Depot- and Gender-related Differences in the Lipolytic Pathway of Adipose Tissue from Severely Obese Patients

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Key Words

Adipocyte • Lipolytic pathway • Adrenergic receptors
• Severe obesity • Gender • Fat depot

Abstract

The present study was performed to analyze in detail gender- and site-related alterations in the adrenergic signal transduction pathway of lipolysis in fat cells isolated from subcutaneous abdominal and visceral fat depots from severely obese patients. The study group consisted of 30 morbidly obese subjects (9 men and 21 women) aged 41.1 ± 1.9 years, with a body mass index (BMI) of 54.7 ± 1.7 kg/m², who had undergone abdominal surgery. Protein levels of hormone-sensitive lipase (HSL) and adrenergic receptors (AR), as well as HSL activity and the lipolytic response to adrenergic agents were analyzed. Both fat depots had similar basal lipolysis, but the capacity of catecholamines to activate lipolysis was greater in visceral fat, both at AR and postreceptor levels. Basal lipolysis and lipolytic activity induced by dibutyryl cyclic AMP were higher in men than in women. However, the visceral depot of women showed a higher maximal stimulation by noradrenaline than that of men, in accordance with higher β_1 - and β_3 -AR protein levels.

In conclusion, the main gender-related differences were located in the visceral depot, with women exhibiting a higher sensitivity to catecholamines associated with an increased provision of β -AR, while men showed an enhanced lipolytic capacity at the postreceptor level.

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Introduction

Lipolysis in fat cells is mainly regulated by the adrenergic system. Catecholamine effects are modulated through four different G-protein-coupled adrenergic receptor (AR) subtypes. β -ARs (β_1 , β_2 and β_3) stimulate lipolysis by increasing the formation of cyclic AMP (cAMP), which leads to the stimulation of protein kinase A (PKA) and the phosphorylation of both the accessory protein perilipin and hormone-sensitive lipase (HSL). In contrast, the α_2 -AR subtype inhibits lipolysis by decreasing cAMP formation. Thus, the net effect of catecholamines on adipose tissue is dependent on the functional balance between β - and α_2 -AR mediated signal transduction [1-3]. Although HSL is considered the rate-limiting enzyme

catalysing lipolysis, the presence of an additional triglyceride lipase in adipose tissue has been recently identified [4-6]; this could also play a role in the lipolytic process, acting in coordination with HSL.

Energy homeostasis can be affected by alterations in the lipolytic pathway contributing to both the development and pathogenesis of obesity [7]. Several differences in the rate of lipolysis have been observed between various fat depots from humans [8-12], with higher lipolytic activity in the visceral adipose tissue than in the subcutaneous one. Numerous studies have emphasized the idea that visceral fat deposition is strongly correlated to the metabolic complications of obesity, such as hypertension, cardiovascular disease, and noninsulin-dependent diabetes mellitus [13, 14].

Moreover, variations in lipolytic activity have also been shown to be gender dependent [15]. Thus, differences between men and women have been observed in the sensitivity to adrenergic stimulation [16, 17], as well as in the rate of lipolysis induced by agents acting at different steps of the lipolytic pathway [16-19].

Previous studies have focused on changes at certain steps of the lipolytic pathway in either only one fat depot or in several, making comparisons between adipose tissues from different anatomical locations. However, in some studies data on men and women have either been analyzed together or genders have been studied in separate works. Thus, the aim of this study was to analyze in detail the different steps of the adrenergic pathway, in order to evaluate the overall effect of both gender and fat depot on the adipocyte lipolytic capacity. For this purpose, we analyzed protein levels of HSL and of the adrenergic receptors, as well as HSL activity and the lipolytic response to different adrenergic agents acting at different steps of the lipolytic pathway in adipocytes isolated from subcutaneous abdominal and visceral adipose tissues from morbidly obese men and women.

Materials and Methods

Materials

Antibodies for β -ARs and α_{2A} -AR were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies for HSL were kindly supplied by FB Kraemer [20]. ($\pm$)-Noradrenaline bitartrate salt, (-)-isoproterenol (+)-bitartrate salt (isoprenaline), dobutamine hydrochloride, terbutaline hemisulfate salt, forskolin, dibutyryl cAMP (Bt₂cAMP), bovine serum albumin (fraction V), *Clostridium histolyticum* collagenase type II and triglyceride GPO-Trinder (n° 337) were obtained from Sigma (St Louis, Mo). RS79948 hydrochloride was obtained from TOCRIS

Fig. 1. Western blot analysis of HSL, β_1 -, β_2 -, β_3 -, and α_2 -AR protein levels in subcutaneous (s) and visceral (v) adipose tissue depots of severely obese men (M) and women (W).

(Madrid, Spain). Glycerol tri[9,10(n)-³H]oleate and [¹⁴C]oleic acid were supplied by American Radiolabeled Chemicals (St Louis, Mo). Aprotinin and leupeptin were supplied by Roche Diagnostics (Basel, Switzerland). Routine chemicals were provided by Amersham Pharmacia Biotech (Piscataway, NJ), Panreac (Barcelona, Spain), and Sigma.

Subjects and adipose tissue biopsies

The study group consisted of 30 morbidly obese -non-diabetic- subjects (9 men and 21 women) aged 41.1 $\pm$ 1.9 years (23-60 years), with a body mass index (BMI) of 54.7 $\pm$ 1.7 kg/m² (40-81 kg/m²), who were undergoing weight reduction surgery by biliopancreatic diversion [21]. None of them used any regular medication. The study protocol was approved by the Ethics Committee of the Son Dureta University Hospital (Palma de Mallorca, Spain) and all subjects gave their written consent to participate in the study. Fasting plasma glucose, insulin, triglycerides and cholesterol were measured by the hospital's routine laboratory. Fat biopsies were removed in each subject from both abdominal subcutaneous and visceral (omental) adipose tissues and were obtained within 20 min after the start of general anaesthesia. Tissue biopsies were immediately frozen in liquid nitrogen and stored at -80°C for subsequent analysis. Fresh adipose tissue samples were taken in Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4), containing 10 mM HEPES and 6 mM glucose for adipocyte isolation and subsequent lipolysis assay.

Adipocyte isolation and lipolysis assay

Adipocytes were isolated using the method described by Robdell [22] with some minor modifications. Briefly, tissue samples were minced at room temperature and incubated for 35 min with 1.5 g/L of collagenase in 10 mL buffer (pregassed with 95% O₂-5% CO₂, pH 7.4), containing 10 mM HEPES, 6 mM glucose and 30 g/L of bovine serum albumin at 37°C in a shaking bath. Cells were filtered through a nylon mesh (250 μ m) and subsequently washed three times with a collagenase-free buffer.

Table 1. Biometric and serum biochemical parameters of severely obese subjects. Results are the mean $\pm$ S.E.M of 9 men and 21 women. Data were analyzed by Student's t test. *NS* non significant.

	Men	Women	p
Age (y)	41.9 $\pm$ 3.4	40.7 $\pm$ 2.3	NS
Body mass index (kg/m ²)	57.8 $\pm$ 2.3	53.4 $\pm$ 2.2	NS
Glucose (mmol/L)	6.11 $\pm$ 0.48	5.75 $\pm$ 0.21	NS
Insulin (μ U/mL)	28.9 $\pm$ 4.4	23.2 $\pm$ 2.8	NS
Triglycerides (mmol/L)	1.59 $\pm$ 0.12	1.54 $\pm$ 0.16	NS
Cholesterol (mmol/L)	3.64 $\pm$ 0.27	3.81 $\pm$ 0.36	NS

Cell counts were performed with an improved Neubauer hemocytometer. The mean adipocyte diameter was calculated by direct microscopy from the measurement of 50 cells in each tissue sample. Assuming that fat cells are spheres, the mean fat cell volume was calculated from these values.

Measurements of lipolytic activity were performed by incubating isolated adipocytes (25,000 cells/mL) at 37°C in a shaking bath in 0.5 mL of KRB buffer containing 10 mM HEPES, 6 mM glucose, and 30 g/L of bovine serum albumin in the presence of increasing concentrations of lipolytic agents under an atmosphere of 5% CO₂ in O₂. The ligands and concentrations used in this study were: noradrenaline (β_1 -, β_2 -, β_3 - and α_2 -AR agonist) from 10⁻⁹ to 10⁻⁵ M and isoprenaline (β_1 -, β_2 -, and β_3 -AR agonist) from 10⁻¹⁰ to 10⁻⁶ M, dobutamine (β_1 -AR agonist) from 10⁻¹⁰ to 10⁻⁴ M, terbutaline (β_2 -AR agonist) from 10⁻¹⁰ to 10⁻³ M, forskolin (stimulating adenylate cyclase) 10⁻⁵ M, and Bt₂cAMP (cAMP analogous resistant to phosphodiesterase and stimulating PKA) 10⁻³ M. The maximal effective concentrations of forskolin and Bt₂cAMP were assessed by preliminary dose-effect analysis. Moreover, lipolytic responses to increasing doses (from 10⁻⁹ M to 10⁻⁵ M) of noradrenaline were measured with 10 μ M of the α_2 -AR antagonist RS79948.

After 90 min of incubation, the reaction was stopped in a water ice bath and aliquots (200 μ L) of the cell-free medium were taken to determine the glycerol concentration, as an index of lipolysis. The glycerol content of the deproteinized aliquots was determined enzymatically with a chemical kit.

HSL activity measurement

Adipose tissue samples were homogenized in a buffer containing 0.25 M sucrose, 10 mM HEPES, 1 mM EDTA, 1 mM dithiothreitol, and 0.005% heparin (pH 7.5) and then centrifuged (400xg for 10 min at 4°C). The fat-free infranatant was used to determine HSL activity as previously described [23].

The infranatant was also used to determine the protein content [24] to standardize HSL activity.

HSL, β_1 -, β_2 -, β_3 - and α_2 -AR protein level determination

A small piece of frozen tissue (~500 mg) was homogenized in phosphate-buffered saline buffer (137 mM NaCl, 3 mM KCl, 6.5 mM Na₂HPO₄, and 3.5 mM KH₂PO₄) with freshly added protease inhibitors (1 μ g/mL aprotinin, 1 μ g/mL leupeptin, and 10 μ g/mL phenylmethyl sulfonyl fluoride). The homogenate was centrifuged at 16,000xg for 20 min at 4°C, and the fat-free infranatant was removed and stored at -20°C. After protein

content determination [24], varying amounts of total protein from the homogenates (50 μ g for HSL, β_1 -, β_2 -, and α_2 -ARs, and 35 μ g for β_3 -AR) were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10% polyacrylamide), and electrotransferred on a nitrocellulose filter as described elsewhere [25]. Development of the immunoblots was performed using an enhanced chemiluminescence Western blotting analysis system. Rabbit polyclonal antibodies against HSL, β_1 -, β_2 -, and β_3 -ARs, and goat polyclonal antibody against α_2 -AR were used as primary antibodies. Bands in films were analysed by scanner photodensitometry and quantified using the Kodak 1D Image Analysis Software 3.5 for windows. Representative western blots are shown in Figure 1.

Statistical analysis

The lipolytic activity was expressed as nmoles of glycerol per 10⁶ cells/90 min and percentage stimulation over basal lipolysis levels (basal lipolysis was set to be 100% and was calculated for each individual). All AR agonists caused a concentration-dependent stimulation of glycerol release which reached a plateau at the highest agonist concentrations. Dose-response curves for agonists were fitted with nonlinear regression analysis for sigmoid curves, as previously described [26], using the GraFit computer program (Leatherbarrow R. J. GraFit version 4, Erithacus Software, Staines, UK, 1998). This data processing allowed us to calculate the maximal stimulation of the glycerol release over basal lipolysis induced by each agonist. Likewise, the statistical differences between groups in the maximal capacity values were obtained using the same program and the procedure described by Motulsky and Ransnas [26] to compare parameters of curves fitted by nonlinear regression.

Protein mass (integrated optical density units per microgram of total tissue protein) data are expressed relative to the mean value of the male subcutaneous group, which was set as 100%. Differences between genders in biometric and serum biochemical parameters were tested using Student's t-test. Adipocyte volume, protein levels of HSL, β_1 -, β_2 -, β_3 - and α_2 -ARs, HSL activity, basal lipolysis and maximal lipolysis induced by forskolin and Bt₂cAMP were assessed by two-way analysis of variance (ANOVA) and also by Student's t test as post hoc comparison, but only when an interactive effect of depot and gender (DxG) was shown. Analyses were performed using SPSS 11.5 software for windows. A p-value of less than 0.05 was considered statistically significant.

Fig. 2. Noradrenaline-stimulated lipolysis in subcutaneous (sc) and visceral (vs) adipocytes isolated from morbidly obese subjects. Data are means $\pm$ S.E.M. Glycerol release rates are expressed as nmol glycerol/(10⁶ cells $\times$ 90 min) and are minus basal lipolysis values. Significant differences were assessed using ANOVA ($p < 0.05$): D effect of adipose tissue depot.

Results

The biometric and serum biochemical parameters of the subjects included in the study are shown in Table 1. No statistical differences were found between men and women in any of the parameters considered. Fasting glucose and insulin levels were similar to the ones described for severely obese patients [27, 28].

Different AR agents were used to stimulate lipolysis in isolated fat cells -noradrenaline (β_1 -, β_2 -, β_3 - and α_2 -AR agonist), isoprenaline (β_1 -, β_2 -, and β_3 -AR agonist), dobutamine (β_1 -AR agonist), terbutaline (β_2 -AR agonist), and RS79948 (α_2 -AR antagonist). Figure 2 shows the mean concentration-response curves for noradrenaline in subcutaneous and visceral fat depots from obese men and women. Noradrenaline increased glycerol release in a concentration-dependent manner. The stimulation of lipolysis induced by noradrenaline was 1.5-fold more effective in isolated fat cells from the visceral depot than subcutaneous one and tended to be higher in men than in women, without reaching statistical significance. Adipocyte volume, basal lipolysis and the maximum action of lipolytic agents, expressed as percentage of stimulation over basal lipolysis levels, are shown in Table 2. Adipocyte volume was higher in both adipose tissues from men than in those from women. Basal lipolysis was higher in men than in women. All agonists stimulated glycerol release

in a concentration-dependent manner. In both genders, visceral adipose tissue showed higher lipolysis stimulation by noradrenaline and dobutamine than the subcutaneous one. Moreover, the visceral depot of women showed a higher maximal stimulation by noradrenaline than that of men. In subcutaneous fat from both genders, noradrenaline acted as a partial agonist with values for intrinsic activity of $<77\%$ of isoprenaline action.

As an index of lipolytic potencies of fat cells, agents by-passing the receptor step such as forskolin and Bt₂cAMP were tested. The lipolytic activity induced by forskolin and Bt₂cAMP at their maximal effective concentrations as we assessed with preliminary experiments (10^{-5} M and 10^{-3} M, respectively) is shown in Table 3. The glycerol released after forskolin stimulation did not show significant differences either depot- or gender-dependent. The maximal lipolytic activity induced by Bt₂cAMP was greater in men than in women, being similar in both adipose depots.

The visceral depot showed higher β -AR levels than the subcutaneous one (Table 4), although values did not reach statistical significance. Gender differences were found in β_1 - and β_3 -AR levels, since women showed a greater protein content than men.

Visceral fat showed higher HSL mass levels (Table 5) than the subcutaneous one, in both men and women. On the other hand, HSL activity values were greater in

Table 2. Cell volume, basal lipolysis and adrenergic stimulated lipolytic activity of subcutaneous and visceral adipocytes isolated from severely obese subjects. Results are the mean $\pm$ S.E.M. Basal lipolysis is expressed as nmol glycerol/(10⁶ cells x 90 min). Maximal action of lipolytic agents is expressed as percentage stimulation over basal lipolysis levels (which were set to be 100%), and was obtained by curve fitting analysis performed by nonlinear regression using the GraFit computer program and curves were compared using the

	Men		Women		ANOVA (p)
	Subcutaneous	Visceral	Subcutaneous	Visceral	
Fat cell volume (pl)	897 $\pm$ 147	1006 $\pm$ 89	743 $\pm$ 46	698 $\pm$ 70	<i>G</i> (0.010)
Basal lipolysis	1021 $\pm$ 301	815 $\pm$ 116	677 $\pm$ 92	559 $\pm$ 43	<i>G</i> (0.024)
Maximal stimulation					
Noradrenaline	93.0 $\pm$ 10.3	153 $\pm$ 18	92.9 $\pm$ 0.9	196 $\pm$ 3* ^o	
RS79948	128 $\pm$ 35	174 $\pm$ 58	139 $\pm$ 9	189 $\pm$ 36	
Isoprenaline	157 $\pm$ 22	192 $\pm$ 20	155 $\pm$ 12	196 $\pm$ 11	
Dobutamine	65.2 $\pm$ 12.9	148 $\pm$ 72	61.2 $\pm$ 8.2	114 $\pm$ 12*	
Terbutaline	133 $\pm$ 18	163 $\pm$ 23	131 $\pm$ 18	173 $\pm$ 14	
Noradrenaline Intrinsic Activity	76.3 $\pm$ 9.4	85.3 $\pm$ 9.7	71.1 $\pm$ 6.0	101 $\pm$ 10	<i>D</i> (0.010)

procedure described by Motulsky and Ransnas [26]. Lipolytic responses to increasing doses of noradrenaline were measured with and without 10 μ M of the α_2 -AR antagonist RS79948. Significant differences in fat cell volume, basal lipolysis and noradrenaline intrinsic activity were assessed using ANOVA ($p < 0.05$): *G* effect of gender, *D* effect of adipose tissue depot. Significant differences in maximal lipolysis over basal lipolysis: * visceral vs subcutaneous, o women vs men.

Table 3. Lipolytic activity induced by forskolin and Bt₂cAMP in subcutaneous and visceral adipocytes isolated from severely obese subjects. Results are the mean $\pm$ S.E.M. Lipolytic activity was expressed at their maximal effective concentrations (10⁻⁵ and 10⁻³ M, respectively) as a percentage over basal lipolysis (which was set to be 100%). Data were analyzed by two-way analysis of variance (ANOVA). Significant differences ($p < 0.05$): *G* effect of gender. *NS* non significant.

	Men		Women		ANOVA (p)
	Subcutaneous	Visceral	Subcutaneous	Visceral	
Forskolin	242 $\pm$ 50	216 $\pm$ 39	252 $\pm$ 30	306 $\pm$ 36	<i>NS</i>
Bt ₂ cAMP	331 $\pm$ 60	279 $\pm$ 68	201 $\pm$ 19	219 $\pm$ 32	<i>G</i> (0.032)

the subcutaneous depot than in the visceral one.

Discussion

In this study, both depot- and gender-dependent effects on the lipolytic capacity of adipocytes from morbidly obese subjects were investigated. Our results point to a higher lipolytic activity in visceral than subcutaneous adipose tissue, due to a lower inhibition of lipolysis via α_2 -AR and increased lipolytic activity through β_1 -AR. Moreover, in both tissues, men were able to counteract the lower lipolytic capacity at the level of adrenergic receptors - compared to women - by a higher lipolytic capacity at the postreceptor level. Thus, the

variations in lipolytic capacity between genders are determined at or beyond the level of PKA.

Depot-dependent differences in lipolytic capacity in adipose tissue depots from severely obese patients

Similarly to previous reports by other authors [8, 11, 12, 15] we found marked variations in lipolytic capacity between subcutaneous and visceral adipose tissues from obese subjects. In fact, it has been proposed that in obese subjects the regional variations in the action of catecholamines on lipolysis are enhanced, due to differences in the activity of the different ARs, insulin receptors and HSL function [10]. The higher lipolytic response to noradrenaline observed in the visceral

Table 4. Adrenergic receptor protein levels in subcutaneous and visceral adipose tissues from severely obese subjects. Results are the mean $\pm$ S.E.M. Protein arbitrary units (AU) mean values of subcutaneous depot from men were set as 100%. Data were analyzed by two-way analysis of variance (ANOVA). Significant differences ($p < 0.05$): *G* effect of gender. *NS* non significant.

	Men		Women		ANOVA (p)
	Subcutaneous	Visceral	Subcutaneous	Visceral	
Protein level (AU)					
$\beta 1$	100 $\pm$ 15	112 $\pm$ 18	137 $\pm$ 13	163 $\pm$ 13	<i>G</i> (0.009)
$\beta 2$	100 $\pm$ 14	114 $\pm$ 10	130 $\pm$ 22	163 $\pm$ 20	<i>NS</i>
$\beta 3$	100 $\pm$ 10	145 $\pm$ 23	199 $\pm$ 32	216 $\pm$ 28	<i>G</i> (0.030)
$\alpha 2$	100 $\pm$ 9	99.8 $\pm$ 10.3	119 $\pm$ 10	127 $\pm$ 9	<i>NS</i>

Table 5. HSL protein levels and enzyme activity of subcutaneous and visceral adipose tissues from severely obese subjects. Results are the mean $\pm$ S.E.M. Protein arbitrary units (AU) mean values of subcutaneous depot from men were set as 100%. HSL activity was

	Men		Women		ANOVA (p)
	Subcutaneous	Visceral	Subcutaneous	Visceral	
Protein levels (AU)	100 $\pm$ 16	161 $\pm$ 18	151 $\pm$ 18	178 $\pm$ 15	<i>D</i> (0.037)
Enzyme activity (U/g protein)	156 $\pm$ 36	136 $\pm$ 20	153 $\pm$ 17	94.6 $\pm$ 9.6	<i>D</i> (0.014)

expressed as μ U/g of tissue protein, 1 μ U was equal to 1 μ mol nonesterified fatty acids released per hour of incubation at 37°C. Data were analyzed by two-way analysis of variance (ANOVA). Significant differences ($p < 0.05$): *D* effect of adipose tissue depot.

compared with the subcutaneous fat depot could be explained in part by a higher provision of β -ARs in the former, but also by a greater inhibitory effect via α_2 -AR in the latter. The results obtained when increasing doses of noradrenaline were tested in the presence of the α_2 -AR antagonist RS79948 are in agreement with intrinsic activity data and point to differences in α_2 -AR function, possibly contributing to the observed depot-dependent variations. Moreover, changes at the postreceptor step must also be considered, mainly at the level of the rate limiting enzyme catalyzing lipolysis, HSL, which showed higher protein levels in visceral than in subcutaneous adipose tissue. Thus, although both tissues had a similar basal lipolysis, visceral fat had a greater capacity to activate lipolysis in response to catecholamines, as a result of a signal transduction system which was more focused toward lipid mobilization, both at AR and postreceptor steps.

In agreement with previously reported data, HSL activity was higher in the subcutaneous adipose tissue compared to the visceral one [29]. In contrast, the HSL mass was higher in the visceral fat, which might be a sign of the regulation of the enzyme at the post-translational level [28, 30] or of the presence of a truncated form of the enzyme with reduced activity which has been found in human adipose tissue [31]; regretfully, the

conditions we used to detect HSL mass did not allow the differentiation between these two forms.

Gender differences in the lipolytic capacity of adipocytes from subcutaneous and visceral fat depots

In the present study, a gender dimorphism was found at the level of different elements of the adrenergic pathway leading to lipolysis. The markedly increased basal rate of lipolysis in both fat depots from men compared to women correlated with the men's larger fat cells, in accordance with previously reported results [28, 32, 33], and also with a higher lipoprotein lipase activity [34]. Moreover, the lipolytic capacity and the effect of noradrenaline on lipolysis in fat depots of obese subjects were also influenced by gender. The lower maximal stimulation over basal lipolysis induced by noradrenaline in the visceral fat of men could be due to a higher resistance to the action of catecholamines compared to women, in a similar way as has been reported in the subcutaneous fat from obese patients [28]. Thus, the inability of adipose tissue from men to increase the lipolytic capacity over basal lipolysis like women do together with their increased basal lipolysis might be related with the fact that men are more prone to accumulate fat in the abdominal region than women, and

could explain, at least in part, gender differences in the resistance to weight reduction.

In both adipose tissues, women showed a provision of ARs more focused toward lipid mobilization, with higher levels of β_1 - and β_3 -AR expression and, in the visceral fat, an increased response to dobutamine. An enhanced lipolytic function of β -ARs, especially β_3 -AR, has been proposed to be one of the mechanisms responsible for the increased catecholamine-induced lipolysis in the visceral fat with obesity [12, 35]. In addition to the differences in the amount of different ARs, other elements of the lipolytic pathway at the postreceptor level must be taken into account to be differentially regulated in men and women. The study carried out by incubating adipocytes with forskolin (stimulating adenylate cyclase) and Bt_2cAMP (which activates PKA) confirmed the existence of a gender dimorphism also at the postreceptor level. Thus, the lipolysis induced by Bt_2cAMP was 1.7- and 1.3-fold higher in subcutaneous and visceral depots from men, respectively, compared to women, which would indicate that the lower activity at the postreceptor step in women was due to differences in the capacity at the PKA or subsequent steps. In this way, it is worth noticing the strong correlation found in visceral fat between Bt_2cAMP -stimulated lipolysis and both HSL activity ($r=0.537$, $p=0.015$) and fat cell size ($r=0.499$, $p=0.021$), in accordance with that found by other authors [7, 28, 29].

In summary, the main gender-related differences are

located in the visceral adipose tissue, with women exhibiting a higher sensitivity to catecholamines associated to an increased provision of β -ARs, while men showed an enhanced lipolytic capacity at the postreceptor level. In men, the increased fat cell size, the basal rate of lipolysis and the noradrenaline-induced glycerol release per cell would be in accordance with an attempt to limit cellular hypertrophy and would be of importance for the metabolic disorders associated to obesity.

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