).

From these measures, the following parameters were calculated whereby all formulas were adopted from Wołczuk et al. (2011), except the magnification ratio (MR), which was taken from Kisielinski et al. (2002) as a measure of surface enlargement via the villus architecture in relation to the smooth inner surface of an intestinal segment:

$$A_V = \pi\, W_V H_V, \quad (1)$$

$$d_V = \left(\frac{1000}{(W_{VB} + D_V)}\right)^2, \quad (2)$$

$$S_{SM} = C_M L_S, \quad (3)$$

$$MR = \frac{(W_V H_V) + \left(\frac{W_V}{2} + \frac{W_C}{2}\right)^2 - \left(\frac{W_V}{2}\right)^2}{\left(\frac{W_V}{2} + \frac{W_C}{2}\right)^2}, \quad (4)$$

$$S_R = (C_M L_S) + \left((\pi\, W_V H_V)\left(\frac{1000}{(W_{VB} + D_V)}\right)^2\right)(C_M L_S), \quad (5)$$

where A_V is villus area (µm^2), d_V is villus density (no. mm^{-2}), S_{SM} is smooth mucosal surface (mm^2) and S_R is resorptive surface (mm^2). For reasons of comparability, the resorptive surface was expressed

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per centimeter segment length and additionally normalized to the animals' individual body mass.

Data analysis and statistics

All results are given as means±s.e.m. unless otherwise stated.

For determination of the change in I_{sc}, the difference between the constant basal I_{sc} measurement before and the constant I_{sc} measurement after the respective nutrient administration was calculated (ΔI_{sc}, see Fig. 1, arrows). Single values of each of the four mucosal segments were averaged per animal.

The overall torpor incidence is calculated per individual hamster and given as the relative number of torpor bouts per observation interval.

Data were tested for normal distribution with the Shapiro–Wilk test. Two group comparisons were tested for significance via *t*-test. Three group comparisons were analyzed via one- or two-way ANOVA and a *post hoc* Holm–Šidák test for normally distributed data and via one-way Kruskal–Wallis ANOVA and a *post hoc* Dunn's test for non-normally distributed data. Potential correlations were determined with the Pearson product moment correlation. A *P*-value <0.05 was considered as significant; a *P*-value <0.1 was considered a trend. The statistical analysis was performed with SigmaPlot© 14.0 (SyStat Software, San Jose, CA, USA).

RESULTS

Both experiments followed the same paradigm with the ultimate aim to acclimate Djungarian hamsters either to a forced and acute (LP-FR) or to a voluntary and long-term (SP-AL) decrease in energy uptake in comparison to non-acclimated control hamsters (LP-AL). While experiment 1 was conducted to analyze the respective effects on the animals' small intestine mucosal electrogenic transport capacity, experiment 2 focused on potential adjustments in small intestine morphology, i.e. the size of the resorptive surface.

Experiment 1: Effect of short photoperiod or food restriction on mucosal electrogenic transport

Body mass, food intake and fur index

The hamsters of group LP-AL-1 had a stable average body mass of 35.0±1.3 g. In contrast, food restriction as well as short photoperiod exposure led to a reduction in body mass with final values of 25.6±0.4 g in group LP-FR-1 and 27.5±0.8 g in group SP-AL-1, whereby the body mass reduction proceeded faster under the food restriction regime (Fig. 2A). Both groups LP-FR-1 and SP-AL-1 showed a significantly higher relative body mass reduction compared with group LP-AL-1, but did not differ from each other (Fig. 2B).

Compared with the fur index of the LP-AL-1 control group (median 1.5, 5th quantile 1.0, 95th quantile 1.5), only group SP-AL-1 significantly increased the median fur index to 3.0 (5th quantile 2, 95th quantile 3.5; Kruskal–Wallis one-way ANOVA H_2=30.1, P<0.001; *post hoc* Dunn's test P<0.001). Group LP-FR-1 stayed at a median fur index of 1.5 (5th quantile 1.0, 95th quantile 1.5).

Forced acute as well as voluntary long-term acclimations led to a significantly lower absolute daily food intake of hamsters in groups LP-FR-1 and SP-AL-1 compared with untreated hamsters of group LP-AL-1. However, the forced reduction of food intake by approximately 40% in group LP-FR-1 also resulted in a significantly lower mean daily food intake than the voluntary, short photoperiod-induced reduction of food intake in group SP-AL-1 (Fig. 3A). In contrast, the body mass-related food intake did not differ among the treatment groups (Fig. 3B).

Intestinal size

Comparison of the absolute length of the small intestine revealed no significant differences between the three treatment groups. However, when relating intestinal length to body mass, hamsters of both groups SP-AL-1 and LP-FR-1 showed significantly higher relative small

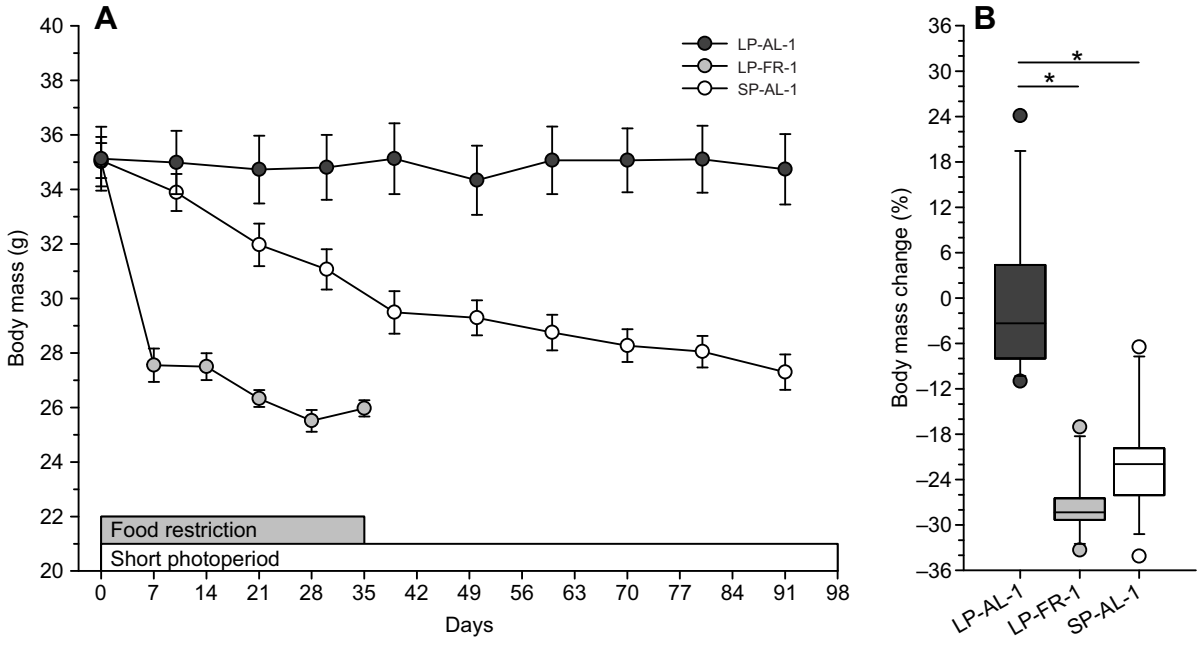

Fig. 2. Body mass development of the three treatment groups of experiment 1. (A) Course of absolute body mass of the three treatment groups (means±s.e.m.). Group LP-AL-1 (n=12) was exposed to a long photoperiod (16:8 LD) under *ad libitum* feeding for 97±3 days. Group LP-FR-1 (n=11) was exposed to a long photoperiod under food restriction for 35 days (gray bar). Group SP-AL-1 (n=15) was exposed to a short photoperiod (8:16 LD) under *ad libitum* feeding for 199±3 days (white bar). (B) Relative body mass change of the three treatment groups at the day of euthanization. Box plots show the median and the 25th and 75th quantiles, while the whiskers represent the 5th and 95th quantiles. Dots mark outliers. Significant differences are marked by asterisks (Kruskal–Wallis one-way ANOVA, H_2=26.7, P<0.001; *post hoc* Dunn's test *P<0.001).

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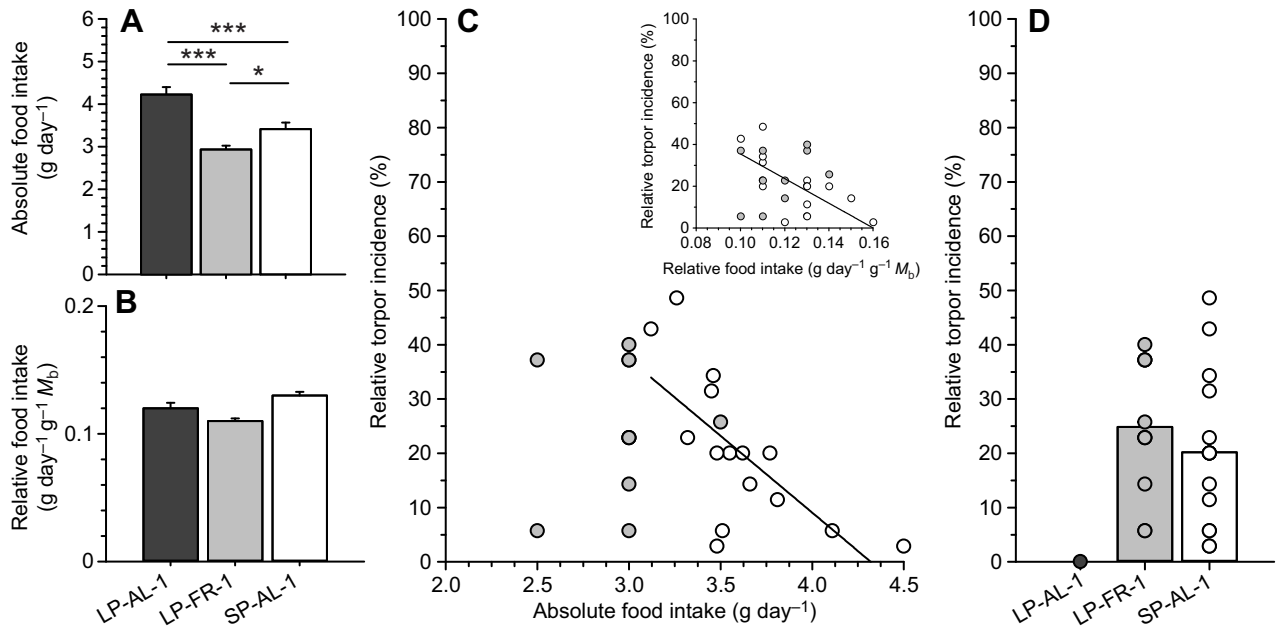

Fig. 3. Daily food intake of the three treatment groups of experiment 1 in relation to torpor expression. Mean (+s.e.m.) (A) daily food intake and (B) body mass-related daily food intake during the last week of the experiment under long photoperiod and *ad libitum* feeding (LP-AL-1, n=12), long photoperiod and food restriction (LP-FR-1, n=11) as well as short photoperiod and *ad libitum* feeding (SP-AL-1, n=15). Asterisks mark significant differences (one-way ANOVA $F_{2,35}$=21.2, P<0.001; *post hoc* Bonferroni test *P<0.05, ***P<0.001). (C) Relationship between food intake and relative incidence of torpor expression; group LP-FR-1 (gray circles) showed fasting-induced torpor and group SP-AL-1 (white circles) showed spontaneous daily torpor. Correlation in group SP-AL-1 was identified via a Pearson product moment correlation (R=−0.8, P<0.001). The inset shows the respective relationship between relative torpor incidence and body mass-related food intake, which was also correlated in group SP-AL-1 (Pearson product moment correlation, R=−0.65, P=0.008). (D) Comparison of the mean relative incidence of torpor expression between the treatment groups. Circles mark the individual torpor incidence for each animal. M_b, body mass.

intestine lengths than hamsters of the control group LP-AL-1, whereby the measurements in group LP-FR-1 were again significantly higher compared with those in group SP-AL-1 (Table 1).

Mucosal electrogenic transport

The measurements of mucosal electrogenic transport were performed under basal conditions, whereby I_{sc} and G_t did not differ between the treatment groups (LP-AL-1: I_{sc}=3.39±0.43 µEq cm^{-2} h^{-1}, G_t=37.45±2.27 mS cm^{-2}; LP-FR-1: I_{sc}=2.83±0.18 µEq cm^{-2} h^{-1}, G_t=39.07±2.41 mS cm^{-2}; SP-AL-1: I_{sc}=4.02±0.38 µEq cm^{-2} h^{-1}, G_t=37.5±2.12 mS cm^{-2}).

Administration of 5 mmol l^{-1} alanine increased the mean I_{sc} in all treatment groups in a comparable manner. After administration of 5 mmol l^{-1} glucose, the increase in I_{sc} over the small intestine mucosa was significantly higher in group LP-FR-1 compared with that of both groups LP-AL-1 and SP-AL-1 (Fig. 4A). An administration of an additional 10 mmol l^{-1} glucose did not further increase the change in I_{sc} in any of the

Table 1. Length and dry mass of the complete small intestine in the three treatment groups of experiments 1 and 2

		Experiment 1		Experiment 2	
		n	Mean±s.e.m.	n	Mean±s.e.m.
Absolute small intestine length (cm)	LP-AL	12	27.6±0.4	10	32.7±0.6[d]
	LP-FR	11	28.3±0.7	7	31.5±0.8
	SP-AL	15	27.1±0.6	10	28.6±1.1[d]
Relative small intestine length (cm g^{-1} M_b)	LP-AL	12	0.8±0.03 [a,b]	10	0.9±0.03[e,f]
	LP-FR	11	1.1±0.02 [a,c]	7	1.2±0.05[e]
	SP-AL	15	1.0±0.02 [b,c]	10	1.1±0.03[f]
Absolute small intestine dry mass (mg)	LP-AL	–	–	10	230.3±10.7[g]
	LP-FR	–	–	7	199.6±11.2
	SP-AL	–	–	10	188.1±16.0[g]
Relative small intestine dry mass (mg g^{-1} M_b)	LP-AL	–	–	10	6.5±0.2
	LP-FR	–	–	7	7.4±0.4
	SP-AL	–	–	10	6.6±0.5

The relative small intestine length and the relative small intestine dry mass are normalized per gram body mass (M_b). The small intestine dry mass has been determined from a small piece of tissue of each proximal, medial and distal small intestine segment (1±0.1 cm), extrapolated to the complete length or the respective segment, and summed for the complete length of the small intestine. Dry masses could not be determined in experiment 1.
All comparisons per parameter and within experiment only, same letters indicate difference between respective groups.
One-way ANOVA $F_{2,35}$=30.1, P<0.001, *post hoc* Bonferroni comparison with [a,b]P<0.001 and [c]P=0.042.
One-way ANOVA $F_{2,24}$=5.7, P=0.009, *post hoc* Bonferroni comparison with [d]P=0.008.
One-way ANOVA $F_{2,24}$=7.5, P=0.003, *post hoc* Bonferroni comparison with [e]P=0.003 and [f]P=0.03.
Kruskal–Wallis one-way ANOVA H_2=8.2, P=0.017, *post hoc* Dunn's comparison with [g]P=0.019.

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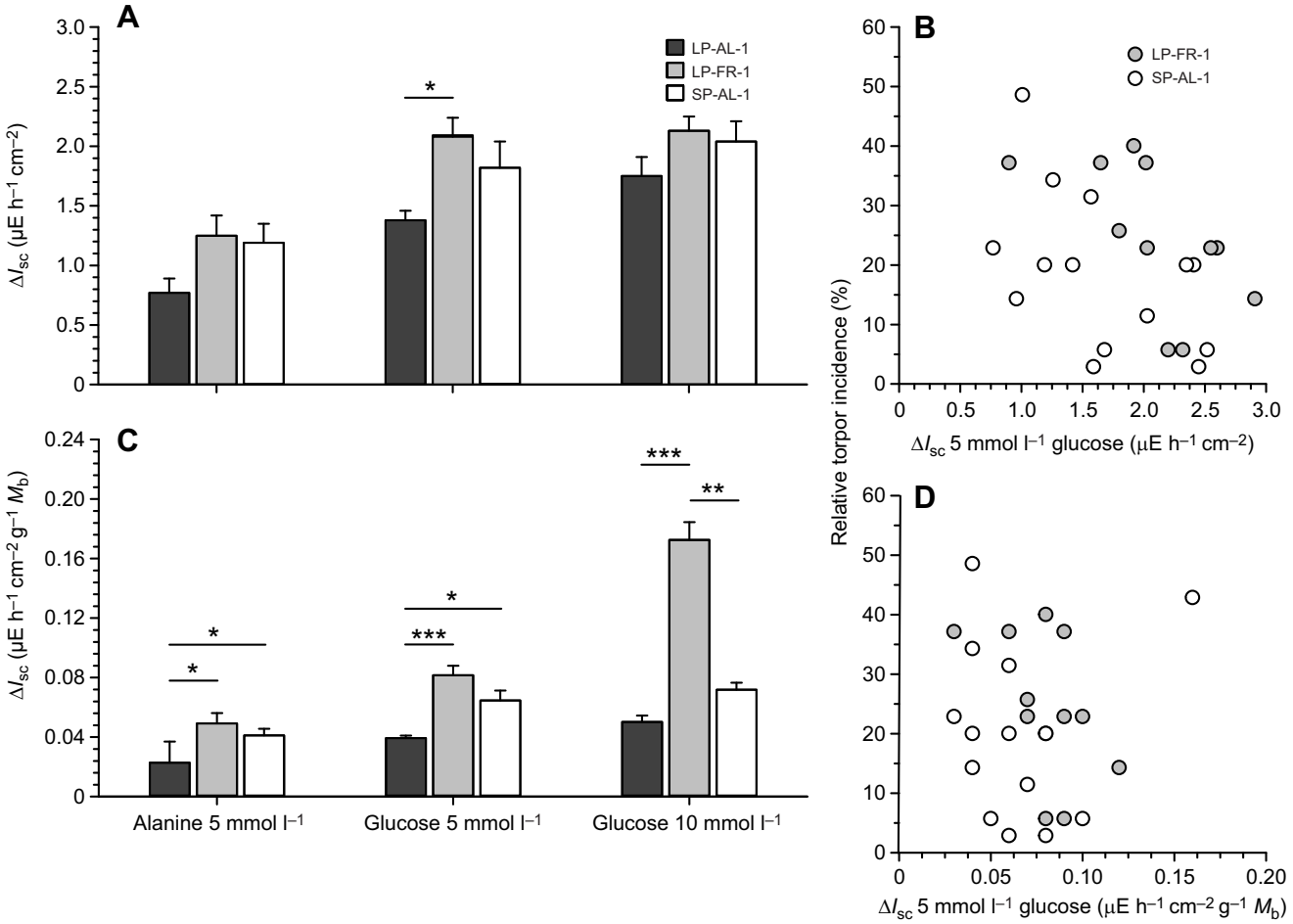

Fig. 4. Changes in short-circuit current (ΔI_{sc}) over the hamster small intestine mucosa after nutrient administration in the three treatment groups of experiment 1. Mean (+s.e.m.) change in (A) short-circuit current I_{sc} and (C) I_{sc} related to individual body mass (M_b) induced by the administration of 5 mmol l^{-1} alanine, 5 mmol l^{-1} glucose and 10 mmol l^{-1} glucose in 15-min intervals. Comparison between the treatment groups under long photoperiod and *ad libitum* feeding (LP-AL-1, n=12), long photoperiod and food restriction (LP-FR-1, n=11) as well as short photoperiod and *ad libitum* feeding (SP-AL-1, n=15). Significant differences are marked by asterisks. For A: Kruskal–Wallis one-way ANOVA H_2=8.0, P<0.018; *post hoc* Dunn's test *P=0.014. For C, alanine 5 mmol l^{-1}: Kruskal–Wallis one-way ANOVA H_2=10.3, P=0.006; *post hoc* Dunn's test LP-AL-1 versus LP-FR-1 *P=0.011 and LP-AL-1 versus SP-AL-1 *P=0.029; glucose 5 mmol l^{-1}: Kruskal–Wallis one-way ANOVA H_2=15.2, P<0.001; *post hoc* Dunn's test LP-AL-1 versus LP-FR-1 ***P<0.001 and LP-AL-1 versus SP-AL-1 *P=0.036; glucose 10 mmol l^{-1}: Kruskal–Wallis one-way ANOVA H_2=27.5, P<0.001; *post hoc* Dunn's test LP-AL-1 versus LP-FR-1 ***P<0.001 and LP-FR-1 versus SP-AL-1 **P=0.002. Relationship between (B) absolute and (D) relative short-circuit current change after 5 mmol l^{-1} glucose administration and expression of fasting-induced torpor in LP-FR-1 animals as well as spontaneous daily torpor in SP-AL-1 animals. A trend towards a correlation in group LP-FR-1 (B) was identified via a Pearson product moment correlation (R=−0.56, P=0.061).

treatment groups. When expressing the short-circuit current changes in relation to the hamsters' body mass, groups LP-FR-1 and SP-AL-1 showed significantly higher changes compared with group LP-AL-1 after both 5 mmol l^{-1} alanine and glucose administration (Fig. 4C). The higher dose of 10 mmol l^{-1} glucose resulted in a significantly higher I_{sc} change in group LP-FR-1 only (Fig. 4C).

The analysis of the respective G_t revealed no significant differences, neither among the treatment groups nor among the administered nutrients (data not shown).

Torpor expression

In response to short photoperiod exposure, 15 out of 21 hamsters in group SP-AL-1 expressed spontaneous daily torpor. The torpor season started after 8±2 weeks in short photoperiod (mean±s.d.). In group LP-FR-1, 11 out of 12 hamsters showed fasting-induced torpor after 2±1 weeks of food restriction and thus during the 40% restriction period. For further analyses, only torpor-expressing hamsters were used. The comparison of the overall torpor incidence revealed no significant difference between the two torpor-expressing groups during the respective interval of observation (Fig. 3C).

In group SP-AL-1, the overall relative incidence of spontaneous daily torpor expression was correlated with the short photoperiod-induced body mass changes, whereby hamsters with a high relative body mass reduction showed a high torpor incidence (Table 2). Furthermore, torpor incidence negatively correlated with the hamsters' mean absolute and body mass-related daily food intake during the last week of the experiment, i.e. the hamsters with the highest food intake showed the lowest torpor expression (Fig. 3B).

Neither spontaneous daily torpor incidence in group SP-AL-1 nor fasting-induced torpor incidence in group LP-FR-1 showed any significant correlation with the change in absolute or body mass-related I_{sc} after administration of alanine or glucose (Fig. 4B,D); however, a trend was found towards a negative correlation between fasting-induced torpor incidence and 5 mmol l^{-1} glucose-induced change in absolute I_{sc} (Fig. 4B).

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Table 2. Summary of correlations between relative torpor incidence (%) and morphological as well as physiological parameters of experiments 1 and 2

	Spontaneous daily torpor		Fasting-induced torpor	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Final body mass (g)	R=–0.50, P=0.057	R=–0.65, P=0.040	R=–0.54, P=0.069	
Body mass change (%)	R=0.60, P=0.012			
Absolute food intake (g day^{-1})	R=–0.78, P<0.001	–		–
Relative food intake (g day^{-1} g^{-1} M_b)	R=–0.66, P=0.008	–		–
Absolute small intestine length (cm)		R=–0.78, P=0.007		
Absolute small intestine mass (mg)	–	R=–0.62, P=0.058	–	
ΔI_{sc} after 5 mmol l^{-1} glucose (µE h^{-1} cm^{-2})		–	R=–0.56, P=0.061	–
Relative medial resorptive surface (mm^2 cm^{-1} g^{-1} M_b)	–	R=0.75, P=0.012	–	

The normally distributed data were analyzed via Pearson product moment correlation. Experiment 1 includes n=15 hamsters that expressed spontaneous daily torpor under short photoperiod and food *ad libitum* (SP-AL-1) and n=11 hamsters that expressed fasting-induced torpor under long photoperiod and food restriction (LP-FR-1). In experiment 2, n=10 hamsters expressed spontaneous daily torpor while n=7 hamsters expressed fasting-induced torpor. Empty cells indicate no correlation, dashes indicate that the respective data have not been measured.

Experiment 2: Effect of short photoperiod or food restriction on mucosal histology

Body mass and fur index

In comparison to group LP-AL-2, both food restriction and short photoperiod acclimation led to significant body mass reduction in groups LP-FR-2 and SP-AL-2, respectively (Fig. 5A). Neither of the two groups under long photoperiod differed in their median fur index (LP-AL-2: 1.0, 5th quantile 1.0, 95th quantile 1.5; LP-FR-2: 1.0, 5th quantile 1.0, 95th quantile 1.5), while group SP-AL-2 showed a significantly higher median fur index of 3.0 (5th quantile 2.0, 95th quantile 4.0; Kruskal–Wallis one-way ANOVA H_2=21.5, P<0.001; *post hoc* Dunn's test P<0.01 against both LP groups).

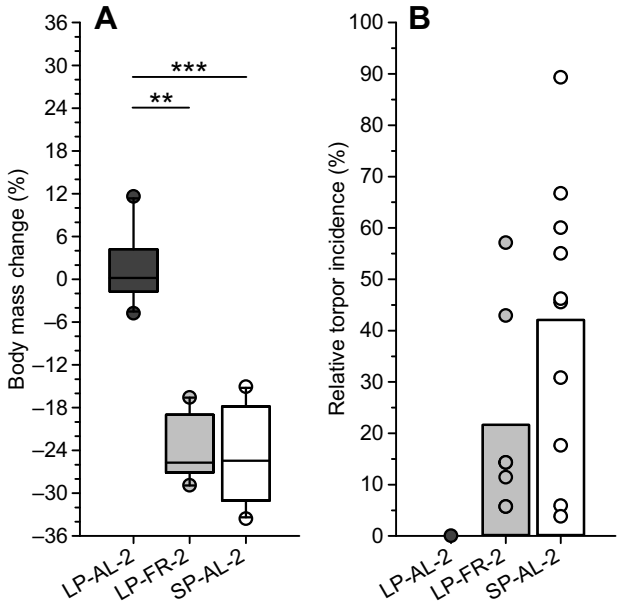

Fig. 5. Body mass change and torpor expression in the three treatment groups of experiment 2. Group LP-AL-2 (n=10) was exposed to a long photoperiod (16:8 LD) under *ad libitum* feeding for 38±1 days. Group LP-FR-2 (n=7) was exposed to a long photoperiod under food restriction for 36±1 days. Group SP-AL-2 (n=10) was exposed to a short photoperiod (8:16 LD) under *ad libitum* feeding for 115±8 days. (A) Median relative body mass change in the three treatment groups at the day of euthanization. Boxes further comprise the 25th and 75th quantiles, whiskers mark the 5th and 95th quantiles, while dots represent outliers. Significant differences are marked by asterisks (Kruskal–Wallis one-way ANOVA H_2=18.3, P<0.001; *post hoc* Dunn's test **P<0.01 and ***P<0.001) (B) Comparison of the mean incidence of fasting-induced and spontaneous daily torpor expression between the treatment groups LP-FR-2 and SP-AL-2, respectively. Hamsters of group LP-AL-2 did not show torpor at all. Circles mark the individual torpor incidence for each animal.

Intestinal size

Compared with group LP-AL-2, short photoperiod-exposed hamsters of group SP-AL-2 had a significantly shorter small intestine, while food-restricted hamsters of group LP-FR-2 did not differ from both other groups (Table 1). In contrast, both group LP-FR-2 and SP-AL-2 showed significantly longer small intestines than group LP-AL-2, when related to body mass, while not differing among each other (Table 1). In experiment 2, absolute small intestinal dry mass was additionally determined, which was significantly higher in group LP-AL-2 compared to group SP-AL-2 (Table 1). Food restriction of group LP-FR-2 did not lead to significant changes in intestinal dry mass. When related to body mass, small intestinal dry mass no longer differed between any of the treatment groups (Table 1).

Mucosal resorptive surface area

When comparing the proximal and medial part of the small intestine within the treatment groups, the magnification ratio (the amount of surface enlargement via the villus architecture in relation to the smooth inner surface of an intestinal segment) was significantly larger in the proximal than in the medial intestinal part of all three groups (Fig. 6A).

In the proximal part of the small intestine, short photoperiod exposure (SP-AL-2) but not food restriction (LP-FR-2) resulted in a significantly lower magnification ratio compared with long photoperiod exposure and *ad libitum* food supply (Fig. 6A). This difference disappeared in the medial part, but here, group LP-FR-2 exhibited a significantly lower magnification ratio than group LP-AL-2 (Fig. 6A).

The resorptive surface area per centimeter of the proximal segment differed significantly among all three groups, whereby group LP-AL-2 showed the largest, and SP-AL-2 the smallest, area (Fig. 6B). For the medial part, the surface difference persisted between group LP-AL-2 and LP-FR-2 only (Fig. 6B).

With the relationship of the small intestinal resorptive surface area to the hamsters' body mass, all described differences, but significantly lower values in group SP-AL-2 compared with LP-FR-2 were eliminated (Fig. 6C).

Torpor expression

The incidence of spontaneous daily torpor expression of group SP-AL-2 under short photoperiod exposure did not differ

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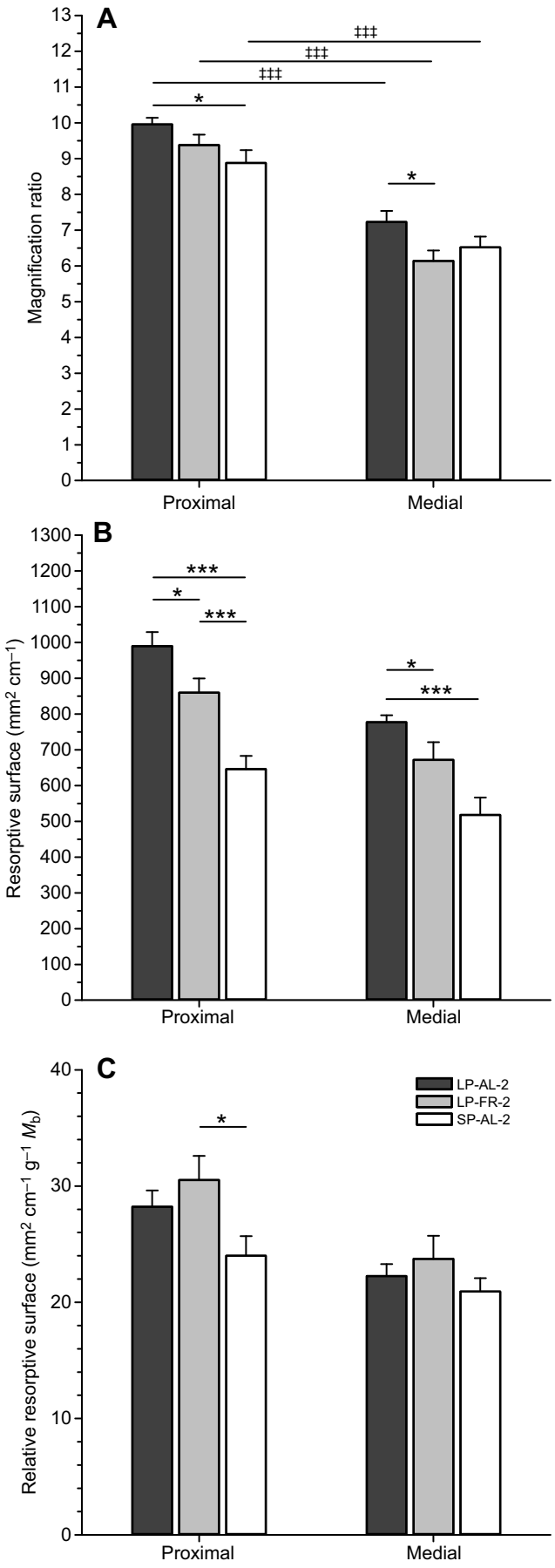

Fig. 6. Small intestine measures in the three treatment groups of experiment 2. (A) Mean magnification ratio (+s.e.m.) of the proximal and medial segment of the small intestine in animals under long photoperiod and *ad libitum* feeding (LP-AL-2, n=10), long photoperiod and food restriction (LP-FR-2, n=7) as well as short photoperiod and *ad libitum* feeding (SP-AL-2, n=10). Significant differences were identified with two-way ANOVA (group $F_{2,48}$=6.1, P=0.004; segment $F_{1,48}$=100.9, P<0.001; interaction $F_{2,48}$=1.0, P=0.370) and *post hoc* Holm–Šidák test with ‡‡‡P<0.001 for significant differences between intestinal parts within one group as well as *P<0.05 for significant differences between groups per segment. (B) Comparison of resorptive surface per centimeter of the proximal and medial segment of the small intestine between the three treatment groups (two-way ANOVA: group $F_{2,48}$=38.2, P<0.001; segment $F_{1,48}$=33.3, P<0.001; interaction $F_{2,48}$=2.3, P=0.109; *post hoc* Holm–Šidák test with *P<0.05 and ***P<0.001). (C) Comparison of resorptive surface per centimeter normalized to individual body mass (two-way ANOVA: group $F_{2,48}$=4.5, P=0.016; segment $F_{1,48}$=17.4, P<0.001; interaction $F_{2,48}$=0.8, P=0.444; *post hoc* Holm–Šidák test with *P<0.05).

significantly from that of fasting-induced torpor of group LP-FR-2 under food restriction (Fig. 5B).

In group SP-AL-2, but not in group LP-FR-2, torpor incidence was negatively correlated with the absolute small intestine length and dry mass; in other words, short photoperiod-acclimated hamsters with an overall smaller small intestine expressed more spontaneous daily torpor (Table 2).

In addition, analyses revealed a positive correlation between the body mass-specific resorptive surface area of the medial part of the small intestine mucosa and the expression of spontaneous daily torpor in group SP-AL-2 (Table 2).

DISCUSSION

The present study investigated potential changes in small intestine morphology and physiology of Djungarian hamsters as a short-term adjustment to food restriction-related energy shortage and as a long-term seasonal acclimation in anticipation of a winter-related energy shortage. This approach should clarify whether the hamsters' small intestine showed signs of short photoperiod acclimation in favor of energy assimilation and whether these changes might be related to individual spontaneous daily torpor incidence. Furthermore, it should be examined whether an improvement of energy assimilation from food can also be found in food-restricted hamsters under long photoperiod that express fasting-induced torpor.

Seasonal acclimation of small intestine in response to short photoperiod

Hamsters in short photoperiod conditions showed the expected voluntarily reduced food intake and, consequently, body mass (Knopper and Boily, 2000), as well as the winter molt (Scherbarth and Steinlechner, 2010). With these and other changes, the hamsters reached an energy-adjusted winter set point (Steinlechner et al., 1983), visible via a body mass-related food intake comparable to that of hamsters under long photoperiod.

In contrast, seasonal acclimation of the hamsters' small intestine revealed a more inconsistent picture, which might relate to subtle differences in diet, housing or photoperiodic history, as the animals originated from different breeding colonies. However, both experiments have in common that the body mass-specific small intestine length of short photoperiod-acclimated hamsters was significantly higher compared with hamsters under long photoperiod. Thus, a smaller body could be sustained by a relatively longer small intestine. According to Clemens and Stevens (1980), the intestinal length of mammals positively correlates with their meal retention time and should lead to a higher nutrient uptake capacity

(Hammond and Wunder, 1995). Thus, the relative increase in the present study might contribute to a more efficient nutrient uptake during short photoperiod acclimation (Karasov and Diamond, 1988; Silby et al., 1990). However, this rather indirect mechanism to increase energy yield is considered to be a side effect of the acclimation process, with less importance for the hamsters when compared with other rodent species. The closely related *Phodopus roborowski* (Chi and Wang, 2011) or representatives of the genera *Apodemus* (Eto et al., 2016; Zhu et al., 2011), *Peromyscus* and *Microtus* show higher absolute intestinal lengths in winter than in summer (Derting and Noakes, 1995; Green and Millar, 1987; Hammond, 1993). Additionally, these taxa had a higher absolute small intestine mass under winter conditions, which is a more reliable estimate for changes in nutrient resorption capacity (Hammond and Wunder, 1995). Although the present study demonstrated the opposite effect for the absolute dry mass of the small intestine, this difference disappeared when related to the hamsters' body mass. In accordance with the reduced absolute dry mass, the small intestine of hamsters under short photoperiod showed a lower mucosal magnification ratio and absolute mucosal surface area. These differences mainly resulted from a decrease in villus height, villus area and smooth mucosal surface, but not from villus width and density (see Table S1). Again, the body mass-specific mucosa surface of hamsters under short photoperiod was comparable to that of hamsters under long photoperiod. The present study also investigated the physiological response of the Djungarian hamster small intestine mucosa on a seasonal scale, whereby the mucosal electrogenic transport capacity for alanine and glucose did not differ between long and short photoperiod-acclimated hamsters. It was, however, significantly higher in the hamsters under short photoperiod when related to their body mass.

Together, these changes provide evidence for a seasonally mediated reduction of the hamsters' small intestine mucosal tissue, presumably to save energy as the intestinal tract is a highly energy demanding organ system (Stevens and Hume, 1995). This phenomenon was already observed in different mammalian species such as striped field mice (*Apodemus agrarius*), elephant shrews and Chilean mouse-opossums (*Thylamys elegans*) as a strategy to maintain a positive energy budget during times of low food availability (Borkowska, 1995; Bozinovic et al., 2007; Carey, 2005; Karasov and Diamond, 1983; Woodall, 1987). When morphological and histological parameters of the present study are related to the Djungarian hamsters' body mass, mucosal dry mass and surface were comparable, while intestinal length and transport capacity were even higher than in long photoperiod conditions. Consequently, it is unlikely that the intestinal reduction in response to short photoperiod conditions had a negative effect on the nutrient uptake capacity of Djungarian hamsters. Rather, the changes rater seem to be part of a seasonally mediated precise trade-off between energy conservation and preservation of an optimized energy assimilation capacity, adjusted to the hamsters' winter set point (Steinlechner et al., 1983).

Seasonal acclimation of small intestine and spontaneous daily torpor expression

As soon as short photoperiod-exposed Djungarian hamsters have reached their winter-adjusted set point of food intake and body mass (Steinlechner et al., 1983), they start to express spontaneous daily torpor to flexibly respond to changes in energy availability and to maintain a long-term energy balance (Heldmaier and Lynch, 1986; Heldmaier and Steinlechner, 1981). A further reduction of food availability below the precisely regulated set point resulted in a negative energy balance, for which the hamsters compensated with an increased torpor expression (Diedrich et al., 2015; Ruf et al., 1993). Interestingly, the *ad libitum* food intake during short photoperiod negatively correlated with torpor incidence (Ruf et al., 1991). Although this relationship could be confirmed in the present study and a higher torpor incidence was additionally associated with a lower body mass, these relationships could not explain the prevalent variability in torpor incidence alone (e.g. Ruf and Heldmaier, 1992; Ruf et al., 1993). In the present study, a high torpor incidence was assumed to also result from a low or suboptimal energy uptake efficiency of the mucosal resorptive surface size and/or nutrient resorption capacity (Karasov and Diamond, 1988). Short photoperiod-acclimated hamsters of experiment 2, but not experiment 1, showed a negative correlation between absolute but not relative small intestine size and torpor incidence. Furthermore, body mass-related medial but not proximal mucosal resorptive surface area positively correlated with torpor incidence, which contradicts the initial hypothesis. Finally, the electrogenic transport capacity for neither alanine nor glucose correlated with spontaneous daily torpor incidence. Based on these inconsistent results, the seasonal acclimations of Djungarian hamsters' small intestine neither explain their high interindividual variability in torpor expression nor help to distinguish whether overall energy availability determines torpor incidence or whether a predefined torpor proneness (Ruf et al., 1993) determines energy uptake under *ad libitum* conditions.

Most studies on the relationship between seasonal intestinal function and regulated hypometabolic episodes to save energy have been conducted on hibernating species. Carey (1990) investigated thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) and demonstrated that normothermic squirrels during their hibernation season in winter had a lower jejunal wet mass, villus height and surface area compared with their counterparts during their non-hibernation season in spring. However, when normalized to mucosal surface, winter-acclimatized animals showed a higher nutrient resorption rate and a higher short-circuit current after alanine or glucose administration. Similar results were obtained from other fat-storing hibernators such as alpine marmots (*Marmota marmota*; Hume et al., 2002) and mouse-eared bats (*Myotis myotis*; Paksuz, 2014), presumably as a compensatory response to mucosal atrophies during the hibernation season because of the ceased food intake (Carey, 1990; Carey and Sills, 1996; Stevens and Hume, 1995). In contrast, food-storing hibernators that do not cease food intake show more diverse strategies. While intestinal acclimation of European hamsters (*Cricetus cricetus*) resembles that of fat-storing hibernators (Weitten et al., 2016), Syrian hamsters (*Mesocricetus auratus*) preserve their intestinal tissues during the hibernation season (Weitten et al., 2013).

At first sight, Djungarian hamsters showed seasonal intestinal acclimation similar to that of true hibernators as they decreased mucosal tissue to save energy and increased nutrient resorption capacity to optimize energy yield from food. However, the hamsters do not express multiday deep hibernation bouts, but show shallow torpor bouts on a daily base; they do not store and even decrease internal fat stores during winter acclimatization and they do not cease feeding but actively forage for food (Heldmaier et al., 2004). Thus, a comparison of intestinal acclimation between hibernators and daily heterotherms would require a more comprehensive data set of internal and external factors, which emphasizes that daily torpor is more than just a short form of hibernation (Ruf and Geiser, 2015). The complexity and diversity of seasonal acclimatization strategies becomes apparent once again when regarding species such as *Phodopus roborovskii*, *Apodemus speciosus* and *Peromyscus leucopus*, which are capable of torpor expression (Eto et al., 2014; Tannenbaum and Pivorun, 1988;

Chi et al., 2016), but increase their intestinal tissue during winter acclimatization (Chi and Wang, 2011; Derting and Noakes, 1995; Eto et al., 2016; Zhu et al., 2011; Green and Millar, 1987).

Acute acclimation of small intestine in response to food restriction

Morphological and physiological responses of the hamster small intestine were also investigated after a short-term acclimation to a reduced food and thus energy availability under long photoperiod. The most obvious result of the food restriction was a fast decrease in body mass to values comparable to those during short photoperiod conditions (Diedrich et al., 2015).

Food restriction did not lead to intestinal atrophy, but also resulted in a significantly higher relative length of the small intestine compared with the *ad libitum* fed hamsters. Furthermore, both absolute and relative dry mass of the small intestine did not differ between the food-restricted and *ad libitum* fed animals. Finally, food restriction led to a reduction in the absolute size of the small intestine mucosa, but in relation to body mass, the size was comparable to that of the *ad libitum* fed hamsters, at least in the proximal part of the small intestine. Under acute conditions of energy shortage without a long-term and voluntary acclimation period, the hamsters appear to protect the integrity of the small intestine as long as possible to be able to resorb nutrients efficiently when they are available again. Additional evidence comes from the higher body mass-related electrogenic transport capacity for alanine and glucose. In contrast to the present study, food restriction has generally been shown to reduce intestinal mucosal mass and to impair mucosal activity and transport function (Ferraris and Carey, 2000). The studies reviewed by Ferraris and Carey (2000) were, however, characterized by complete food removal, while the hamsters of the present study received a small food portion every day. The absence of intestinal content has been proven to be responsible for food restriction-induced atrophies (Debnam and Levin, 1975; Gleeson et al., 1972; Hughes and Dowling, 1980; Karasov and Diamond, 1983; Kotler et al., 1981). Thus, long-term moderate restriction might prevent the described pathological effects, as it induces adjustments in terms of maximizing energy yield in relation to the decreasing body size. Although this process showed similarities to that of the seasonal acclimation, several facts point out a clear difference between the acute and long-term acclimation, which will be discussed in the light of torpor expression.

Acute and seasonal acclimation of small intestine related to torpor expression

Food-restricted hamsters showed a fast body mass reduction and the first bouts of fasting-induced torpor occurred after only 1 week of food restriction. Spontaneous daily torpor bouts occurred after a voluntary, gradual decrease in food intake as well as body mass not before 7 weeks of short photoperiod exposure. Many considerable differences between the two types of torpor were shown previously (Diedrich and Steinlechner, 2012; Diedrich et al., 2015), and it was concluded that spontaneous daily torpor is a flexible way to maintain a long-term energy balance, whereas fasting-induced torpor serves as an emergency response to acute energetic challenges after a forced reduction of energy reserves. The present experiments show additional differences regarding the seasonal and acute acclimation of the hamsters' small intestine. Although both food-restricted and short photoperiod-acclimated hamsters showed a higher body-mass related electrogenic transport capacity for 5 mmol l^{-1} alanine and glucose, an additional 10 mmol l^{-1} glucose administration induced a further drastic increase in hamsters under food restriction. These results indicate that the acute energetic challenge induces a flexible increase in physiological transport capacity rather than a reduction and reorganization on the morphological level. This strategy could then be used not only to maximize energy yield from the reduced amount of food (Ferraris and Carey, 2000), but also to preserve the intestinal summer condition when sufficient food resources are available again. Although thirteen-lined ground squirrels are hibernators and differ in many seasonal adjustments from Djungarian hamsters, a study from Carey (1992) supports this theory. While short-term fasting in the squirrels resulted in an increased secretory response of the small intestine mucosa, no changes in mucosal architecture could be measured.

Another difference between the acute and long-term seasonal acclimation strategy was the hamsters' use of torpor in relation to the small intestine morphological and physiological parameters. While spontaneous daily torpor incidence correlated with several morphological parameters, the expression of fasting-induced torpor in food-restricted hamsters tended to negatively correlate with the absolute electrogenic transport for 5 mmol l^{-1} glucose. In other words, with a higher glucose uptake capacity from a given reduced amount of food, an individual hamster has no need for a high incidence of fasting-induced torpor to compensate for the energetic deficit. Thus, it can be assumed that fasting-induced torpor is not integrated as much in the overall acclimation strategy in response to food shortage, when compared with the seasonal spontaneous daily torpor (Heldmaier and Lynch, 1986). So far, it has been stated that Djungarian hamsters use different acclimation strategies to save energy, whereby a high torpor expression occurs together with a lower energy uptake (Ruf et al., 1991, 1993). However, a study on Chilean mouse-opossums indicates that increased torpor expression is used together with increased energy conservation via the reduction of intestinal and other organ mass when energy uptake is limited (Bozinovic et al., 2007). Thus, the present study emphasizes once more the incredible variety and flexibility of acclimatization mechanisms in mammals facing acute or seasonal energetic challenges.

Conclusions

The present study shows, for the first time, that the small intestine morphology and physiology of Djungarian hamsters adjusts to short-term energy shortage as well as to long-term seasonal and anticipatory winter-related energy shortage. Especially in short photoperiod-acclimated hamsters, these responses appeared to be precisely regulated and to occur in close relation to changes in food intake and body mass to reach an energy-efficient winter set point. Intestinal morphology and physiology did not seem to be maximized, but were optimized in a complex trade-off between energy conservation and energy assimilation. However, proneness for the energy-saving spontaneous daily torpor did not appear to be integrated in the intestinal adjustment. By contrast, the present study revealed evidence that fasting-induced torpor under long photoperiod conditions is correlated negatively with the small intestine transport capacity for glucose. Thus, hamsters with a higher nutrient uptake capacity might have a reduced need for employing torpor during periods of acute food restriction to compensate for the energy deficit. This important finding implies that fasting-induced torpor is used as a last emergency response towards a severe energetic challenge, which is comprehensible as torpor has not only energetic advantages but also partly life-threatening disadvantages (Diedrich and Steinlechner, 2012; Geiser and Drury, 2003; Wojciechowski and Jefimow, 2006). Additional work is needed to complete the picture of acute and seasonal

acclimatization in the Djungarian hamster regarding the small intestine. This could include the quantification of mucosal glucose uptake via transepithelial flux measurements or the investigation of intestinal non-electrogenic glucose transporters that show fast changes in response to glucose availability (Kellett et al., 2008). Finally, energetic challenges such as reduced ambient temperature and food together with short photoperiod would represent a more natural winter situation (Gross et al., 1985; Hammond, 1993), evoking more pronounced intestinal changes to supplement energy-saving mechanisms such as daily torpor.

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Competing interests
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Author contributions
Conceptualization: B.S., G.B., S.S., V.D.; Methodology: E.P., B.S., G.B., S.S., V.D.; Software: E.P., V.D.; Validation: B.S., V.D.; Formal analysis: E.P., E.H., V.D.; Investigation: E.P., V.D.; Resources: S.S., V.D.; Data curation: E.P., B.S., V.D.; Writing - original draft: S.S., V.D.; Writing - review & editing: E.P., A.H., E.H., B.S., G.B., S.S., V.D.; Visualization: V.D.; Supervision: A.H., S.S.; Project administration: S.S., V.D.; Funding acquisition: A.H., S.S., V.D.

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Data availability
Data are available from the figshare digital repository: https://doi.org/10.6084/m9.figshare.13656527.v1

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