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Titel | Title

Do food supplements affect body condition and the timing of reproduction in female Common hamsters (Cricetus cricetus) in the subsequent season?

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Abstract

Hibernating mammals such as the Common hamster (Cricetus cricetus) are facing seasonal weather changes by reducing their body temperature and metabolism to save energy in this critical phase. Common hamsters are food caching animals that especially rely on external food stores during this period, although differences in caching behaviour between males and females can be seen. In this study, free-ranging hamsters were provided with food supplements in form of 500g sunflower seeds shortly before hibernation onset. Subcutaneously implanted data loggers recorded their body temperature between winter immergence and vernal emergence. Regular observations of the focal hamsters' burrows in spring ensured that we caught them right at their first emergence after the hibernation period. We compared body mass and fat changes between supplemented and unsupplemented hamsters and their correlation with hibernation duration and the euthermy phases. We focused on female reproductive timing. The results showed that additional food stores had an impact on male body condition, female emergence and the timing of reproduction. Supplemented male hamsters gained body mass during hibernation, while the other groups lost mass, females more than males. The change in fat proportion over winter was highest in supplemented females, who lost more than the other groups. Supplemented females emerged earlier than unsupplemented ones and started earlier to reproduce. These results indicate differences in the use of food supplements between the sexes, which is also illustrated by the differences in food caching behaviour. Furthermore, the influence of supplementation on the timing of reproduction shows the extent to which females can adapt to an appropriate food supply.

Introduction

In seasonal environments, animals must face critical factors such as the periodical drop of ambient temperature during winter, food shortage or drought (Humphries et al. 2003, Ruf & Geiser 2015). To cope with such extreme situations, animals need to adapt to these conditions by applying special strategies.

Several heterothermic mammalian and bird species use torpor to overcome such difficult conditions (Geiser 2004, Ruf & Geiser 2015). This strategy can be distinguished into two patterns: daily torpor and hibernation (Geiser 2004). Daily torpor lasts for less than 24 hours with continued foraging and occurring often throughout the year (Geiser 2004, Ruf & Geiser 2015). During hibernation, torpor lasts for consecutive days or weeks with short arousal episodes in between and is often seasonal (Geiser 2004, Ruf & Geiser 2015). Sufficient energy reserves should be available for survival during the hibernation period. Some species mainly achieve this either internally, by storing body fat or externally, by hoarding food prior to the beginning of hibernation (Humphries et al. 2003).

One of those mammalian species performing hibernation to save energy by reducing its body temperature and metabolic rate during winter is the Common hamster *Cricetus cricetus* (Wassmer 2003, Siutz et al. 2012). In general, this species relies on food stores during winter. But differences can be found between the sexes, for example regarding caching behaviour. While males mainly feed above ground during the active season, females cache food and primarily feed underground (Siutz et al. 2012).

Additionally, adult males show higher body fat proportions before immergence than females (Siutz et al. 2012). Studies also demonstrated that males immerge approximately one month earlier into their hibernaculum than females and juveniles (Siutz et al. 2016).

Regarding hibernation patterns, differences have been also found between the sexes (Siutz et al. 2016). Compared to males, females started hibernation later and for shorter periods, therefore spending less time in torpor. Nevertheless, hibernation expression is highly variable within individuals (Wassmer 2004). Wassmer (2004) showed that body temperature patterns during the hibernation period, could vary from deep torpor bouts with alternating short arousals to only few torpor bouts with long euthermic periods.

After hibernation, in spring, male hamsters usually emerge before females (Weinhold & Kayser 2006). The reproductive period then starts as soon as females appear above ground (Franceschini-Zink & Millesi 2008).

Common hamsters have a promiscuous mating system, where females, unlike other mammalian hibernators, can produce up to 3 litters per season until August or even September (Franceschini-Zink & Millesi 2008, Hufnagl et al. 2011, Lebl & Millesi 2008). As studies have shown, females can have a postpartum oestrus (Franceschini-Zink & Millesi 2008). They can mate right after parturition. Therefore, lactation can occur at the same time as gestation. This strategy enables females to increase their reproductive output in a limited active period.

After the breeding season, in contrast to males, due to maternal investment, females have a shorter time to prepare themselves for hibernation in their burrow. Hence, the timing of reproduction has a considerable effect on reproductive output and body condition of the mothers before immergence in autumn. For female hamsters it is an advantage to start reproduction earlier in the season, as they can enhance their reproductive output through more litters and a higher number of juveniles in total (Francheschini-Zink & Millesi 2008). But the timing of reproductive events can also affect their juveniles, as they need time to structurally grow and accumulate food and body fat until immergence for the winter period. Therefore, it is beneficial for juveniles to be born early in the season as they have more time to prepare themselves for their first hibernation Francheschini-Zink & Millesi 2008).

In addition, access to high quality food could also have an impact on those criteria.

As studies in European ground squirrels (*S. citellus*) have shown, females with a higher emergence mass have earlier-born litters that are larger than later-born litters in the season and were also nursed for longer time spans (Millesi et al. 1999). This affected the mothers' condition. Millesi et al. (1999) demonstrated that the longer the lactation duration lasted, the higher the mass loss during lactation. In consequence, the start of prehibernation fattening was delayed and immergence mass was lower than that of females with shorter lactation durations.

Another effect of having a good body condition as a juvenile is demonstrated in the study of Rödel & von Holst (2009) in male European rabbits (*Oryctolagus cuniculus* L.). They showed, that the higher the male nestling body mass, the more offensive interactions they had with other juveniles. In addition, these males also expressed more agonistic behaviour and were more successful fighters as adults in intra-sexual conflicts.

To investigate the effects of body condition and reproductive timing in our study, we supplemented free-ranging Common hamsters in Vienna with 500 g sunflower seeds each, before immergence into their hibernaculum. The seeds have a high energy content. We analysed the effect of this food supplements on male and female body condition and the timing of reproduction in spring and compared it to the data of unsupplemented hamsters from previous years. This study was part of a project investigating hibernation patterns in supplemented and unsupplemented Common hamsters. Therefore, results of hibernation patterns are not shown in here, as we only focused us on differences in body condition and reproductive timing.

Material & Methods

Study site and period

The study was conducted at the surrounding area of the Kaiser-Franz-Josef hospital in the south of Vienna, within the 10th district. Our 2.3 ha sized focal site was surrounded by hospital buildings, parking spaces and visitor pathways. The vegetation of this urban area consisted mostly of meadows with some trees and bushes.

Data were collected from September to November 2015, before the hamsters immerged into their hibernacula. We captured 52 Common hamsters (21 adults and 31 juveniles) and chose 18 of those individuals as focal animals for food supplementation (11 adults and 7 juveniles). Juveniles were only chosen when they weighed over 200 g to make sure they were in a good body condition for iButton implantation (see ‘Body temperature recordings’). The second part of data collection was carried out after the hibernation period from March until the beginning of July 2016. In this period, fieldwork was performed 3 to 5 times per week, starting in the morning at approximately 05:30 until 11:00 am or in the evening from 05:00 until 09:00 pm.

Field techniques

We applied the capture-mark-recapture method, by using Tomahawk live traps that were baited with peanut butter and placed in front of the hamster's burrow. We checked them every 15 – 20 min to minimize the time in the live trap for the animals. Trapped individuals were then released into cone-shaped cotton sacks with laterally equipped Velcro fasteners, to enable the examination of hamsters without anaesthesia. For each capture, we recorded date, time and weather condition, as well as the parameters sex, age (adult, juvenile), body mass (g) and head, tibia and foot length (mm). Adult hamsters were those that had hibernated at least once, and juveniles were born in the current season. In addition, we documented reproductive status by determining teat and vulval development according to the classification by Franceschini-Zink & Millesi (2008). Therefore, non-recognisable teats and a closed vulva, would indicate that none of the reproductive phases have taken place yet. In contrast to this, extremely swollen teats would imply that the female is lactating. An extremely open vulva could either demonstrate previous mating or parturition.

To mark each individual hamster permanently, we injected a transponder chip (PIT tag, Datamars) subcutaneously. For direct observations, the hamsters' fur was marked with different symbols using commercial hair dye (red for females, black for males). All in all, this whole procedure described above, did not last longer than 10 minutes. At the end, the hamsters were released in front of their burrows.

Supplementation

Each focal hamster was supplemented with 500g sunflower seeds in late autumn, before the hibernation period started. Sunflower seeds were chosen because of their high quality and good storability. We presented the seeds right in front of the focal hamster's burrow and waited there until they collected all the seeds. By this, we ensured that only the focal animals themselves and not another hamster cached their supplements. In total, we supplemented 18 Common hamsters - 11 females (9 adults and 2 juveniles) and 7 males (2 adults and 5 juveniles).

Body temperature recordings

Before hibernation, temperature loggers (iButtons) were implanted subcutaneously in the hamsters' neck region under isoflurane anaesthesia at a vetenary clinic. These data loggers had a diameter of 15 mm and were covered with Elvax (ethylene vinyl acetate resins) and paraffin. More details of this procedure can be found in Siutz et al., 2018. The whole process of trapping, implantation and releasing the hamster again at its burrow lasted approximately 2 to 3 hours.

During winter (December – March), we monitored the hamster burrows regularly by covering the holes with leaves, to determine potential surface activity. This monitoring enabled us to recapture the focal animals shortly after vernal emergence.

We implanted iButtons in all our supplemented hamsters. Therefore, we had 11 females and 7 males with implantations. 14 of those individuals could be recaptured in spring, but only 13 iButtons were used for analysis, because one female had lost its temperature logger.

One of the focal males changed his burrow shortly before immergence. Hence, we could not be sure that he still had the supplements at his disposal over the hibernation period. Since this is also confirmed by the hamster's hibernation pattern and body condition, we regarded him as non-supplemented. Therefore, we had 6 individuals in each sex with temperature recordings and supplementation.

iButton recordings were used to measure the hamsters' body temperature (Fig. 1). It also helped us to identify the date of immergence, which is indicated by termination of surface activity and blocking the burrow entrance. In this time, body temperature slowly starts to decline under 35°C. This pre-hibernation euthermy ends when the first deep torpor bout (DTB) shows up (body temperature under 20°C). Hibernation duration is defined as the period from the start of the first DTB to the end of the last DTB. The recordings also helped us to define the emergence date. Emergence only appeared after post-hibernation euthermy, which is defined as the period after the last DTB until emergence, where body temperature stays above 30°.

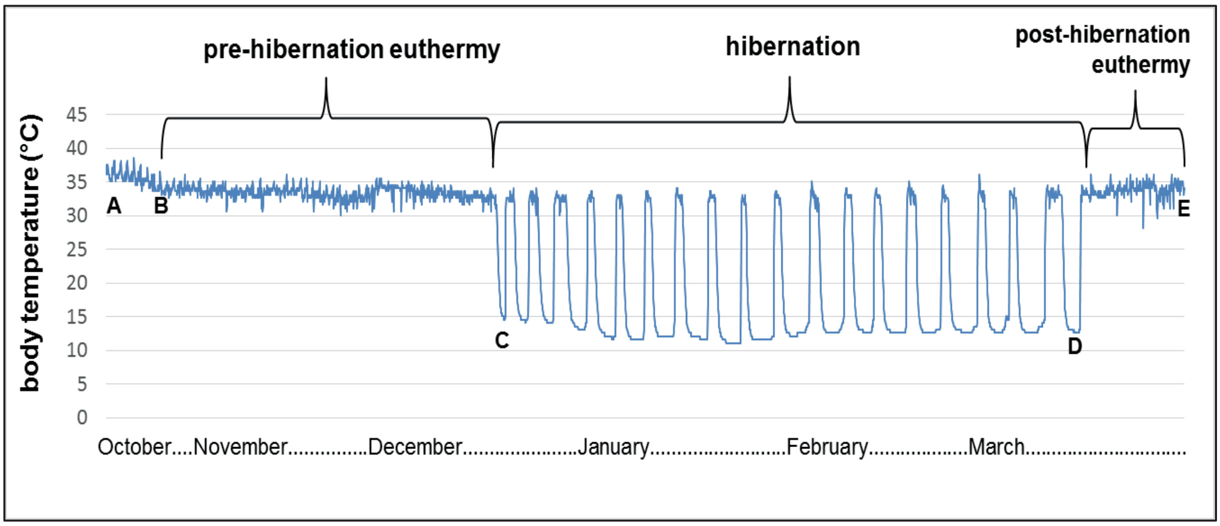

Figure 1: iButton recording of a hibernation pattern of a Common hamster. A: last capture; B: immergence; C: first deep torpor bout (DTB) / hibernation onset; D: last deep torpor bout (DTB) / hibernation end; E: emergence

Although iButton recordings helped us to determine those dates, we mostly relied on documentation of closed (hamster still in burrow) and opened (hamster already emerged) burrow entrances to define immergence and emergence.

Comparison of supplemented and non-supplemented hamsters

To compare the supplemented hamsters from autumn 2015 with non-supplemented ones, we used hibernation and reproduction data from the same study site but from previous years (2013 - 2015). Those data showed that environmental conditions such as weather and temperature, were similar in all those years. They were also collected and provided from the Department of Behavioural Biology, University of Vienna.

Calculations

First conception date was based on observed mating and subsequent body mass gain. Earlier studies (Franceschini et al. 2007, Franceschini-Zink & Millesi 2008) have shown that gestation can be assumed to have an average duration of approximately 20 days, which we used to additionally estimate the conception date. Parturition was identified by a rapid body mass loss, combined with increased teat size, indicating lactation. We could not trap first litter juveniles of all our focal females. Due to this lack of data, it was not possible to obtain valid results on natal emergence dates and total juvenile number in first litters.

Body fat was calculated using a non-invasive method. Calculations were based on the morphometric measurements body mass and head, foot and tibia length, which were integrated in a multiple regression model (Siutz et al. 2012).

Statistical analysis was performed using IBM SPSS Statistics 24. Shapiro-Wilk test was used to test normality of the data distribution. To compare different groups with each other (supplementation, sex), we applied Student's t test for unpaired samples for normally distributed data. Correlations were done using Pearson for normally and Spearman-Rho for not normally distributed data. Results are shown as means ± standard deviation (SD).

Ethical statement

All procedures performed on common hamsters were approved by the Austrian Ministry of Science, Research and Economy, City of Vienna (MA22-22484/10, MA22-310/11), the Ethical Committee for Animal Welfare (GZ BMWFW-66.006/0013-WF/VI3b/2015) and the University of Vienna (2015-010).

Results

Male and female body condition

Body mass change

The comparison of relative body mass change between male and female Common hamsters not only revealed a significant sex difference between supplemented animals ($t = -4.880$, $p = 0.001$), but also between supplemented males and unsupplemented females ($Z = -2.475$, $p = 0.013$) (Fig. 2, Tab. 1). Within males, relative body mass change differed significantly between supplemented and unsupplemented individuals ($t = 2.530$, $p = 0.022$) (Fig. 2). Other group comparisons (supplemented – unsupplemented females, supplemented females – unsupplemented males, unsupplemented females – unsupplemented males) displayed no significance in body mass change (Z = -0.589, p = 0.556; t = -1.345, p = 0.196; Z = -0.434, p = 0.664) (Fig. 2). Only supplemented males gained body mass during hibernation, while the other groups lost body mass. Males in general emerged with a higher body mass in autumn than female hamsters. Supplemented hamsters had a higher body mass change over winter than their unsupplemented counterparts (Fig. 2).

Table 1: Body mass (Mean ± SD) of male and female Common hamsters with and without additional food stores.

	n	Immergence body mass (g)	Emergence body mass (g)	Relative body mass change (%)
Suppl. females	6	294.7 ± 41.0	259.5 ± 53.7	-12.2 ± 10.4
Unsuppl. females	13	330.2 ± 82.8	282.6 ± 59.9	-3.0 ± 25.9
Suppl. males	6	339.3 ± 62.8	419.7 ± 63.8	25.0 ± 15.5
Unsuppl males	14	329.1 ± 59.1	323.2 ± 6.5	0.3 ± 21.3

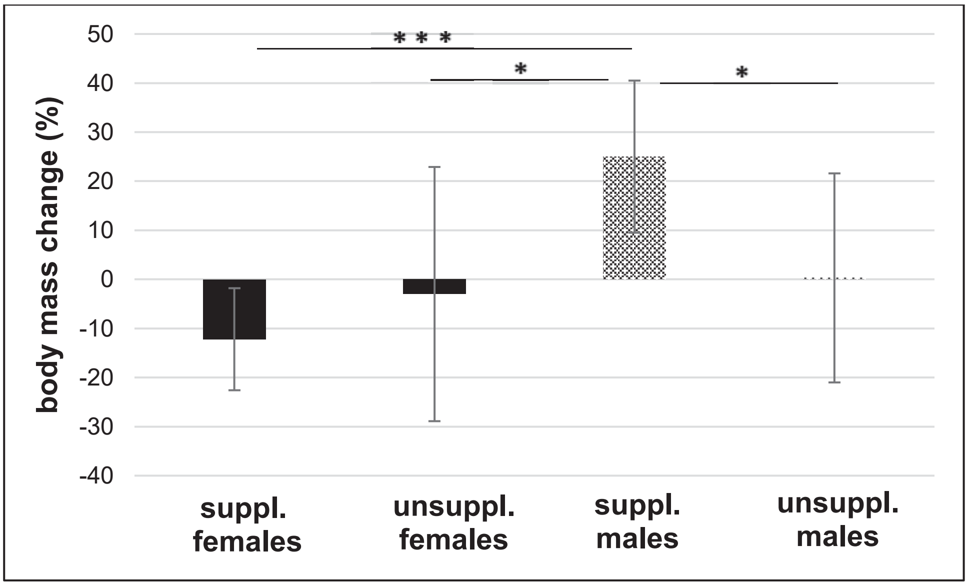

Figure 2: Relative body mass change (Mean ± SD) of male and female Common hamsters with and without additional food stores (*p ≤ 0.05, ***p ≤ 0.001).

Proportions of body fat

Regarding changes in body fat proportions over winter, a significant difference was found between supplemented male and female hamsters (t = -2.941, p = 0.016) (Tab. 2, Fig. 3). Females with additional food stores lost nearly half of their fat proportions over winter. When comparing this fat change over winter with unsupplemented females, the latter only lost one third. Supplemented males on the other hand, lost less fat than their supplemented counterparts (Fig. 3).

We received a significant result, when comparing supplemented females and unsupplemented males with each other (t = -2.943, p = 0.011). Looking at fat change within females, the results were also significant (t = -2.144, p = 0.055). Fat change did not differ significantly within the male groups and between supplemented males and unsupplemented females (t = 0.774, p = 0.454; t = 0.173, p = 0.866). As well as between unsupplemented male and females (t = 0.441, p = 0.666) (Fig. 3).

Table 2: Body fat proportions (Mean ± SD) in male and female Common hamsters with and without additional food stores.

	n	Fat at immergence (%)	Fat at emergence (%)	Fat change (%)
Suppl. females	6	25.3 ± 5.6	14.4 ± 4.3	-10.8 ± 3.6
Unsuppl. females	7	20.2 ± 8.8	17.1 ± 6.0	-3.1 ± 8.2
Suppl. males	5	14.8 ± 5.1	12.5 ± 5.6	-2.3 ± 6.0
Unsuppl. males	9	16.3 ± 5.6	11.9 ± 4.6	-4.5 ± 4.4

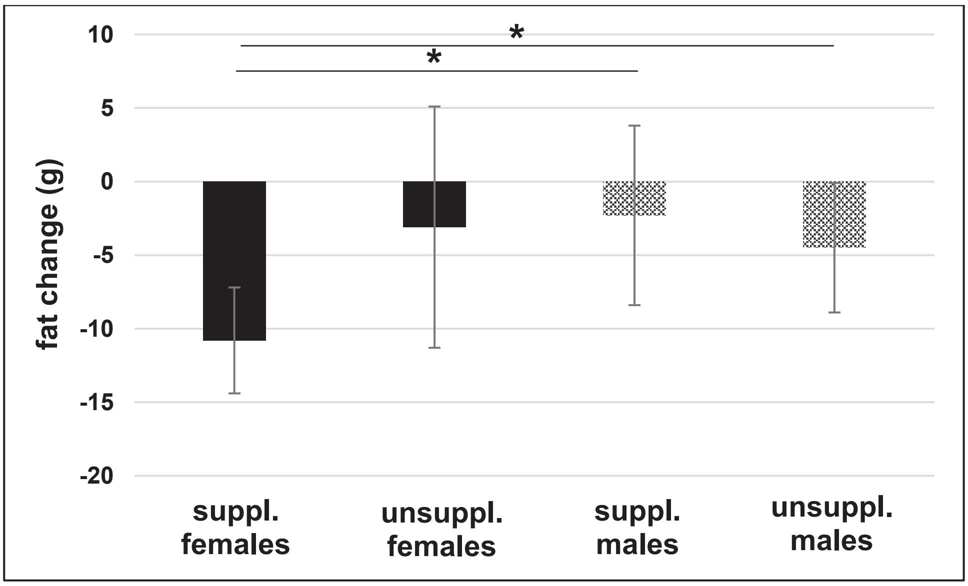

Figure 3: Fat change (Mean ± SD) of male and female Common hamsters with and without additional food stores (*p≤ 0.05).

Correlations between body mass change and hibernation phases

Pre-hibernation euthermy

Unsupplemented female Common hamsters showed a significant negative correlation between relative body mass change and post-hibernation euthermy (r = -0.678, p = 0.045, Fig. 4). The more days they spent in pre-hibernation euthermy, the higher their body mass loss. No such relationship was found in supplemented females (r = 0.540, p = 0.269, Fig. 4) and in neither of the male groups (supplemented/unsupplemented males: r = 0.016/0.372, p = 0.976/0.211).

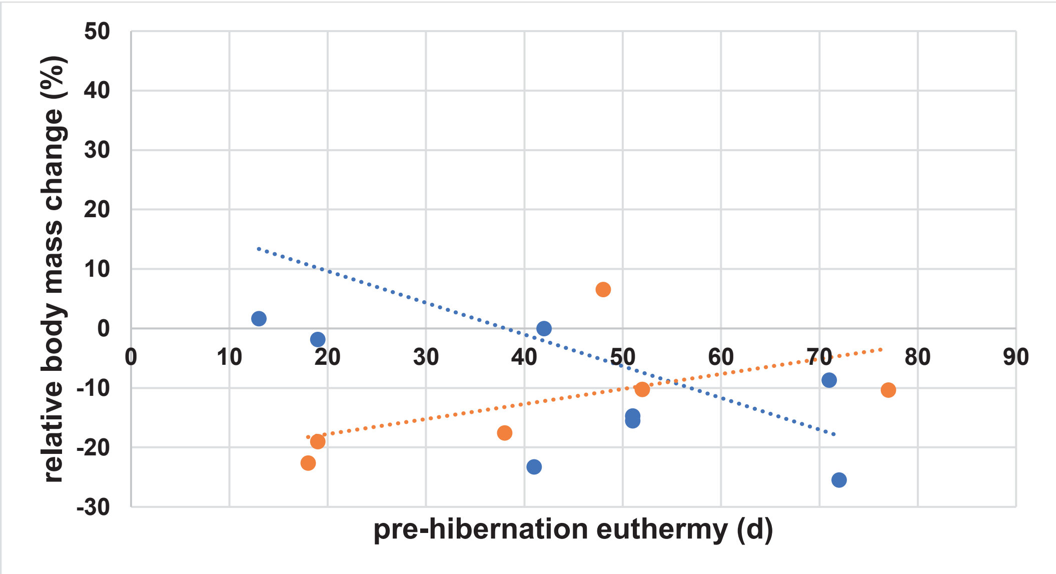

Figure 4: Correlation between post-immergence euthermy (days) and relative body mass change (%) in supplemented (orange dots, n = 6) and unsupplemented (blue dots, n = 9) female Common hamsters.

Hibernation duration

We could not find any significant correlation between relative body mass change and hibernation duration in females without supplements (r = 0.500, p = 0.170, Fig 5), while supplemented ones showed a strong negative tendency (r = -0.799, p = 0.057, Fig. 5), indicating that the longer the hibernation period lasted, the more weight they lost over winter.

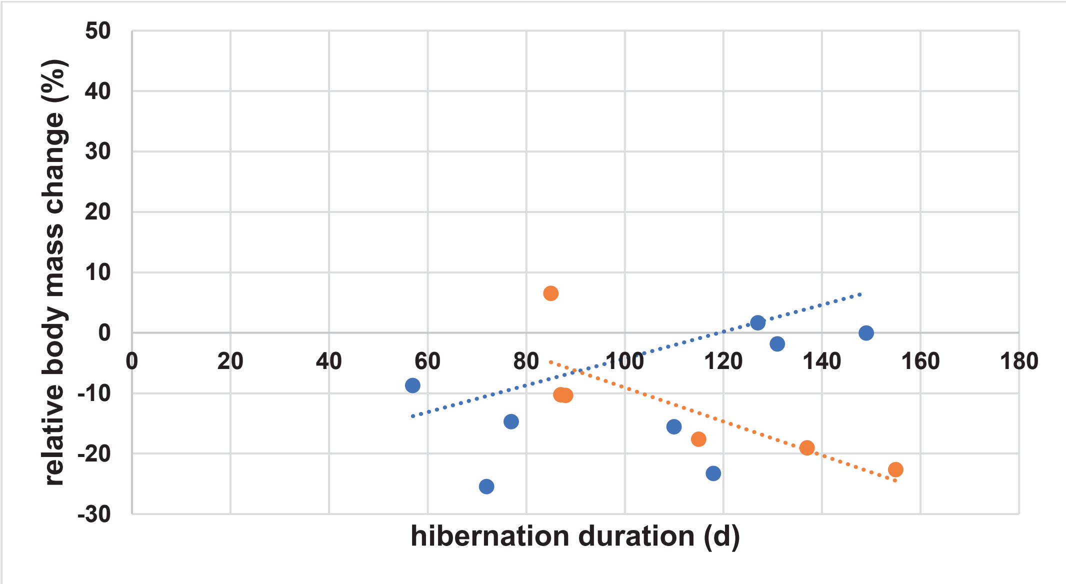

Figure 5: Correlation between hibernation duration (days) and relative body mass change (%) in supplemented (orange dots, n = 6) and unsupplemented (blue dots, n = 9) female Common hamsters.

We found no significant relationships among post-hibernation euthermy, hibernation duration and mass changes in male Common hamsters (supplemented/unsupplemented males: r = -0.296/-0.293, p = 0.568, p = 0.330).

Post-hibernation euthermy

As seen before, also post-hibernation euthermy did not correlate with relative body mass change in unsupplemented females (r = 0.417, p = 0.265, Fig. 6), while the correlation in supplemented ones, was marginally significant (r = 0.792, p = 0.060, Fig. 6). This means the longer the duration of post-hibernation euthermy, the lower mass loss during winter.

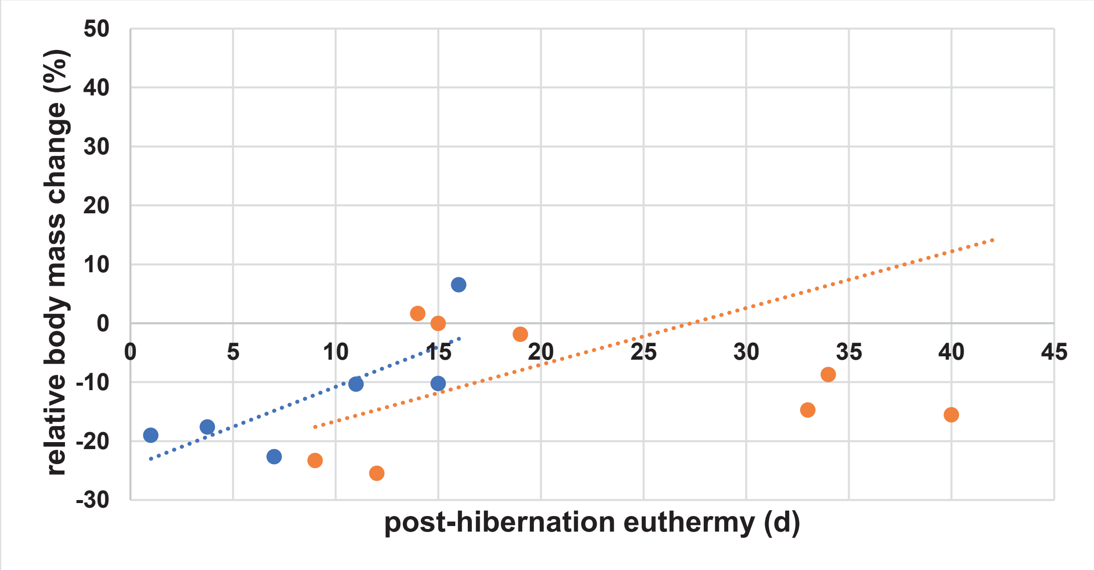

Figure 6: Correlation between pre-emergence euthermy (days) and relative body mass change (%) in supplemented (orange dots, n = 6) and unsupplemented (blue dots, n = 9) female Common hamsters.

Correlations among male hamsters, did not reveal any significant results (supplemented/unsupplemented males: $r = 0.199/0.237$, $p = 0.706/0.435$).

Correlations between fat change and hibernation phases

Both male and female hamsters, neither showed a significant relationship between absolute fat change and pre-hibernation euthermy (supplemented/unsupplemented: males: $r = 0.368/0.330$, $p = 0.542/0.386$; females: $r = 0.127/-0.654$, $p = 0.810/0.111$), nor with hibernation duration (supplemented/unsupplemented: males: $r = 0{,}679/-0.410$, $p = 0.207/0.273$; females: $r = -0.319/0.539$, $p = 0.538/0.212$). In addition,post-hibernation euthermy was not correlated significantly to absolute fat change in both sexes (supplemented/unsupplemented: males: $r = -0.045/0.301$, $p = 0.943/0.431$; females: $r = 0.729/0.605$, $p = 0.101/0.150$).

Female reproductive timing

Vernal emergence date and body mass

In contrast to immergence date and the total days spent inside the hibernaculum, where supplemented and unsupplemented females did not differ (t = -0.345/-1.637, p = 0.734/0.124), supplemented ones emerged significantly earlier in spring than those without additional food stores (Fig. 7, t = -2.555, p = 0.016). Females without supplements emerged approximately ten days later, around mid-April.

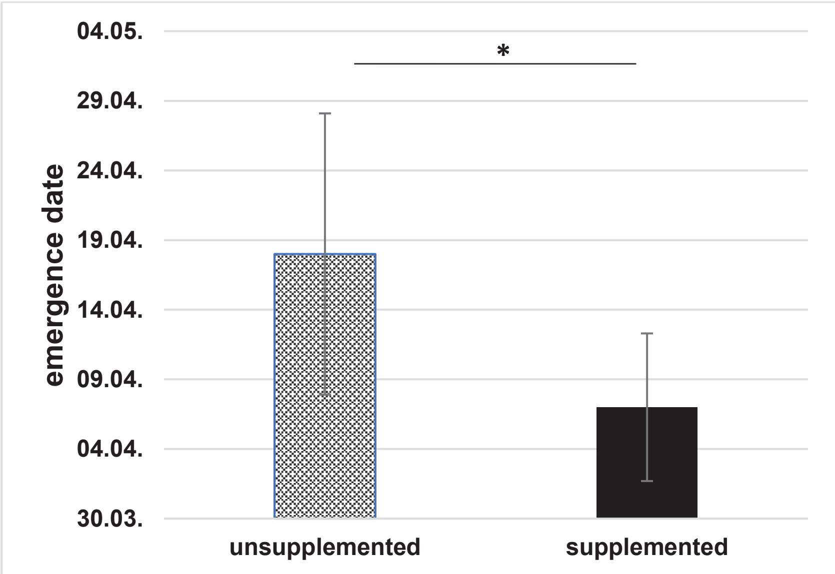

Figure 7: Emergence date (Mean ± SD) of supplemented (n = 6) and unsupplemented (n = 24) female Common hamsters (*p≤ 0.05).

Body mass at emergence did not differ significantly between the groups (Fig. 8, Z = -0.997, p = 0.319). Both emerged with body masses ranging between 250 to 300 g.

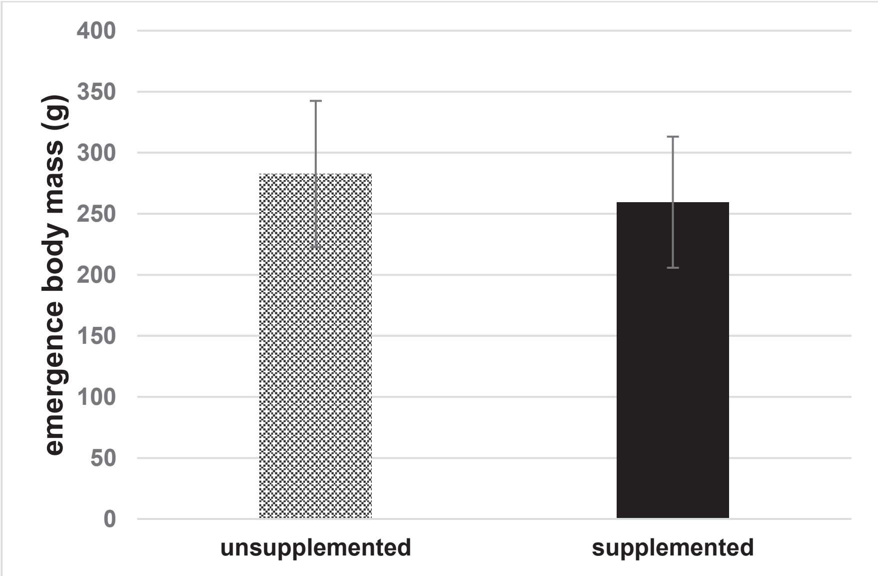

Figure 8: Emergence body mass (Mean ± SD) of supplemented (n = 6) and unsupplemented (n = 23) female Common hamsters.

In addition, we could not find any difference in teat and vulval development at emergence between the female groups. They emerged with non-recognisable teats and vulva openings between 0.5 and 1.5.

Timing of reproduction

Our results revealed a difference between supplemented and unsupplemented female hamsters in the date of first conception in the studied season (Fig. 9, $t = -2.252$, $p = 0.032$). Here again, supplemented females significantly preceded the others approximately ten days, starting to reproduce between the 12th and 14th of April. Females without additional food stores started with reproduction between the 22nd and 24th of April.

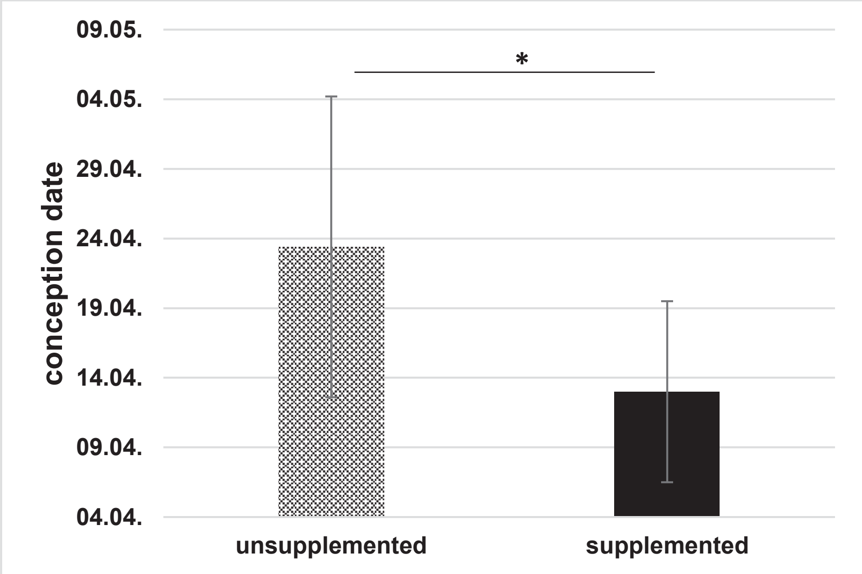

Figure 9: Conception date (Mean ± SD) of supplemented (n = 6) and unsupplemented (n = 26) female Common hamsters (*p≤ 0.05).

Consequently, supplemented hamsters also significantly preceded the unsupplemented group in the date of first parturition (t = -2.175, p = 0.039), giving birth around the 3rd of May (Fig. 10). Unsupplemented hamsters gave birth around the 13th of May.

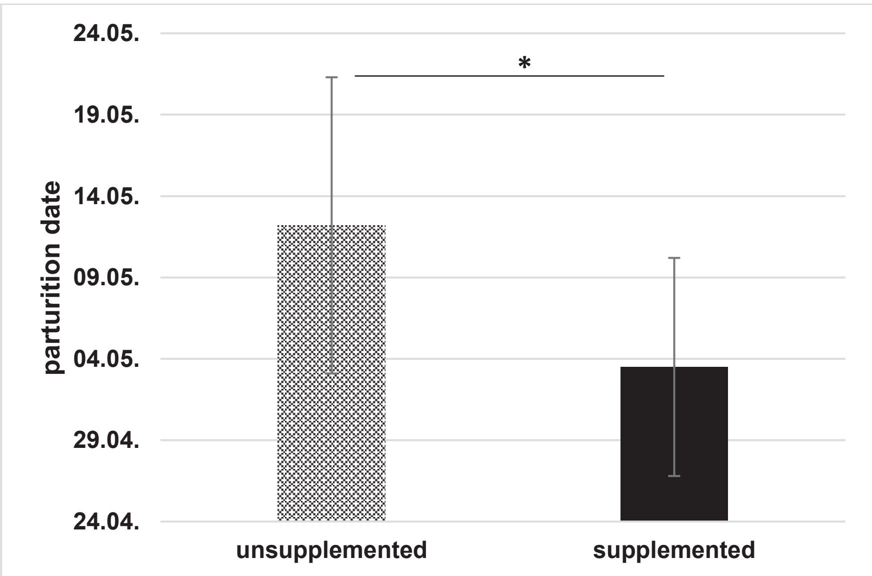

Figure 10: Parturition date (Mean ± SD) of supplemented (n = 6) and unsupplemented (n = 23) female Common hamsters (*p≤ 0.05).

Discussion

Our results have shown that supplementation of free-ranging Common hamsters with high-energy food reserves before immergence, affected body condition in males, and the timing of female vernal emergence and reproduction. While both supplemented and unsupplemented females, as well as unsupplemented males lost body mass during the hibernation period, supplemented males were the only group that clearly gained body mass during this time.

As Siutz et al. (2018) have demonstrated, supplemented males immerged later into their hibernacula and had a longer euthermic phase before hibernation onset than their unsupplemented counterparts, which led to a delayed start of hibernation. They also showed fewer and shorter torpor bouts and expressed more shallow torpor bouts (STBs) of shorter duration during post-hibernation euthermy, therefore spending more time in shallow torpor (Siutz et al. 2018). During STBs, body temperature ranged between 20 and 30°C for less than 24 hours (Wassmer & Wollnik, 1997) and in this period, the minimum body temperature of supplemented males was also higher than in unsupplemented ones (Siutz et al. 2018). This indicates that supplemented hamsters could have used the extended euthermic period with the described patterns to gain body mass due to the availability of additional food stores and to compensate energy expenditure.

This might also be reflected in their overall fat change over winter, indicating that supplemented males lost less fat than the unsupplemented ones, although these differences were not significant in our study.

Overall, supplemented males displayed a shortened hibernation period compared to unsupplemented ones and they also hibernated for a shorter period than supplemented females (Siutz et al. 2018). The latter is opposite to the usual pattern in adult Common hamsters, where adult males enter their hibernaculum first and started to hibernate earlier and for longer periods than females (Siutz et al. 2016). Siutz et al. (2016) also found no differences in body mass changes over winter between the sexes. Studies in European ground squirrels, *Spermophilus citellus,* have also shown that males, which in general have a shorter hibernation duration than females, have a lower overwinter mass loss than females (Millesi et al. 1999b).

Furthermore, Humphries et al. (2003) demonstrated that free-ranging Eastern chipmunks with additional food supplements reduced their torpor expression during hibernation, hence spending more time in euthermy in this period than unsupplemented chipmunks. They even showed a higher temperature during torpor than the unsupplemented group.

Supplemented male hamsters tended to emerge earlier in spring than the other male group (Siutz et al. 2018). Together with a better body condition due to a higher body mass, those males are assumed to be more competitive during intrasexual conflicts (Grulich 1986, Lebl & Millesi 2008). In addition, testes growth could be already fully completed until the emergence of the first female (Lebl & Millesi 2008) compared to later emerging males, which could lead to a higher reproductive success.

In contrast to males, female common hamster did not show any significant changes in body mass over winter or differences in their hibernation patterns between the groups (Siutz et al. 2018). Both lost body mass and fat over winter. This indicates that the supplemented females either did not use the additional energy reserves during hibernation at all or at least only partly, so that the supplements must have been still available in their hibernaculum after emergence.

This finding can be explained by the different foraging behaviours between the sexes. Female Common hamsters show food caching behaviour during their active season, whereas males rather rely on body fat by feeding above the ground (Siutz et al. 2012). This is also due to the larger home ranges of males, as they are searching for oestrus females and therefore frequently change their burrows, not allowing them to cache food for a longer period in the same place (Kayser & Stubbe 2003). Unsupplemented females significantly lost more body mass, the more time they spent in pre-hibernation euthermy. In comparison, the supplemented females lost less body mass, the longer they stayed in euthermy before hibernation onset. Although this result is not significant, it could indicate that they might have consumed part of their energy-rich food during this period, as assumed in the supplemented males (Siutz et al 2018).

Correlations between relative body mass change and pre-emergence euthermy suggest a lower body mass loss, the longer supplemented and unsupplemented females stayed in euthermy before emergence, indicating the consumption of cached food in this time. A similar correlation has been shown in Siutz et al. (2016) where hamsters showed lower mass loss or even gained weight, the longer the euthermic period prior to emergence lasted.

Interestingly, our results did not indicate any significant difference in body mass at emergence between females of both groups, confirming, as mentioned above, that most of our supplemented food was most likely not consumed over winter.

All our focal females emerged in April, but supplemented females appeared significantly earlier than the unsupplemented group, which emerged approx. ten days later. Body temperature analyses revealed they had shortened their post-hibernation euthermy phase compared to the unsupplemented ones (Siutz et al. 2018). The difference in the onset of the active season is also reflected in the timing of reproduction. Our results demonstrate an earlier start of conception and parturition in supplemented female hamsters. Earlier studies on populations in close distance to that investigated in this study found significant correlations between spring emergence date, first conception and first parturition date (Franceschini & Millesi 2008). The earlier females emerged, the earlier they started with reproduction and correspondingly the earlier their first litter emerged. Female common hamsters emerge with open vaginas, indicating that the activation of the ovarian cycles have been already completed inside the hibernaculum (Siutz et al. 2016). This was also the case in our study. Both female groups started with reproduction approx. one week after emergence, showing that the latencies between emergence and first conception did not differ (Siutz et al. 2018). The timing of vernal emergence is therefore critical for subsequent reproductive success.

Our results suggest that the availability of additional high-quality food reserves at the time of emergence not only significantly affected the emergence date itself, but also the timing of conception and parturition. It appears that body mass at emergence did not influence the timing of reproduction, as seen in other hibernating species such as European ground squirrels (*Spermophilus citellus*) (Millesi et al. 1999a), where a lower emergence body mass resulted in a delayed oestrus, which, as Millesi et al. (1999a) suggested, is also reflected in smaller and late-emerging litters.

An earlier start into the active season, should give female hamsters more time to produce additional litters and thereby increase their reproductive output. Unfortunately, in our study, we were unable to determine litter number and size of our studied females. Nevertheless, previous studies indeed found significant positive correlations between emergence date, litter number and total offspring per season (Franceschini-Zink &

Millesi 2008). This study showed that early emerging females produced more litters and had more total offspring per season than those that delayed the onset of the active season. Body mass at emergence did not affect those parameters.

However, this high reproductive output did not affect the duration of the reproductive period in general (Franceschini-Zink & Millesi 2008). All females had a similar reproductive time span from the beginning of the first conception until the termination of reproduction in the respective year, no matter when they had emerged. Although successful females had a longer active and post-reproductive period, body mass before hibernation did not vary compared to females with a lower reproductive output, indicating that they were able to compensate the costs of their reproductive effort, by accumulating enough food (Franceschini-Zink & Millesi 2008). High variations within female individuals however are present. We can also expect these results for our supplemented females, but this must be investigated in further studies.

Females at one point need to end reproduction, not only because male hamsters are not available for reproduction throughout the season, as they too need to start preparing for hibernation, but also due to seasonal changes, hibernators are confronted with a limited active period. An example of how crucial the optimal start into the active season can be, is shown in a study by Hufnagl et al. (2011). Due to exceptional weather conditions, with a long and cold winter and snow cover until late March, emergence and the start of reproduction was clearly delayed for 2 weeks than in the years before. Litter size in the studied females was significantly lower, as was the reproductive output in general compared to the previous years. Especially litter size in the first and second litter per season differed from previous years. The first litters had fewer but heavier pups than the second ones. This drastic impact of harsh weather conditions furthermore led to a shortened active period with a shorter post-reproductive period.

To produce more offspring within a limited active period, female Common hamsters can have a postpartum oestrus, where they can be pregnant with their second litter, while still lactating (Grulich 1986, Franceschini-Zink & Millesi 2008). This would give them, in addition to early emergence, the opportunity to maximise their reproductive output. Even though we could not investigate this fact in our study, findings in literature demonstrate that early emergence has a significant effect on successfully rearing a postpartum litter (Franceschini-Zink & Millesi 2008). Interestingly, also Hufnagl et al.

(2011) suggested that even a late emergence in the season, due to very cold weather conditions led to 86% of the female hamsters successfully rearing a postpartum litter than in the preceding years where only 27% of the females had success. Not only adult hamsters, but also juveniles have very limited time to develop and prepare themselves for their first hibernation. An earlier birth date could be therefore advantageous for them to gain weight and to grow, as later in the season food availability may also decline. First-litter juveniles for example showed higher body mass and body fat content prior immergence than second-litter juveniles (Pluch et al. 2013). More time for achieving a better body condition would give them also the possibility to already reproduce in their first year, although this is not seen very often (Franceschini & Millesi 2008; Niethammer 1982). In addition, they also have a better chance to survive over winter (Siutz & Millesi 2012). Being more developed than later born juveniles in the following season, could also have a positive effect on their competitive behaviour. This would enable them to have better access to food and better chances of successful mating, following a higher reproductive outcome. As studies in male European rabbits have shown (Rödel & Holst 2009), male juveniles with higher nestling body mass, showed more escalated offensive behaviour against other juveniles.

As our focal female hamsters did not show any difference in body mass at emergence, we suspect that the additional food, that we have supplemented before immergence, has been not used during the hibernation period. It seems that, due to the availability of additional food stores in the hibernaculum, supplemented females were able to shorten their post-hibernation period and therefore emerging earlier in spring than the others. Having already enough food in their burrow, would save them time to search for food not only during reproduction, but also during gestation and lactation, reducing the time spent above ground and therefore reducing predation risk. Due to the lack of information of the reproductive output in our studied animals, we can only assume that the additional food stores might have been also available at least for the first litter to consume.

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Appendix

Zusammenfassung

Winterschlafende Säugetiere wie der Feldhamster (Cricetus cricetus) bewältigen die Herausforderungen saisonaler Wetteränderungen, wie niedrige Temperaturen und Nahrungsmangel, durch die Reduktion der Körpertemperatur und des Stoffwechsels, um Energie zu sparen. Zusätzlich legen Feldhamster Futtervorräte an, die eine wichtige Rolle als Energiereserve in der Winterschlafphase spielen. Hierbei zeigen Männchen und Weibchen unterschiedliche Verhaltensweisen, wenn es um die Nahrungsbeschaffung geht. Während Männchen in der aktiven Phase hauptsächlich Nahrung oberirdisch direkt aufnehmen, lagern Weibchen diese hauptsächlich in ihrem Bau ein.

In dieser Studie wurden freilebende Feldhamster vor dem Beginn des Winterschlafs mit 500 g Sonnenblumenkernen als zusätzlichen Nahrungsvorrat versorgt. Subkutan implantierte Temperaturdatenlogger zeichneten die Körpertemperaturen der Tiere vom Abtauchen in ihren Bau während der gesamten Winterschlafperiode, bis hin zum ersten Wiederfang der Tiere im Frühling auf. Die Baue der Tiere wurden regelmäßig beobachtet, um das erste Auftauchen nach der Winterperiode festzustellen. Ziel war es, die Auswirkungen der zusätzlichen Nahrungsvorräte auf die Körperkondition, die Winterschlafmuster sowie den ersten Reproduktionsstart zu analysieren, mit besonderem Fokus auf die Unterschiede im Beginn der Reproduktion zwischen Weibchen mit und ohne zusätzlichen Vorrat.

Unsere Ergebnisse zeigten, dass Männchen mit zusätzlichem Nahrungsvorrat während des Winterschlafs an Körpergewicht zugenommen, während Männchen und Weibchen der anderen Gruppe Gewicht verloren hatten. Weibchen verloren insgesamt mehr Körperfett als Männchen, insbesondere die zugefütterten Weibchen, die nahezu die Hälfte ihres Körperfetts eingebüßt hatten. Weibchen ohne Nahrungszusatz verloren mehr Gewicht, je länger sie in der euthermen Phase vor Beginn des Winterschlafs verblieben, währen Weibchen mit Nahrungszusatz weniger Gewichtsverluste in dieser Phase zeigten.

Interessanterweise tauchten die supplementierten Weibchen mit ähnlichem Körpergewicht wie nicht supplementierte Weibchen auf, obwohl sie deutlich früher aus

ihrem Bau im Frühling herauskamen, nämlich um etwa zehn Tage. Diese begannen auch entsprechend früher mit der Fortpflanzung. Der Zeitpunkt der ersten Paarung und Geburt lag bei den zugefütterten Weibchen signifikant früher in der Saison, was ihnen potenziell erlaubte, mehr Nachkommen innerhalb der begrenzten aktiven Periode zu produzieren. Frühere Geburten bieten den Jungtieren mehr Zeit zur Vorbereitung auf den ersten Winter, was sowohl ihr Überleben als auch ihre spätere Konkurrenzfähigkeit steigert.

Während Männchen die ergänzte Nahrung zur Verbesserung ihrer Körperkondition nutzten, scheinen Weibchen ihre Vorräte primär für die frühe Fortpflanzung und die Versorgung der ersten Würfe einzusetzen.

Die Studie konnte zeigen, dass ein größerer Nahrungsvorrat geschlechtsspezifische Auswirkungen auf den Winterschlaf, die Körperkondition und die Reproduktion von Feldhamstern hat. Weibchen profitieren insbesondere durch einen früheren Eintritt in die Fortpflanzungssaison, was langfristig ihre Reproduktionsleistung erhöht. Diese Ergebnisse zeigen die Bedeutung von ausreichenden Nahrungsressourcen für die Anpassung an saisonale Bedingungen und das Überleben von Winterschlaf-Säugetieren.