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Regional variation of white adipocyte lipolysis during the annual cycle of the alpine marmot

Nathalie Cochet [a,*], Roger Meister [a], Gregory L. Florant [b], Hervé Barré [a]

[a] *Laboratoire de Physiologie des Régulations Energétiques, Cellulaires et Moléculaires, Université LYON I, UMR 5578, bat 404 CNRS-UCB Bd du 11 Novembre 1918, F-69622, Villeurbanne Cedex, France*

[b] *Department of Biology, Colorado State University, Fort Collins, CO, 80523, USA*

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Abstract

During winter, hibernating animals rely on their lipid stores for survival. In vitro lipolytic activity of isolated adipocytes from gonadal and subcutaneous white adipose tissue (WAT) was studied in captive alpine marmots (*Marmota marmota*) at two different times of their yearly cycle. During the summer, when marmots were eating, adipocyte responsiveness and sensitivity to isoprenaline and noradrenaline were higher in gonadal than in subcutaneous WAT. During hibernation, when marmots were spontaneously fasting, both the response and sensitivity to catecholamines decreased in gonadal WAT to the level of subcutaneous WAT. A similar pattern of response was also observed when lipolysis was stimulated with glucagon but the lipolytic rate was three times lower than with catecholamines. Adenosine deaminase (ADA) had a marked stimulatory effect on lipolysis, especially during the 'feeding' period, suggesting that adenosine may be a potent lipolytic modulator in marmot adipocytes. It is concluded that in marmots, lipolysis could be differentially regulated between fat depots during the annual cycle possibly to optimize either the building-up or the use of fat reserves.

Keywords: Isolated adipocytes; Lipolysis; Fasting; *Marmota marmota*; White adipose tissue; Adenosine deaminase; Glucagon; Catecholamines

1. Introduction

Alpine marmots (*Marmota marmota*) have a marked seasonal cycle of food consumption and body mass [24] similar to that reported in yellow-bellied marmots (*Marmota Flaviventris*) [1,14]. During summer time (from April to September), marmots eat and accumulate large amounts of lipids in both internal and subcutaneous adipose depots. Later, in the fall, marmots spontaneously cease to feed just before entering hibernation and keep fasting during the whole hibernation season. Consequently, fat depots prior to hibernation constitute an important energy source for surviving the 5–6 months of fasting during winter. In some hibernators, the amount of lipids depleted during winter can be up to 80% [8]. The relative use of the various body fat stores has been investigated in hibernators. It was earlier thought that subcutaneous fat depot would represent a major compact form of energy storage in hibernators, rather than an insulating layer [37]. More recently, a preferential lipid depletion of internal depot is reported in male Siberian hamsters (*Phodopus sungorus*) on short day exposure [2]. By contrast, lipid depletion is found to be uniform in several fat depots of hibernating ground squirrels (*Spermophilus lateralis* [8]). The contribution of subcutaneous fat to FA mobilization may therefore vary among species.

Such differential lipolytic response of various fat depots may depend on differential hormonal stimulation. In most species, fat cell lipolysis is mainly controlled by catecholamines via the lipolytic β (β1, β2 and β3) -adrenoceptors and the antilipolytic α2-adrenoceptors (reviewed in [26]). These adrenoreceptors regulate, via adenylate cyclase, hormone-sensitive lipase activity which catalyzes triacylglycerol hydrolysis and free fatty acid release. In hibernators, it is likely that the succes-

Abbreviations: WAT, white adipose tissue; ADA, adenosine deaminase; EC_{50}, efficient concentration to produce 50% of the maximum response.

* Corresponding author. Tel.: +33-0472-431173; fax: +33-0472-431172.

E-mail address: ncochet@physio.univ-lyon1.fr (N. Cochet)

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sion of periods of intensive food intake and subsequent fasting is associated with changes in the adipocyte hormonal environment, adipocyte responsiveness and lipolytic pathways during the yearly cycle. Surprisingly, this aspect has not received considerable attention.

Adenosine may also play a role in the modulation of the fat cell lipolytic rate. Adenosine is produced by adipocytes in vitro [33] and released extracellularly in vivo, in response to sympathetic nerve stimulation [18]. It acts on its own receptor (A1-antilipolytic) which is coupled to the inhibitory G protein subunit. It should be noted that the antilipolytic effect of adenosine on adipocytes is mostly described in non-hibernator species [12,17], and its physiological relevance still remains difficult to establish. For hibernating species like marmots, in which adipose tissue presents a marked insulin-resistance during the hibernation season [5,27,35], adenosine would represent a potential regulating factor of in vivo lipolysis.

The authors hypothesized that the white adipose tissue depots of marmots would respond differently to lipolytic and antilipolytic agents at different times of the year. As such, the present study investigates the changes in lipolytic rates of white adipocytes from gonadal and subcutaneous depots of captive marmots during each season.

2. Materials and methods

2.1. Animals

The experiment was performed on eleven adult Alpine Marmots of both genders (5 males and 6 females), trapped in the French Alps (Bonneval-sur-Arcs, Savoie) and kept in captivity in the laboratory for 2 years before the experiments. From May to November, marmots were caged in a room exposed to outdoor conditions of photoperiod and temperature which ranged from 15 to 25°C. During the hibernation season, marmots were placed in a cold dark room with an ambient temperature of 6 ± 1°C. Food (rodent laboratory food, Genthon, France) and water were provided ad libitum after the emergence from hibernation (March–April) and were removed when the animals ceased to feed in late fall (November).

2.2. Adipose tissue sampling

Marmots were biopsied at two different times of their yearly cycle: (a) in winter, when all were both spontaneously fasting and hibernating; (b) in summer, when all were both active and eating (Fig. 1). During the summer period (from mid-July to early September), that was named 'feeding' period, marmots were kept overnight without any food, as a precaution before the surgery (the Marmot is a diurnal species and used to eat during the day, not at night). During the winter (from mid-January to mid-February), that was named 'fasting' period, hibernating marmots were taken out from the cold room and kept overnight at 20°C for complete arousal (body temperature close to 37°C). In both cases, 'feeding' as 'fasting' marmots were maintained under a physiological fast. Euthermic marmots were then tranquilized with 20 mg kg^{-1} of zoletil 100 (Reading laboratories, Virbac, France) and few gram samples of gonadal (internal depot chosen for its easy accessibility and its known sensitivity to hormones) and inguinal subcutaneous WAT were surgically removed. After the winter surgeries, marmots were kept a few days at room temperature (20°C) to recover, and then, placed back into the cold room.

2.3. Adipocyte isolation

Adipocytes were isolated as described by Rodbell [31] with slight modifications. Adipose tissue from each depot was cut into thin pieces in a Krebs–Hepes buffer (Hepes 5 mM, pH 7.4), containing 5% (wt/vol.) fatty acid free bovine serum albumine, 6 mM glucose and maintained at 37°C. Adipose tissue pieces were rinsed twice with buffer and digested in polypropylene vials with collagenase (0.3 mg ml^{-1}) during 30 min in a shaking water bath at 37°C. The two different suspensions were then filtered and isolated cells were rinsed

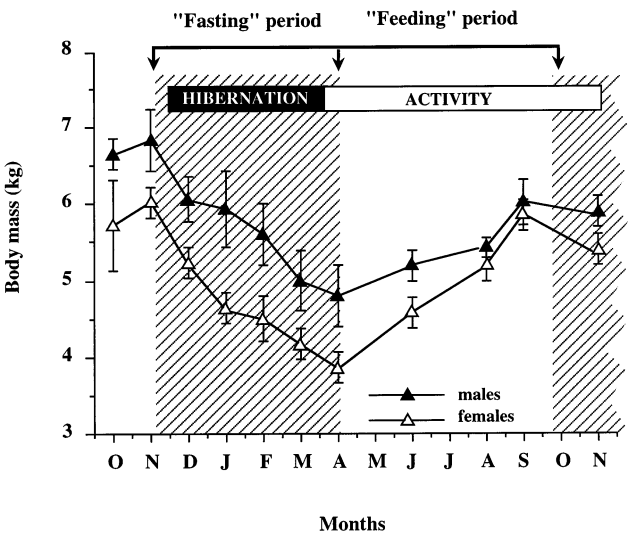

Fig. 1. changes in body mass of alpine marmots over the yearly cycle. Body mass of females ($n = 6$, open triangles) and males ($n = 5$, filled triangles) are expressed in kilograms. The striped background indicates when marmots were spontaneously fasting. Frames on the top indicate when marmots were hibernating (filled) or active (open). Marmots were sampled at two different times of their yearly cycle: in winter (mid-December to mid-February), when they were both spontaneously fasting and hibernating (named the 'fasting' period) and in summer (mid-July to early September) when marmots where both active and eating (named the 'feeding' period).

three times with the buffer at 37°C. A slightly diluted adipocyte suspension was prepared. An aliquot was sampled and stored in chloroform/methanol (2:1, vol:vol) to determine extemporaneously the cellular lipid content using the method of Folch et al. [16]. The cell content of the remaining suspension was immediately evaluated according to Di Girolamo et al. [10]: siliconized calibrated tubes (internal diameter: 1.6 mm; length: 95 mm) were filled with an aliquot of each suspension and sealed. After a light centrifugation, the ratio of the packed cell volume to the total suspension volume was determined. Then, adipocytes were diluted in the Krebs Hepes buffer to obtain a 30–33 µl isolated cells per ml suspension. Aliquots of this final adipocyte suspension (300 µl) were incubated in 2 ml Eppendorf polypropylene vials in a shaking water-bath for 45 min at 37°C, in the presence of noradrenaline, isoprenaline or glucagon at various concentrations. Basal lipolysis was measured with or without adenosine deaminase (ADA, 0.4 U ml^{-1}), which inactivates adenosine. At the end of the incubation time, tubes were rapidly spun and the upper cellular layer was discarded by vacuum suction. Lipolytic activity was quantified by dosage of the glycerol released in the incubation medium. Using the cellular lipid content and the suspension cell content, results were expressed in nmol of glycerol released per 100 mg of cellular lipids per hour.

2.4. Statistics

A total of 11 marmots were studied at each of the two periods: seven animals were sampled twice, i.e. once during the 'fasting' period and once during the 'feeding' period. Two more animals were sampled once during the 'fasting' period only and two others were sampled once during the 'feeding' period only. In a first time, statistics on male and female data were performed separately. In the absence of any significant effect of the gender, both male and female data collected on the same period were pooled together. The adipocyte response to each lipolytic stimulator was compared between depots and periods at each concentration using a one repeated factor 3-way ANOVA with Bonferoni/Dunnett posthoc tests. Differences were considered significant when $P < 0.05$. The half-maximal responses (EC_{50}) with their 95% confidence intervals have been determined by fitting lipolysis values to a sigmoidal equation as described by Honnor et al. [22] and calculated with the software Graphpad Prism according to the method of Motulsky and Ransnas [29].

2.5. Chemicals

Collagenase P and fatty acid free Bovine serum albumine were obtained from Boheringer–Mannheim. Noradrenaline ((−) arterenol bitartrate salt), isoprenaline ((−) isoproterenol hydrochloride crystalline), adenosine deaminase suspension from calf spleen and glycerol reagent kit (Triglyceride GPO-trinder) were purchased from Sigma Chemicals, France. Glucagon was from Novo Nordisk.

3. Results

3.1. Body mass changes during the cycle

Marmot body mass fluctuated during the experimental period which was studied, confirming that the captive animals maintained a yearly cycle of food consumption and body mass (Fig. 1). Marmot body mass peaked just before the first 'fasting' period (males: 6.8 ± 0.9 kg, $n = 5$; females: 5.8 ± 0.6 kg, $n = 6$), decreased during hibernation and was minimal at the end of the 'fasting' period (males: 5.0 ± 0.9 kg, -30%; females: 3.9 ± 0.5 kg, -33%). Body mass increased again during the following spring and summer to peak in late September (males: 5.9 ± 0.2 kg, females: 5.6 ± 0.2 kg). The absence of a significant difference in the % of body mass loss between males and non-pregnant females after the hibernation season might suggest that the degree of mass loss is not sex-related in marmots.

3.2. Basal lipolysis and effect of adenosine deaminase

In gonadal WAT, basal lipolytic rates of adipocytes were similar for the two periods considered (Fig. 2). In subcutaneous WAT, the basal lipolytic rate of adipocytes was significantly higher during the 'fasting' period (including hibernation) than during the 'feeding' period. Despite trends, basal rates were not statistically different between depots at any period. In the two depots, ADA (0.4 U ml^{-1}) markedly increased the basal lipolytic rate during both 'feeding' and 'fasting' periods ($P < 0.0001$), suggesting that marmot WAT could be highly sensitive to adenosine (Fig. 2). Compared to the basal rate, the stimulatory effect of ADA was 82 (gonadal WAT) to 160 times (subcutaneous WAT) higher during the 'feeding' than the 'fasting' period (52 and 47 times, respectively, Fig. 2). Consequently, when ADA was added, the lipolytic rate of gonadal adipocytes was significantly higher during the 'feeding' than the 'fasting' period while no different rate was observed with subcutaneous adipocytes between these two periods.

3.3. Effect of isoprenaline

Isoprenaline, a general β-adrenoceptor agonist, significantly increased lipolysis in both gonadal and subcutaneous WAT ($P < 0.0001$, Fig. 3 A and B). The amplitude of the response differed however significantly

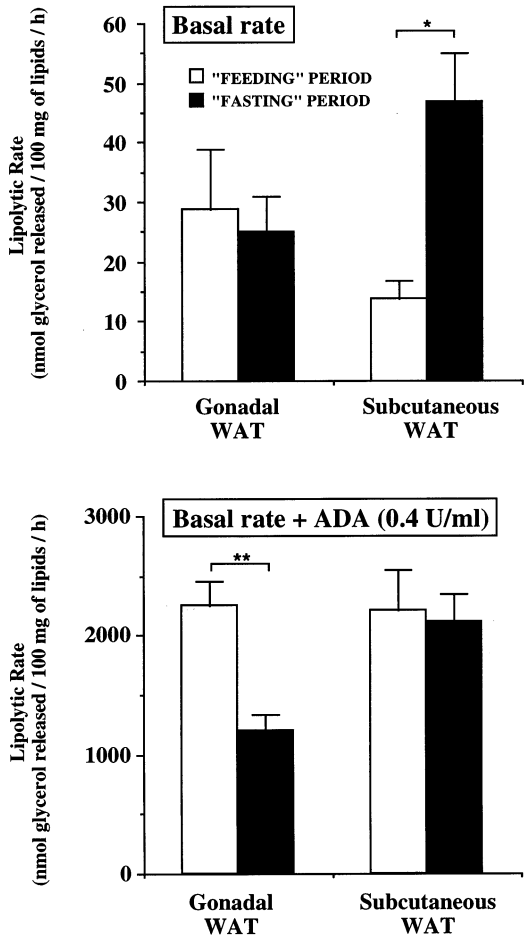

Fig. 2. in vitro basal lipolytic rate of gonadal and subcutaneous WAT depots during the 'feeding' (open bars, $n = 9$) and the 'fasting' (filled bars, $n = 9$) periods. Basal rates were measured in the absence or the presence of ADA (0.4 U ml^{-1}) in the incubation medium and expressed in nmol of glycerol released per 100 mg of cell lipids per hour. Effect of ADA on basal lipolysis, $P < 0.0001$. * $P < 0.05$; ** $P < 0.01$.

according to the depot and the period of the yearly cycle. The main difference between depots occurred during the 'feeding' period with a significantly higher lipolytic rate in gonadal than in subcutaneous adipocytes, in the range of 0.1–1 μM isoproterenol ($P < 0.05$, Fig. 3 A and B). The response of gonadal adipocytes (Fig. 3A) was significantly higher during the 'feeding' than during the 'fasting' period in the range of 1–10 μM ($P < 0.01$) while the opposite was observed in subcutaneous WAT (Fig. 3B). Gonadal adipocytes showed a significantly lower EC_{50} value for isoproterenol than subcutaneous adipocytes during the 'feeding' period (0.04 vs. 0.45 μM, Table 1). These data therefore indicate that during the 'feeding' period, gonadal WAT is more sensitive and shows a higher maximal response to β agonists than the subcutaneous depot. During the 'fasting' period, by contrast, the lipolytic response to β agonists decreased in the gonadal depot but increased in the subcutaneous one and there was no difference in sensitivity (similar EC_{50}).

3.4. Effect of noradrenaline

Noradrenaline, a β and α2-adrenoceptor agonist, also significantly increased lipolysis in both depots ($P < 0.0001$, Fig. 3 C and D), and the maximal rates of lipolysis were similar to those observed with isoprenaline. In gonadal WAT, the adipocyte response to noradrenaline 1 and 10 μM noradrenaline was higher during the 'feeding' than during the 'fasting' period ($P < 0.001$ and $P = 0.02$, respectively; Fig. 3C). The adipocyte response to 1 μM noradrenaline was also significantly higher in gonadal than in subcutaneous WAT ($P < 0.01$) when marmots were fed (Fig. 3 C and D). The EC_{50} values (Table 1) were not significantly different between depots or periods. Moreover, the increase in subcutaneous WAT lipolytic activity observed with isoprenaline during the 'fasting' period was not observed with noradrenaline (Fig. 3 B and D).

3.5. Effect of glucagon

Increasing doses of glucagon stimulated the lipolytic rate of both gonadal and subcutaneous adipocytes ($P < 0.0001$, Fig. 3 E and F), although this effect was 3–6-fold lower than that observed with catecholamines. It should be noted however that at low concentrations of glucagon (0.001 μM), the lipolytic rate of both depots was higher than that obtained with low concentrations of noradrenaline (0.01 μM), regardless of whether the animal was fasting or not. As seen previously with catecholamines, the 'feeding' period was associated with a higher lipolysis in the gonadal WAT as compared with the 'fasting' period, but this effect was only significant at 1 μM glucagon ($P = 0.04$). Gonadal adipocytes were more responsive to high doses of glucagon (1 μM) than the subcutaneous ones in the 'feeding' period ($P = 0.03$).

4. Discussion

This study reports differential variations in the lipolytic activity of gonadal and subcutaneous WAT during the annual cycle of alpine marmots. During the 'feeding' period, the adipocyte responsiveness to catecholamines or glucagon was higher in gonadal than in subcutaneous WAT, while during the 'fasting' period, there was no difference between the two depots. Finally, adenosine may be an important modulator of marmot adipocyte lipolysis on account of its marked antilipolytic effect in vitro.

The variation of lipolysis between WAT depots observed for the first time in marmot resembles that described in various rodent species including rats

[25,34] and hamsters [11,30]. By analogy with the mechanisms proposed in these studies, variations in lipolysis may be linked to a different balance in the population of β and α2-adrenoceptors [11,19] and/or a defective coupling of adenylate cyclase Gs subunits to β-receptors [11]. A lower β-receptor density and/or activity may possibly account for the lower sensibility to catecholamines of subcutaneous versus gonadal WAT in fed marmots. The lower stimulation of lipolysis exerted by noradrenaline as compared with isoprenaline, a general β-receptor agonist (Fig. 3 A vs. C and Fig. 3 B vs. D) indicates that the inhibitory action of the hormone through α2-adrenoreceptors may be predominant at low doses (0.01–0.1 μM) in both depots. These results therefore suggest that the difference between the two depots observed in fed marmots might be predominately linked to differences in β-adrenoceptor density and activity.

The 'fasting' period was associated with a lowering of the responsiveness of gonadal WAT to β-agonists (Fig. 3 A and C) and an increase of that of subcutaneous WAT (Fig. 3B). There was however no major change in

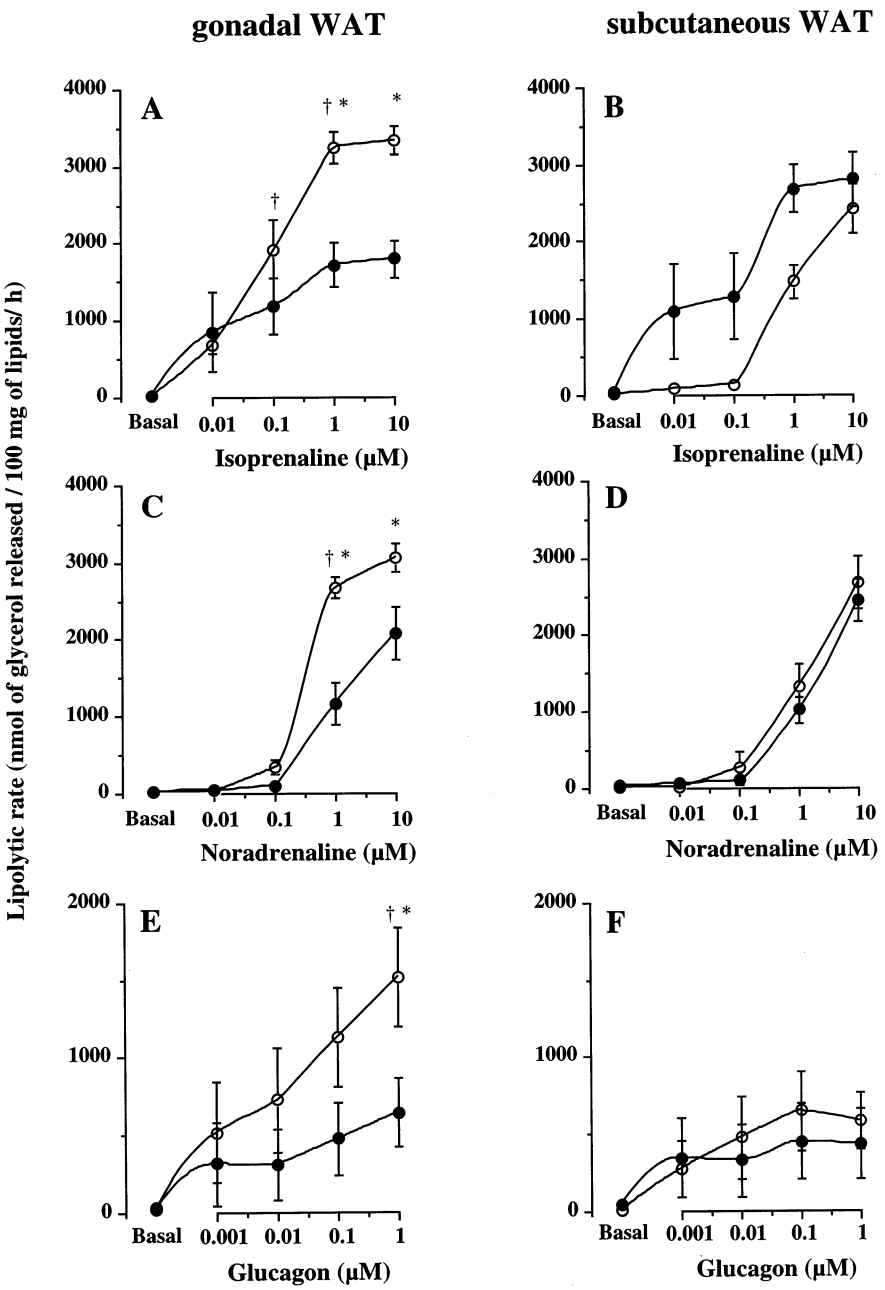

Fig. 3. In vitro lipolytic rate of gonadal and subcutaneous WAT measured during the 'feeding' (open circles) and the 'fasting' (filled circles) periods. Dose–response curves were obtained by stimulating isolated white adipocytes with isoprenaline, noradrenaline and glucagon. † Significant difference between depots over the same period. * Significant difference between periods within a same depot ($P < 0.05$).

Table 1
Changes in marmot white adipocyte sensitivity to isoprenaline and noradrenaline between the 'feeding' and the 'fasting' periods. Effective concentration producing a half-maximal response (EC_{50}) and its 95% confidence interval is reported for both catecholamines in gonadal and subcutaneous WAT, at both period. EC_{50} and confidence intervals are expressed in μM

	Gonadal WAT		Subcutaneous WAT	
	'Feeding'	'Fasting'	'Feeding'	'Fasting'
Isoprenaline				
EC_{50} (μM)	0.04	0.12	0.45[a]	0.18
95% Confidence interval (μM)	0.02–0.08	0.024–0.5	0.24–1.2	0.04–0.7
Noradrenaline				
EC_{50} (μM)	0.35	1.05	0.55	0.9
95% Confidence interval (μM)	0.09–1.5	0.6–2.0	0.2–1.7	0.45–1.8

[a] EC_{50} significantly different from gonadal WAT over the same period.

adipose tissue sensitivity to catecholamines in response to fasting and hibernation. Present results on marmot gonadal WAT are in accordance with those obtained on epididymal adipocytes of 72 h-fasting rats in which isoproterenol only weakly stimulated lipolysis as compared with fed animals [3]. Contrasting results have however been obtained in fasting rats with a stimulation [6,7,9,32], a reduction [20] or no change [21] of the sensitivity of adipose tissue to catecholamines. Nevertheless, in these studies, the effects of fasting could not be related to a decrease in the number or the affinity of adrenergic receptors [9,20]. Present results on marmots during the hibernation season indicate that the response to fasting clearly differs between fat depots, suggesting regional regulation of the tissue activity. It is rather surprising to observe such down-regulation of the responsiveness of marmot gonadal WAT to catecholamines during fasting and hibernation at a time when fat stores should be markedly mobilized. It may possibly be linked to a continuous stimulation of the tissue by endogenous catecholamines. The fact that basal lipolysis in presence of ADA was also lower in gonadal than in subcutaneous WAT during the 'fasting' period (independently from any β-adrenergic stimulation) suggests that the drop in gonadal WAT lipolysis likely involves post-receptor mechanisms. Recent experiments [28,36] report an involvement of minor components of the fat cell endogenous lipid droplet, phosphatidycholine and protein, as potent regulators of the hormone-sensitive lipase activity and lipolysis. In marmots, it would be interesting to determine if the fasting-refeeding cycle induces marked changes in the concentration of those minor components in gonadal fat cell droplets, supporting for the seasonal variation of lipolysis in this depot that was observed in the study. It is tentatively speculated that such drop of the lipolytic activity in the gonadal WAT during the hibernation fast would contribute to the sparing of triglycerides for a later mobilization, after the emergence, in the early spring. At this time of the year, food is not available yet in the wild and this period is therefore critical for marmots since they have to mate while they keep starving.

Maximal stimulation of lipolysis by glucagon was weaker than that measured with catecholamines in both depots. Low concentrations (0.001 and 0.01 μM) of glucagon were however more effective than low concentrations of noradrenaline (0.01 and 0.1 μM), indicating that the sensitivity of marmots WAT to glucagon is higher than to catecholamines. As for catecholamines, the responsiveness of gonadal WAT to glucagon tended to be higher in the 'feeding' versus the 'fasting' period. At low and more physiological concentrations of glucagon, there was however no obvious difference between the two depots. As demonstrated in hedgehogs (*Erinaceus europeatus*) and marmots, glucagon secretion is not abolished at low body temperature [15,23]. Glucagon may therefore provide a basal and continuous supply of fatty acids all along the marmot cycle, when catecholamine plasma levels are low. This hypothesis is in agreement with the absence of marked variations in glucagon plasma level between euthermia, deep hibernation and arousal found in *M. flaviventris* [15].

Present data showed that adenosine deaminase massively increased lipolysis in marmot adipocytes, indicating that adenosine produced by adipocytes may potentially play a role in the regulation of lipolysis. Such a powerful antilipolytic effect of adenosine on white fat cells was also described in vitro using N6-phenylisopropyladenosine, a non ADA-degradable agonist of adenosine, in various hibernators [4] and in *M. flaviventris* [13]. Because ADA induced a higher stimulation of lipolysis in subcutaneous than in gonadal WAT depots, it is suggested that the antilipolytic effect of adenosine is higher in subcutaneous adipocytes and may contribute at least in part to the lower responsiveness of subcutaneous adipocytes to lipolytic hormones. Interestingly, ADA also induced a higher activation of lipolysis in fed than in fasting marmots. These results contrast with those obtained in *M. flaviventris* [13] showing no variation in vitro of the effect of adenosine with the time of the year. There may therefore be species or WAT depot specificities to account for such difference. In the present study, the higher stimulation exerted by ADA indicates that lipolysis was more inhibited by adenosine during the 'feeding' period (Fig. 2).

Such phenomenon could be related to either a higher release of adenosine in fed than in fasting marmots or to a lower antilipolytic effect of adenosine during the 'fasting' period. In vitro data suggest that the antilipolytic effect of adenosine is decreased during fasting in mammals [32]. Although further experiments are required to demonstrate that the same indeed occurs in marmots, these results suggest that when marmots are building up fat reserves prior to hibernation, there may be a potentially high inhibition of lipolysis by endogenously produced adenosine, contributing therefore to the storage of lipids.

It is concluded that in alpine marmots, the lipolytic activity of WAT adipocytes varies according to the fat depot (gonadal vs. subcutaneous) and the time of the annual cycle considered. During the 'feeding' period, adipocyte responsiveness to isoprenaline, noradrenaline or glucagon was higher in gonadal than in subcutaneous WAT, while during the 'fasting' period, there was no difference between the two depots. The antilipolytic action of adenosine may possibly be involved in this regulation. These results therefore suggest that in marmots, lipolysis could be differentially regulated between fat depots according to the time of the year and/or the fasting-feeding status, possibly to optimize the use of fat reserves. The evidence for a differential lipolysis between gonadal and subcutaneous WAT depots suggests also that those results may not be extended to other 'deep' depots.

Acknowledgements

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