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RESEARCH ARTICLE

Interplay between group size, huddling behavior and basal metabolism: an experimental approach in the social degu

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ABSTRACT

Mammals exposed to low temperatures increase their metabolic rate to maintain constant body temperature and thus compensate for heat loss. This high and costly energetic demand can be mitigated through thermoregulatory behavior such as social grouping or huddling, which helps to decrease metabolic rate as function of the numbers of individuals grouped. Sustained low temperatures in endothermic animals produce changes over time in rates of energy expenditure, by means of phenotypic plasticity. However, the putative modulating effect that huddling exerts on the flexibility of the basal metabolic rate (BMR) due to thermal acclimation remains unknown. We determined BMR values in *Octodon degus*, an endemic Chilean rodent, after being acclimated to either 15 or 30°C during 60 days, both alone and in groups of three and five individuals. At 15°C, BMR of huddling individuals was 40% lower than that of animals housed alone. Moreover, infrared thermography revealed a significant increase in local surface temperatures in huddled animals. Furthermore, individual thermal conductance was lower in individuals acclimated to 15°C than to 30°C, but no differences were observed between single and grouped animals. Our results indicate that huddling prevents an increase in BMR when animals are acclimated to cold conditions and that this effect is proportional to the number of animals grouped.

KEY WORDS: Acclimation, Basal metabolic rate, Huddling, *Octodon degus*, Phenotypic plasticity

INTRODUCTION

Endothermy is defined as the ability to produce endogenous heat, allowing individuals to maintain a positive temperature differential with the environment and remain in homeothermic condition (Hill et al., 2004). Heat production may be modulated through behavioral and physiological changes at different scales and across a wide range of ambient temperatures (Gilbert et al., 2010). For example, animals exposed to temperatures below the thermoneutral zone (TNZ) must compensate for heat losses by increasing their metabolic rate in order to remain homeothermic (Canals, 1998). Thus survival of small mammals at low temperatures may depend on their ability to reduce heat loss and/or to increase metabolic rate, which in many cases involves a large energy cost (Kauffman et al., 2003).

To compensate for the increased energy expenditure caused by exposure to low temperatures, individuals may exhibit behavioral responses such as social grouping or huddling (Canals, 1998; Gilbert et al., 2010). Recently, Gilbert et al. (Gilbert et al., 2012) documented that local heating is crucial in reducing the extent of the cold challenge in huddling rabbit pups. Through thermal images, they demonstrated that at 14°C, the mean surface temperature of the huddle was higher than the mean temperature of isolated pups. Their study demonstrated that local heating when huddling provided each pup with an ambient 'public warmth' in the cold. Thus, huddling behavior reduces energy costs by reducing the metabolic rate and average thermal conductance of each individual in the group, mainly because of the reduction in surface area and altering the thermal environment experienced by animals exposed to the cold. In this sense, however, whilst in some species the benefit is shared in an equitable manner, in others it has been reported that some individuals may benefit more than others when huddling (Bustamante et al., 2002). Apparently, this asymmetry would result in some animals preferably occupying the best location in the group (i.e. the center), while others would be relegated to occupying the periphery a larger proportion of time (Schank and Alberts, 1997). Moreover, this reduction is proportional to the number of individuals in the group to the power of −0.33 (Gilbert et al., 2010; Canals and Bozinovic, 2011). For example, for *Octodon degus* (Molina 1782), a rodent that dwells in semi-arid areas of northern and central Chile, the huddling effectiveness [HE, the maximum energy saving during huddling (Canals et al., 1997)] when these organisms are exposed to temperatures of 0, 5 and 10°C reaches 43%, which constitutes a significant fraction of the body's energy budget (Canals et al., 1998). Therefore, at temperatures at least 5°C below the lower limit of the TNZ, the HE is constant, a phenomenon that was also reported for other mammal species. However, with increasing temperature above this critical value, HE tends to decrease (Canals et al., 1997).

Thus the capacity of endotherms inhabiting seasonal environments depends on their ability to develop physiological and behavioral mechanisms allowing physiological homeostasis (Pigliucci, 2001; Piersma and van Gils, 2011). In this vein, physiological flexibility, the ability to change and modify physiological traits in response to environment cues, is crucial for maintaining homeostasis in changing environments (Piersma and Drent, 2003; McKechnie et al., 2007) and has been demonstrated to occur in response to seasonal variations (Bozinovic and Contreras, 1990; Bacigalupe et al., 2004) and in laboratory thermal acclimation experiments in several mammalian species (Nespolo and Rosenmann, 1997; Nespolo et al., 2001). This physiological flexibility, a particular case of phenotypic plasticity (see Garland and Adolph, 1991; Piersma and Drent, 2003), can cause variations in thermal insulation as well as changes in basal (BMR) and maximum cold-induced metabolic rates ($\dot{M}_{sum}$). Thus, changes in any of these parameters may be indicative of changes in energy expenditure rates (Piersma et al., 1996; Nespolo et al., 1999). In particular, BMR represents the minimum rate of energy necessary to maintain homeostasis and reflects the cost of maintaining high levels of sustained activity. This metabolic trait exhibits high flexibility,

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which has been demonstrated in experiments in the laboratory by thermal acclimation (Nespolo and Rosenmann, 1997; Speakman, 2000). The reaction norm of BMR (i.e. a function that describes the changes in BMR as a function of acclimation temperature) exhibits a negative association, that is, endotherms acclimated to low temperatures commonly exhibited an increase in their BMR. Such an increase in BMR is thought to be related to the increase in maintenance costs of metabolically active organs when animals are faced with the high energy requirements of thermoregulation in the cold (see Cruz-Neto et al., 2003; McKechnie et al., 2007).

Experimental evidence has shown the existence of (1) decreased rates of individual energy expenditure (e.g. resting metabolic rate) in grouped organisms when exposed to temperatures lower than the TNZ and (2) a remarkable physiological flexibility of rates of energy expenditure when acclimated to different temperatures. In the former case, huddling allows energy savings during the grouping behavior, whereas physiological flexibility modifies the rates of energy expenditure in the medium term (e.g. weeks) in order to cope with the different thermoregulatory needs. However, to date, there is no evidence that the use of huddling may affect medium- to long-term rates of energy expenditure in endotherms, i.e. that huddling behavior affects the phenotypic response of individuals acclimated to different temperatures. The aim of the present study was to estimate the effect of social grouping on flexibility in BMR, transepidermal water loss and thermal conductance in *O. degus* or degus, a social rodent that exhibits huddling behavior both in captivity and in the wild (Ebensperger and Wallen, 2002) and dwells in highly seasonal environments of central Chile (Di Castri and Hajek, 1976). We test the hypothesis that the presence of huddling and group size plays a modulating role in the acclimation capacity of BMR in adults. Specifically, we predict that huddling will decrease or prevent an increase in BMR when animals are acclimated to cold conditions and that this effect is proportional to the number of animals grouped.

RESULTS

After thermal acclimation, we found a significant effect of body mass on total BMR. The allometric equation relating BMR with body mass (M_b) was: BMR=7.9$M_b^{0.53}$ (r^2=0.21, $F_{1,25}$=13.88, P=0.009). We also found a significant effect of acclimation temperature ($F_{1,25}$=6.98, P=0.014), the number of individuals grouped ($F_{2,25}$=9.27, P=0.001) and the interaction between these two factors on mass-adjusted BMR ($F_{2,25}$=6.92, P=0.004). The *post hoc* test revealed that mass-adjusted BMR of rodents acclimated individually at 15°C was greater than that observed in rodents acclimated individually at 30°C (Fig. 1). In groups of three, *O. degus* decreased mass-adjusted BMR by 15 and 7% when acclimated at 15 and 30°C, respectively (Fig. 1). Additionally, cold-acclimated animals in groups of three exhibited higher mass-adjusted BMRs than those of warm-acclimated animals in groups of three, but this difference appeared to be smaller than that observed in individually acclimated animals. Compared with those housed alone, in groups of five the decrease in mass-adjusted BMR was ~40% for degus acclimated to 15°C. However, there was no significant energy reduction for degus acclimated to 30°C. Finally, mass-adjusted BMRs of animals in groups of five individuals did not present significant differences between warm- and cold-acclimated groups. Furthermore, the mass-adjusted BMR of cold-acclimated animals in groups of three was similar to that of cold-acclimated animals in groups of five individuals. The same was true for warm-acclimated animals for these two groups. Also, we found a significant effect of acclimation temperature on thermal conductance in *O. degus*

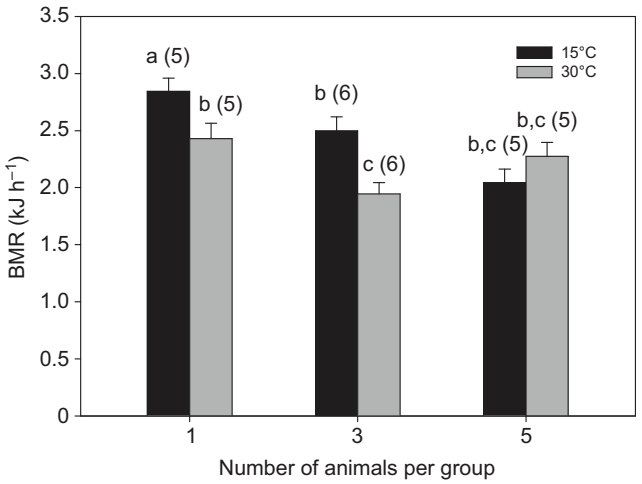

Fig. 1. Mass-adjusted basal metabolic rate in *Octodon degus* as a function of the number of individuals grouped after a thermal acclimation of 2 months to two temperature conditions. The black bars represent the mean ± s.e.m. basal metabolic rate (BMR) of animals acclimated to 15°C, and the white to 30°C. Similar letters denote non-significant difference between treatments by a Fisher *a posteriori* test.

(ANOVA, $F_{1,29}$=6.47, P=0.016; Table 1). Specifically, we found that the average individual conductance of animals was greater in rodents acclimated to 30°C than to 15°C (Fig. 2). However, we found no effect of group size ($F_{2,29}$=1.56, P=0.22) or the interaction between factors ($F_{2,29}$=0.90, P=0.41) on thermal conductance.

We found significant differences in measured temperatures between treatments ($F_{9,71}$=165.9, P<0.0001). The *a posteriori* analyses showed that maximum surface temperature and mean contour temperatures were higher in grouped than isolated animals at 15°C, but not at 30°C. Moreover, at 15°C, the temperature of contact was higher than the contour temperatures of grouped and isolated animals (Fig. 2). We also found that the spatial location of individuals in the group was different between individuals (χ^2=16.94, d.f.=4; P=0.02). Some individuals spent more than five times as long in the center of the group than others. The 95% confidence intervals of the frequencies that each animal spent in the center of the group were: [0.06–0.24], [0.07–0.26], [0.03–0.17], [0.17–0.40] and [0.26–0.51]. Finally, we found that solitary animals consumed significantly more food than grouped animals at 15°C, which in turn consumed more food than animals maintained at 30°C (Kruskal–Wallis ANOVA: $H_{3,20}$=16.63, P=0.001). In descending order, the food intake per animal was as follows: solitary animals at 15°C (29.98±4.87 g day^{-1} animal^{-1}) > grouped animals at 15°C (19.86±2.35 g day^{-1} animal^{-1}) > grouped animals at 30°C (10.47±2.17 g day^{-1} animal^{-1}) = solitary animals at 30°C (8.02±1.70 g day^{-1} animal^{-1}).

DISCUSSION

Our results confirm the previously demonstrated effect of short-term thermal acclimation on energy expenditure rates in endotherms. In fact, the difference in BMR between solitary cold- and warm-acclimated animals can reach ca. 30%, which is in the range of the acclimation magnitudes reported for other rodent species in comparable studies (e.g. Novoa et al., 2005; Nespolo et al., 2001). However, our results demonstrate for the first time that huddling behavior exerts a modulatory effect on thermal acclimation of BMR in endotherms. Increasing the number of individuals to three animals per group and allowing them to huddle during acclimation led to a

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Table 1. Body mass, whole-animal basal metabolic rate (BMR) and wet thermal conductance (C_t) of *Octodon degus* acclimated at either 15 or 30°C for 2 months individually or in groups of three and five

Ambient temperature (°C)	Group size	Body mass (g)	BMR (kJ h^{-1})	C_t (J g^{-1} h^{-1} $°C^{-1}$)
15	1	152.27±12.20	2.78±0.24 (8.7)	0.67±0.25
	3	176.55±9.80	2.59±0.25 (9.6)	1.25±0.84
	5	148.37±17.70	1.96±0.45 (23.1)	1.30±0.38
30	1	192.27±10.37	2.60±0.23 (8.2)	1.50±0.33
	3	157.44±8.16	1.92±0.36 (18.8)	1.72±0.17
	5	146.33±13.60	2.19±0.32 (14.6)	1.97±0.42

Data are presented as means ± s.d. Coefficients of variation (%) are shown in parentheses.

reduction in the difference in BMR between temperatures. When animals were allowed to form groups of five individuals, BMR was lower than solitary animals when acclimated at 15°C, suggesting that degus were acclimated to different effective temperatures. In fact, huddling promotes local heating and reduces the cold challenge, because single degus experienced colder conditions compared with degus huddling in a group of five (Fig. 2), a phenomenon that has been also recently demonstrated in rabbit pups (Gilbert et al., 2012). It is therefore likely that the individual minimum energy requirement decreases in degus that huddle for long periods, as occurs with the energy expenditure of this species when grouped (Canals et al., 1989; Kotze et al., 2008). In this vein, the observed reduction in the acclimation response of individual BMR in grouped degus (ca. 40%) is comparable with the reduction of metabolic rate by means of huddling of grouped individuals in other mammal species [range: 11–50% (see Canals et al., 1989; Canals et al., 1998, Gilbert et al., 2010 and references therein)]. Moreover, the fact that individuals acclimatized at 30°C and maintained in groups of five individuals did not save significant amounts of energy agrees with the idea proposed by Canals et al. (Canals et al., 1998), which states that the huddling efficiency decreases at temperatures near the TNZ of the animal, which for *O. degus* corresponds to the range between 24 and 32°C (Rosenmann, 1977).

Thus huddling allows energy savings over at least two time scales. First, huddling induces metabolic depression of animals while grouped, as has been demonstrated previously (e.g. Canals et al., 1997). Second, our results strongly suggest that in degus acclimated to low temperatures and in groups of three or more individuals, individual energy expenditure (BMR) decreases compared with in degus subjected to the same temperatures but acclimated

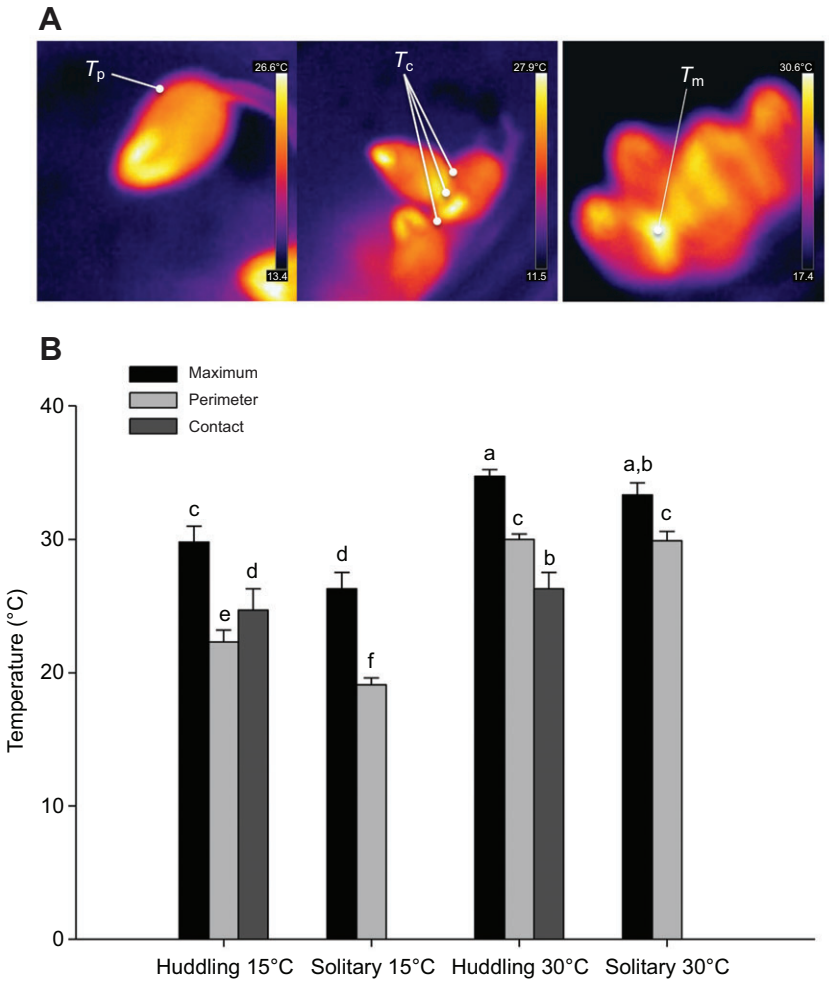

Fig. 2. The effect of group size on surface temperature in *Octodon degus*. (A) Thermal images are shown for isolated and huddling degus exposed to an ambient temperature of 15°C. Examples of measurements of the temperature of the perimeter (T_p), maximum temperature (T_m) and temperature of the contact between individuals (T_c) are shown. (B) Surface temperatures of the back (maximum), perimeter and contact of isolated and huddling *Octodon degus* at 15 and 30°C. Similar letters denote non-significant differences between treatments by an *a posteriori* Fisher test.

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individually. Huddling therefore helps mitigate the excessive expenditure of energy to maintain homeothermy, buffering the decreasing ambient temperature by decreasing its thermogenic response. This is also supported by the observed decrease in food consumption when animals were grouped at low temperatures. Interestingly, the low food consumption may lead to a decrease of the masses of digestive organs (not measured), which suggests that BMR changes may reflect changes in the mass of metabolically active organs. Accordingly, some studies have shown a significant increase in size of metabolically active organs such as the heart and liver when exposed to cold (Hammond et al., 2001; Naya et al., 2010). This possible proximal mechanism, not addressed in this paper, could be considered in future studies.

Energy economy may be beneficial for rodents inhabiting arid or semi-arid environments, where animals are exposed to large temperature variations and heterogeneous distribution of food (Scantlebury et al., 2006). The energy savings observed in the laboratory probably can also have consequences in the field. Thus, decreasing thermogenic response by means of huddling may lead to a decrease in food needs and thus an increase in the allocation of time to activities other than foraging. Alternatively, because energy is limited for animals, the energy saved by huddling may be allocated to other biological functions or activities, while acting as a selective pressure important for life in groups in rodents (Gilbert et al., 2012; Ebensperger and Wallen, 2002; Schradin et al., 2006). For example, in *O. degus*, some activities associated with foraging may be energetically costly, such as digging in dry areas with low humidity (Ebensperger and Bozinovic, 2000), exploration behavior in open spaces at low temperatures after foraging, and building burrows (Torres-Contreras and Bozinovic, 1997; Quispe et al., 2009). All these activities may increase the biological fitness of *O. degus*, particularly in periods of high energy demand such as winter and during the breeding season.

In this way, individuals can regulate the levels of energy expenditure to maintain their functional capabilities in variable thermal environments through plastic and adaptive changes in metabolic rate, and reassign the energy saved to other activities (Nespolo, 2000). However, Bozinovic et al. (Bozinovic et al., 2004) reported that the BMR in *O. degus* in the wild is not altered by seasonality in a Mediterranean environment of central Chile. Indeed, these authors found that the BMR remains unchanged throughout the year. Interestingly, the average BMR values reported for wild degus are very similar to those of cold-acclimated degus in groups of three and five individuals. As *O. degus* is a social rodent that lives in groups of four or more individuals sharing the same burrow system and performs huddling in nature, one might infer that thermoregulatory behavior such as huddling and its long-term effects could be a compensatory mechanism that helps maintain this rodent BMR relatively stable over the year.

There are several factors that can affect metabolic expenditure of animals performing huddling. Among the most important are decreasing body area exposed to the environment (Canals et al., 1998) and the local microclimate (Hayes et al., 1992). In agreement with that, our results revealed that the surface temperature of the entire group exposed to 15°C was higher than the surface temperature of solitary degus, and that such a difference is coupled with the increased temperature of the boundary of each animal when huddled (Fig. 2). An additional hypothesis, the so-called socio-physiological effect (Speakman and Rossi, 1999), states that there would be a decrease in metabolic rate of animals grouped because they tend to decrease their levels of anxiety when in proximity to conspecifics (Martin et al., 1980). However, the socio-physiological factor did not have a significant effect in this study, because individuals grouped in five and acclimated at 30°C did not exhibit a significant reduction compared with the BMR observed in solitary individuals (Fig. 1).

Furthermore, the decrease in energy expenditure individually within the group conducting huddling also has a per capita cost because the fuel consumed by thermogenesis is generated individually, although the benefits are shared by the group (Haig, 2008). In this vein, it is possible that some individuals could benefit more than others in huddling. Our video record revealed that some degus of our group treatments may have benefited more than others and thus decreased their BMR more, which is consistent with the observation of a higher coefficient of variation in BMR in grouped than in non-grouped acclimated degus. According to Bustamante et al. (Bustamante et al., 2002), small individuals of the rodent *Phyllotis darwini* are most favored by the larger individuals huddling, especially at temperatures below the TNZ. Further experimental studies will need to assess whether all individuals in the group performing huddling receive the same benefits, as for example, individuals who are dominant competitors and can occupy the best location in the center of the group (Schank and Alberts, 1997). In this vein, experiments of Bautista et al. (Bautista et al., 2008) showed that the offspring of rabbits (*Orytolagus cuniculus*) share the thermal advantages by moving continuously within the group. It would be interesting to study the effect of body size, ontogeny and social hierarchy within the group on the medium-term (acclimation effect) energy benefits of huddling in adult and juvenile *O. degus*. Furthermore, from a comparison viewpoint, it would also be interesting to perform acclimation experiments in other species that exhibit different degrees of sociability.

Individual thermal conductance of *O. degus* was lower only in individuals acclimated at 15°C, irrespective of group size. Thus the results of this study suggest that compared with the temperature factor, the grouping effect is not so important. It is possible that the metabolic changes due to huddling are less rigid than the changes that occur at the morphological level, such as increased length and density of the fur, which could in turn restrict their movement (Cutrera and Antinuchi, 2004). Future studies will then be necessary to determine the mechanisms that explain the association between metabolic changes and thermal conductance acclimatization *O. degus*.

The convergence of huddling behavior among animals is a well established evolutionary event, emperor penguins being a notable case (Gilbert et al., 2010). As such, it is possible that this phenomenon might also occur in other endotherms, including birds, in which it has been demonstrated can reduce energy expenditure by comparable amounts when huddling (MacKechnie and Lovegrove, 2001; Wojciechowski et al., 2011). In summary, during huddling, animals group together and maintain close bodily contact, which is particularly important for decreasing thermoregulatory costs and for increasing survival times when environmental conditions are harsh. Huddling induces metabolic depression without hypothermia and is mainly attributed to the reduced surface area to volume ratio of the huddling group and to the increase of effective temperature. The novelty of our results is that huddling not only decreases metabolic rate in individuals while grouped, but also provides significant energy savings at the individual level that persists after huddling. Finally, further studies are necessary to assess whether huddling behavior could modulate thermal acclimation in other species of endotherms.

MATERIALS AND METHODS

Thirty-two adult individuals of *O. degus* were trapped with Shermann traps in autumn–winter 2011 in the Quebrada de la Plata, Chile (33°28′S, 70°54′W).

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Individuals were transported to the laboratory in Santiago, Chile, where they were kept in cages of 2.0×2.5×2.0 m (length × width × height) with food and water provided *ad libitum* for 1 month, to minimize the possible effects of the previous thermal experience (see Nespolo and Rosenmann, 1997). Then individuals were randomly divided into two groups of 16 individuals. One group was acclimatized at 15°C and the other at 30°C for 2 months, with water and food provided *ad libitum* and with a photoperiod of 12 h:12 h light:dark. Each acclimation group was divided into three treatments: solitary individuals (n=5), two groups of three (n=6) and one group of five (n=5) individuals in plastic cages (87×33×32 cm, length × width × height). We decided to use a maximum size group of five because five individuals exhibited the largest energy savings by huddling in this species (Canals et al., 1989). All observations and measurements were performed on adult males and females with a body mass (mean ± s.d.) of 173±25.7 g (males) and 147.92±23.76 g (females). No differences in body mass between sexes (P=0.1) or between groups of different size were found (P=0.09).

All protocols were approved by the Institutional Animal Care Committee of the University of Chile.

Respirometry

Following acclimation, BMR was measured as the oxygen consumption rate using a computerized open-flow respirometry system (Sable Systems, Henderson, NV). For BMR determinations, post-absorptive animals were placed individually in stainless steel metabolic chambers (5000 ml) for 12 h in the rest phase (night) in the dark at 30.0±0.5°C, which is within the TNZ zone of this species (Rosenmann, 1977). To calculate thermal conductance, we determined oxygen consumption at two temperatures below the TNZ (20 and 10±1°C) for at least 2 h or until a visual inspection of the recorded data allowed us to determine when steady-state conditions had been achieved. With these values of oxygen consumption, we estimated heat loss using the following equation:

$$C = \frac{\Delta M}{\Delta T}, \quad (1)$$

where C is thermal conductance in J g^{-1} h^{-1} ºC^{-1}, ΔM is the difference between metabolic rates measured at 20 and 10ºC, respectively, and ΔT is 10ºC. Values of oxygen consumption were transformed into Joules using the caloric equivalent of oxygen of 20.17 J ml^{-1} O_2. Briefly, external air (700 ml min^{-1}) was drawn into the metabolic chamber by negative pressure created by a downstream vacuum pump controlled by a Sierra mass flowmeter/controller (Sierra Instruments, Monterey, CA), which was calibrated monthly with a volumetric flowmeter. Before arriving in the chamber, the air was dried using Drierite desiccant and passed through Bev-A-Line tubing (Thermoplastic Processes). The excurrent air from the metabolic chamber passed through Drierite, Baralyme and Drierite to remove water vapor and CO_2 gas before being passed through the O_2 analyzer (model FoxBox, Sable Systems). The open-flow respirometry system was calibrated with a known mixture of oxygen (20%) and nitrogen (80%) that was certified by chromatography (INDURA, Chile). Because CO_2 and water vapor were scrubbed before entering the O_2 analyzer, oxygen consumption ($\dot{V}_{O_2}$) was calculated according to Withers (Withers, 1977):

$$\dot{V}_{O_2} = \mathrm{FR} \times 60 \times \frac{F_{I_{O_2}} - F_{E_{O_2}}}{1 - F_{I_{O_2}}}, \quad (2)$$

where FR is the flow rate (ml min^{-1}) and $F_{I_{O_2}}$ and $F_{E_{O_2}}$ are the fractional concentrations of O_2 entering and leaving the metabolic chamber, respectively. In order to confirm that animals were euthermic after the metabolic trials (T_b=37–38°C), we recorded their rectal body temperature (T_b) with a Cole Palmer 24-gauge copper-constantan thermocouple attached to a Digisense thermometer (model 92800-15). Outputs from the oxygen analyzer (%) were digitized using a Universal Interface II (Sable Systems) and recorded on a personal computer using data acquisition software (EXPEDATA, Sable Systems). Our sampling interval was 5 s. We averaged the O_2 concentration of the excurrent air stream over an entire record period of 30 min after the lower steady state was reached (following Bozinovic et al., 2009). To assess whether changes in thermal/huddling conditions were accompanied by changes in energy intake, we measured food consumption of grouped (n=5) and solitary animals at 15 and 30°C. At the end of the acclimation period, animals were placed in metabolic cages to estimate food intake in 48-h trials. We fed animals with a known amount of pellet (rabbit food, Champion®) and after 48 h we collected the uneaten food. Samples were dried at 90°C to constant mass (±0.001 g), and the intake was calculated by subtracting the uneaten from the total offered food.

Thermography and behavior

In a second experiment, the surface temperature of solitary degus and degus in groups of five was recorded at the end of acclimation periods using a thermal imaging camera FLIRi40 calibrated at FLIR Systems Brasil (2011, wwwFLIR.com). Images were recorded at a height of 1 m above the chamber and at two ambient temperatures of 15 and 25°C. Thermal images were analyzed using the ad hoc software FLIR QuickReport 1.3 SP1, with a fur emissivity 0.98. Surface temperatures were averaged from 10 images for each treatment taken at the end of the respirometry periods. The contour surface temperature of degus was determined by fitting a polygon around the individual animal in the case of isolated pups and around the entire huddle for grouped animals using the option 'isotherm' of the software. This isotherm is the average temperature along the polygon, which defines the limit above and below temperatures observed in the thermal image. The maximum temperature of degus was determined using the option 'area' of the software. After fitting the area of huddled or solitary animals, a unique maximum temperature value was recorded for each thermograph. Furthermore, we determined the temperature of contact between pairs of individuals in grouped animals using the option 'mobile', by fitting a line of contact between paired animals, dividing it into five sections and using the four points inside the sections in order to obtain the average surface temperature of contact between two animals. Then we averaged those temperatures to calculate the average temperature of contact of the entire group. Finally, in order to assess whether grouped animals constantly changed positions, we determine the proportion of time that each animal spent in various positions within the group. For this, we marked each individual with hair dye on the back and recorded ten 2-h films each in a group of five animals kept at 15°C. The measurements were performed at 1 m height using a Sony NP-FV50. The films were analyzed on a personal computer by recording the time each individual spent in the center, on the periphery of the group or isolated while engaged in huddling in groups of five individuals. In order to maximize the time that each animal was analyzed and because the groups are not always formed with the maximum number of individuals (i.e. five), video recordings were analyzed only when the animals spent more than 15 min clustered in groups of at least four individuals. The proportion of time that animals were engaged in such behavior approached 87%.

Statistical analysis

We performed a general lineal model to compare BMR between treatments using log BMR and thermal conductance as the dependent variables; sex, temperature and the number of individuals in the group as fixed factors; and log body mass as the covariate. Because our analyses exhibited a non-significant effect of sex ($F_{1,19}$=0.84, P=0.77 and $F_{1,19}$=0.63, P=0.43, for BMR and C, respectively) this term was dropped from the models. Our analysis also revealed that thermal conductance was not related to body mass in degus ($F_{1,30}$=0.99, r^2=0.03, P=0.33), thus, we only performed an ANOVA to test differences among experimental groups. Then a *post hoc* Fisher's test was performed to determine significant differences in mass-adjusted BMR (least square means calculated from ANCOVA) and thermal conductance between specific groups. To analyze the association between physiological variables and body mass, a simple linear regression was performed. All the data met the assumptions of the ANOVA. Surface and contour temperatures of the different experimental conditions (grouped and solitary at 15 and 30°C) were compared with one-way ANOVAs and then a *post hoc* Tukey's test was performed to check for specific differences among treatments. The proportion of time each animal spent in the center of the group was evaluated by means of counting the number of times each animal was viewed in this position in random analyses. Each tape record was analyzed through random selection of five periods of 6 min by trial (2 h each). We hence analyzed a total of 50 records for which we obtained 239 individual observations. We counted as positive when the subject was surrounded by at least two animals, and negative when

the subject was located at the periphery of the group. Then, we calculated the observed frequency of times that each animal spent in the center of the group and the expected frequency by chance (i.e. total number of observations divided by the number of animals). A chi-square test was used to estimate significant differences among individuals. Food intake was evaluated by means of a nonparametric Kruskal–Wallis test and then a multiple comparison of mean rank was performed. All results are reported as means ± s.e.m. All analyses were performed using Statistica 7.0 software (StatSoft Inc., Tulsa, OK, USA).

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Competing interests
The authors declare no competing financial interests.

Author contributions
P.S. and M.N.-V. conceived and designed the metabolic experiments; P.S., M.N.-V and F.B. designed the thermography experiments. M.N. performed all experiments. All the data and analyzed results were examined carefully and discussed by all authors. The final manuscript was read and approved by all authors.

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