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Research Article

Gender- and site-related effects on lipolytic capacity of rat white adipose tissue

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Abstract. Gender- and site-related differences in the lipolytic capacity, at the different steps of the adrenergic pathway, in gonadal and inguinal white adipose tissue (WAT), were assessed by studying α_{2A}-adrenergic receptor (AR), β_3-AR and hormone-sensitive lipase (HSL) protein levels, and by determining the lipolytic response to different agents. Gonadal WAT showed a lower α_{2A}/β_3-AR ratio, a greater lipolytic capacity in response to AR agonists, and higher HSL activity and protein levels than inguinal WAT. In female rats, we found greater α_{2A}-AR protein levels and α_{2A}/β_3-AR ratio compared to their male counterparts, but, on the other hand, a higher lipolytic response to β-AR agonists and a greater lipolytic capacity at the postreceptor level, including a more activated HSL protein. Thus, the lipolytic capacity was clearly higher in gonadal than in inguinal WAT, at the different steps of the adrenergic pathway studied. Moreover, in both tissues, females showed a greater inhibition of lipolysis via α_2-AR, which was counteracted by the higher lipolytic capacity at the postreceptor level.

Key words. Lipolysis; adrenergic receptor; gender dimorphism; site-related differences; HSL.

The adrenergic system plays a major role in the regulation of lipolysis in white adipose tissue (WAT), through the action of catecholamines. Catecholamines, by activating β-adrenergic receptors (ARs), stimulate lipid mobilization in WAT, mainly through the production of cAMP and the activation of cAMP-dependent protein kinase (PKA) which, in turn, activates hormone-sensitive lipase (HSL), leading to lipolysis [1, 2]. In rodents, β_3-AR is quantitatively the most abundant AR in white adipocytes. [3, 4]. However, catecholamines, acting through α_2-AR, are able to prevent lipid mobilization by inhibiting cAMP production [2]. As β- and α_2-AR coexist in the same fat cell, differences in the number and/or activity of both AR subtypes may determine whether fat is stored or released [5].

Adipose tissue is a heterogeneous organ with marked variations in fat cell metabolism depending on the anatomical location [6–8]. Thus, many authors have reported in rats an increase in catecholamine-stimulated lipolysis in visceral fat depots compared with the subcutaneous ones [9–14]. Regional differences in lipolytic activity have also been observed in humans [15–19], although there are some contradictory results, which may be explained by the anatomical location of the visceral or subcutaneos WAT depot studied, the gender or the body mass index of the subjects [17, 20]. The marked variations in fat cell metabolism depending on anatomical location are of potential pathophysiological interest in human metabolic disorders associated with obesity, such as dyslipidemia, diabetes mellitus, and hypertension, in particular when fat is mainly accumulated in the visceral depot [21–23]. Several mechanisms, affecting various steps

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of the lipolytic cascade, have been proposed to explain the site-specific differences in lipolysis: from the α_2/β-AR balance [16, 24, 25], to steps at the postreceptor level, including adenylate cyclase activity [13], as well as HSL activity and expression [9, 10, 25].

Moreover, variations in lipolytic activity have also been shown to be gender dependent. Thus, sex differences have been observed both in sensitivity to adrenergic stimulation and in the number of ARs (both α_2 and β) [26–28], as well as in the lipolysis induced by agents acting at the adenylate cyclase and the PKA level [26, 27, 29], and also in HSL activity [30]. These gender-dependent differences have been suggested to be due to variations in the hormonal environment [26]. In this respect, several studies suggest a direct effect of sex hormones on the metabolic control of adipose tissue, acting at different steps in the lipolytic pathway. [31–35].

Previous studies have focused on changes at certain steps of the lipolytic pathway, between different fat depots, or between genders, but they have failed to assess the global effect of both factors throughout the whole lipolytic pathway. 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 the anatomical location of the tissue on lipolytic capacity, in adipocytes isolated from gonadal (visceral) and inguinal (subcutaneous) WAT, from 110-day-old male and female rats.

Materials and methods

Materials

All enzymes, substrates, and coenzymes were obtained from Sigma-Aldrich (Madrid, Spain). Antibodies for UCP2, β_3-AR, and α_{2A}-AR were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies for HSL were supplied by F. B. Kraemer [36].

(±)-Norepinephrine bitartrate salt, (–)-isoproterenol(+)-bitartrate salt (isoprenaline), BRL37344, forskolin, dibutyryl cyclic AMP, bovine serum albumin (fraction V), and *Clostridium histolyticum* collagenase type II were obtained from Sigma. Triolein and oleic acid were obtained from Sigma, triolein[9, 10-^{3}H(N)] was obtained from American Radiolabeled Chemical (St. Louis, Mo.), and [1-^{14}C]oleic acid was supplied by Amersham Pharmacia Biotech (Barcelona, Spain).

Routine chemicals used were supplied by Amersham Pharmacia Biotech, Panreac (Barcelona, Spain), Sigma-Aldrich, and Cultek (Madrid, Spain).

Animals

All animals were treated in accordance with accepted standards of humane animal care. Male and female Wistar rats (110 days old), bred in our laboratory, were used. The animals were housed three per cage at 22 °C, with a 12-h light/12-h dark cycle, and ad libitum free access to both drinking water and standard chow pellets (supplied by Panlab, Barcelona, Spain). All animals were killed by decapitation at the start of the light cycle. The inguinal (subcutaneous) and gonadal (visceral) WAT depots were dissected. Lipolysis assays were performed on seven males and seven females, while ten male and ten female rats were used to determine HSL and cytochrome c oxidase (COX) activity, and UCP2, HSL, and AR protein levels.

Preparation of isolated adipocytes and lipolysis assays

Adipocytes were isolated from gonadal and inguinal depots using the method described by Rodbell [37] with minor modifications. Briefly, tissue samples were minced at room temperature and incubated for 30 min with 1.5 g/l of collagenase in 10 ml Krebs-Ringer bicarbonate (KRB) buffer (pregassed with 95% O_2-5% CO_2, 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 though a nylon mesh (250 µm) and subsequently washed three times with a collagenase-free buffer. Cell counts were performed with an improved Neubauer hemocytometer.

Measurements of lipolytic activity were performed by incubating isolated adipocytes (30,000 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% CO_2 in O_2. The ligands and concentrations used in this study were: noradrenaline (β_1-, β_2-, β_3- and α_2-AR agonist) and isoprenaline (β_1-, β_2-, and β_3-AR agonist) from 10^{-10} to 10^{-4} M, BRL37344 (full β_3-AR agonist) from 10^{-9} to 10^{-4} M, forskolin (stimulating adenylate cyclase) 10^{-4} M, and dibutyryl cyclic AMP (Bt_2cAMP) (stimulating PKA) 10^{-3} M. After 90 min of incubation, the reaction was stopped in a water ice bath for 30 min, and aliquots (200 µl) of the cell-free medium were taken to determine the glycerol concentration, as an index of lipolysis. The glycerol content of the deproteinated aliquots was determined enzymatically with a Sigma chemical kit [Triglyceride (GPO-Trinder) no. 337].

Determination of HSL enzyme activity

About 160 mg of frozen (at –75 °C) tissue sample was homogenized in 0.5 ml of 0.25 M sucrose, 10 mM Hepes, 1 mM EDTA, 1 mM dithiothreitol, and 0.005% heparin, pH 7.5 at 4 °C. The homogenates were then centrifuged at 400 g at 4 °C for 10 min. An aliquot of the fat-free infranatant was used to determine HSL protein expression by Western blot, and the rest of the fat-free infranatant was recovered for analysis of HSL activity.

The substrate for the enzyme reaction was obtained by drying 100 µl of triolein[9, 10^{-3}H(N)] solution in toluene (0.66 µM, 11 µCi/µmol) under a stream of nitrogen. Afterwards, 1 ml of a buffer containing 27.5 mM PIPES,

55.5 mM $MgCl_2$, and 0.05% albumin (fatty acid free) was added to the dried triolein[9, 10^{-3}H(N)] and sonicated (20 W) five times, for 30 s, at 4 °C.

Samples (20 µl) were mixed with 20 µl 5 M NaCl. After a preincubation step (5 min at 37 °C), 160 µl of substrate was added to the sample. All samples were incubated in duplicate at 37 °C for 30 min with gentle shaking. The reaction was stopped in ice and the labelled free fatty acids (FFAs) were isolated by addition of 3.5 ml methanol-chloroform-heptane (141 : 125:100) and 1 ml of 0.1 M borate/carbonate buffer (pH 10.5). As internal control for the FFA separation process, 10 µl of [1-^{14}C]-oleic acid (2 mM, 0.33 µCi/ml) were also added to each tube. After centrifugation for 10 min at 850 g, an aliquot of the supernatant was collected for scintillation counting (Beckman LS 3801 scillation counter, Madrid, Spain). The HSL activity was related to the total protein concentration of the homogenate, which was measured using the microcolorimetric method of Lowry et al. [38], using bovine serum albumin as standard.

Western blot for UCP2, HSL, and α_{2A}-AR, and β_3-AR

The different depots were homogenized in HEPES/sucrose buffer (250 mM sucrose, 1 mM HEPES, 0.2 mM EDTA), with a teflon/glass homogenizer, for α_{2A}-AR, β_3-AR, and UCP2 protein level measurement, and then filtered through a 250-µm silk filter. Aliquots of the fat-free infranatants from HSL activity assays were used to determine HSL protein levels. Protein concentration was measured by the method of Lowry et al. [38]. Varying amounts of total protein from the homogenates (30 µg for UCP2, 50 µg for HSL, 25 µg for ARs) were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (12% polyacrylamide for UCP2 and 10% polyacrylamide for HSL and ARs) according to Laemmli [39] and electrotransferred onto a nitrocellulose filter as described elsewhere [40]. Staining with Ponceau S was used to provide visual evidence for correct loading and electrophoretic transfer of proteins to nitrocellulose filters. Blocking and development of the immunoblots were performed using an enhanced chemiluminescence Western blotting analysis system (Amersham). Goat polyclonal antibodies against UCP2, α_{2A}- and β_3-ARs and rabbit polyclonal antibody against HSL were used as primary antibodies. Bands in films were analyzed by scanner photodensitometry and quantified using the Kodak 1D Image Analysis Software.

Autoradiograms of membrane proteins revealed proteins exhibiting an apparent molecular mass of 33, 84, 58, and 55 kDa, for UCP2, HSL, β_3-AR, and α_{2A}-AR, respectively. The difference between the values obtained for β_3-AR, and α_{2A}-AR and the corresponding calculated molecular mass based on the deduced amino acids of rat genes is attributed to the presence of a carbohydrate moiety [41].

COX activity

Different aliquots of the previously obtained homogenates, in HEPES/sucrose buffer, were used for COX (EC 1.9.3.1) activity determinations. COX activity was measured spectrophotometrically [42].

Statistical analysis

Lipolytic activity was expressed as a percentage stimulation over basal lipolysis levels. All AR agonists caused a concentration-dependent stimulation of glycerol release, which reached a plateau at the highest agonist concentrations. Dose-response curves for noradrenaline, isoprenaline, and BRL37344 were fitted with nonlinear regression analysis for sigmoidal curves, as previously described [43], using the GraFit computer programme (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 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 [43] to compare parameters of curves fitted by nonlinear regression. With respect to COX activity, UCP2 and AR levels, HSL levels and activity, basal lipolysis and maximal lipolysis induced by forskolin and Bt_2cAMP, differences between groups 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 tissue and gender (TxG) was shown. The analysis was performed with SPSS 10.0 for Windows.

Results

α_{2A}- and β_3-AR protein content in WAT

Table 1 shows α_{2A}- and β_3-AR protein levels as well as the α_{2A}/β_3-AR ratio. In both genders, the protein content of both ARs was higher in gonadal than in inguinal WAT. There were no significant differences between genders in β_3-AR protein content, although, in inguinal WAT, females showed greater β_3-AR levels than males, which did not reach statistical significance. In both tissues, females showed a higher α_{2A}-AR content compared to their male counterparts. The α_{2A}/β_3-AR ratio was significantly higher in inguinal than gonadal WAT, and in both tissues, the ratio was greater in female rats than in males.

Lipolytic activity in isolated fat cells

In this study, the lipolytic response to stimulation with different AR agonists was evaluated. The overall adrenergic responsiveness was measured with the physiological agonist noradrenaline (β_1-, β_2-, β_3-, and α_2-AR agonist). The contribution of β-ARs to lipolytic activity was determined using isoprenaline (β_1-, β_2-, and β_3-AR agonist),

Table 1. Effect of gender and tissue location on the relative levels of α_{2A}- and β_3-AR protein in gonadal and inguinal WAT.

Parameter	Gonadal WAT		Inguinal WAT		Anova
	males	females	males	females	
β_3-AR	100 ± 9.9	102 ± 19.2	16.9 ± 1.8	29.0 ± 5.2	T
α_{2A}-AR	100 ± 10.7	149 ± 20.5	41.1 ± 12.3	75.0 ± 14.1	T, G
α_{2A}/β_3-AR	1.00 ± 0.08	1.42 ± 0.13	1.91 ± 0.38	2.71 ± 0.56	T, G

AR mean values of the gonadal male rats were set as 100%; the ratio of α_{2A}/β_3-AR of gonadal male rats was set as 1. The data represent means ± SE of ten animals per group. Analysis of variance significance was $p < 0.001$ for T effect of tissue; and $p < 0.05$ for G effect of gender.

and the contribution of β_3-AR – quantitatively the most abundant β-AR subtype in rodent adipocytes [3, 4, 44] – was assessed by stimulating glycerol release with BRL37344 (full β_3-AR agonist). The maximum action of these lipolytic agents, expressed as percentage stimulation over basal lipolysis levels, and also basal lipolysis, is shown in table 2.

Maximum capacity, as well as differences between groups, was analyzed using the Grafit4 computer program. All agonists stimulated glycerol release in a concentration-dependent manner. The stimulation of lipolysis by noradrenaline was higher in gonadal than in inguinal WAT, but, in both depots, was similar between genders. On the other hand, the stimulation with isoprenaline and BRL37344 induced a greater lipolytic response in females than in males and, again, in both genders, it was much higher in gonadal than in inguinal WAT. No significant differences were found in basal lipolysis between genders or depots.

Some postreceptor steps of the lipolytic cascade were also evaluated, using agents that bypass the receptor step, such as forskolin (stimulating adenylate cyclase) and Bt_2cAMP (cyclic AMP analogue resistant to phosphodiesterases, which stimulates PKA) at their maximal effective concentrations (10^{-4} and 10^{-3} M, respectively). Table 3 shows maximal lipolysis, expressed as a percentage over basal lipolysis, induced by forskolin and Bt_2cAMP in adipocytes from the different groups. The glycerol released after forskolin stimulation was similar in both depots, with much higher lipolysis in females compared to their male counterparts. The maximal lipolytic activity induced by Bt_2cAMP was greater in gonadal adipocytes from female rats, compared to the other three groups, which were all at the same level.

HSL activity and protein levels in WAT

Table 4 shows HSL protein content and activity in the four experimental groups. Gonadal WAT showed a much higher HSL activity than inguinal WAT in both genders (3.4-fold in males and 3.8-fold in females). Moreover, in gonadal WAT, we found gender-dependent effects on HSL activity, with a higher value in females compared to males. As far as HSL protein levels were concerned, although there were no significant differences between genders, a similar pattern to HSL activity was shown between tissues, with significantly greater protein levels in gonadal than inguinal WAT.

COX activity and UCP2 protein levels in WAT

To confirm the important metabolic differences between the two fat depots, we decided to study COX activity, as an indicator of respiratory chain capacity, i.e., mitochon-

Table 2. Effect of gender and tissue location on adrenergically stimulated lipolytic activity of isolated fat cells from gonadal and inguinal WATs.

Parameter	Gonadal WAT		Inguinal WAT	
	males	females	males	females
Maximal stimulation				
Noradrenaline	352 ± 96	371 ± 15	47.2 ± 8.0^o	43.4 ± 16.9^o
Isoprenaline	358 ± 26	437 ± 98	93.8 ± 7.5^o	148 ± 21*^{o}
BRL37344	183 ± 42	364 ± 63*	33.8 ± 6.3^o	69.7 ± 3.7*^{o}
Basal lipolysis	760 ± 111	625 ± 64	723 ± 88	798 ± 128

Data are means ± SE of seven animals per group. Lipolytic activity was expressed as a percentage of stimulation over basal lipolysis. Maximal action over basal lipolysis and significant differences were obtained using the GraFit computer program; *males vs females, ^{o}gonadal vs inguinal. Basal lipolysis is expressed as nmol glycerol/(10^6 cells × 1.5 h). Significant differences in basal lipolysis were assessed using ANOVA ($p < 0.05$), but no significant differences were obtained.

Table 3. Effect of gender and tissue location on maximal lipolysis induced by forskolin and Bt_2cAMP, at their maximal effective concentrations (10^{-4} and 10^{-3} M, respectively), in isolated adipocytes from gonadal and inguinal WAT.

Parameter	Gonadal WAT		Inguinal WAT		Anova
	males	females	males	females	
Forskolin	238 ± 40	642 ± 175	200 ± 88	516 ± 198	G
Bt_2cAMP	274 ± 48	778 ± 200*	297 ± 96	277 ± 74º	G, T × G

The data represent means ± SE of seven animals per group. The lipolytic activity was expressed as a percentage over basal lipolysis. ANOVA significance was $p < 0.05$; G effect of gender; T × G interaction of tissue and gender. Post hoc comparison: *males vs females; ºgonadal vs inguinal.

Table 4. Effect of gender and tissue location on HSL activity and protein levels in gonadal and inguinal WAT.

Parameter	Gonadal WAT		Inguinal WAT		Anova
	males	females	males	females	
HSL activity [nmol FFA/ (h × mg protein)]	32.5 ± 1.1	41.3 ± 0.9*	9.6 ± 0.9º	10.8 ± 0.8º	T, G, T × G
HSL protein level (% a.u./mg protein)	100 ± 10	98.3 ± 8.1	30.6 ± 2.5	19.8 ± 3.3	T

HSL protein level mean value of gonadal male rats was set as 100%. The data represent means ± SE of eight animals per group. ANOVA significance was $p < 0.001$; T effect of tissue; G effect of gender; T × G interaction of tissue and gender. Post hoc comparison: *males vs females; ºgonadal vs inguinal. a.u., arbitrary units.

Table 5. Effect of gender and tissue location on COX activity and UCP2 protein levels in gonadal and inguinal WAT.

Parameter	Gondal WAT		Inguinal WAT		Anova
	males	females	males	females	
Specific COX activity (IU/g protein)	72.6 ± 9.9	81.2 ± 10.5	37.9 ± 5.4	29.2 ± 5.3	T
UCP2 protein level (% a.u./g prot)	100 ± 10	114 ± 10	47.9 ± 9.4	33.1 ± 4.3	T

UCP2 protein level mean value of gonadal male rats was set as 100%. The data represent means ± SE of eight animals per group. ANOVA significance was $p < 0.001$; T effect of tissue. a.u., arbitrary units.

drial oxidative metabolism functionality [45], as well as protein levels of UCP2, a UCP1 homologue, which may play a role in preventing mitochondrial production of reactive oxygen species [46, 47]. Gonadal WAT showed greater COX activity and UCP2 protein levels, in both genders, than inguinal WAT. However, there were no gender-dependent differences in either of the two parameters studied (see table 5).

Discussion

In this study, both gender- and site-dependent effects on the lipolytic capacity of rat white adipocytes were investigated. Our results point to a higher lipolytic capacity in gonadal than inguinal WAT at the different steps of the adrenergic pathway studied. Moreover, they indicate an adrenoceptor balance which prevents lipid mobilization in females, counteracted by a greater lipolytic capacity at the postreceptor level, both in gonadal and inguinal WAT.

Differences in lipolytic capacity in rat white adipocytes depending on the anatomical location of the tissue

Similarly to other authors, we found marked variations in lipolytic capacity depending on the anatomical location. The higher lipolytic response to catecholamines, as well as to β-AR agonists, shown in gonadal than in inguinal WAT could be explained, in part, by the larger amount of

β_3-AR and, especially, by the lower α_{2A}/β_3-AR ratio found in this tissue. Moreover, the differences observed between fat depots in the lipolytic response may also be explained by changes at the postreceptor step, mainly at the level of the rate-limiting enzyme catalyzing lipolysis, HSL, which showed, in both genders, higher protein levels and activity in gonadal than in inguinal WAT. Thus, although both tissues had a similar basal lipolysis, gonadal 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 the AR step and at the postreceptor step. Similar results have been reported by other authors [9–14, 18], who have found differences between depots in the expression of the different adrenoceptor subtypes [16, 24, 25], as well as in other steps of the lipolytic pathway [9, 10, 13, 25]. Altogether, these results suggest essential differences in the pattern of gene expression between the different fat depots that could be related to different programs of differentiation and maturation of adipocytes.

As an indicator of the respiratory chain capacity [45], COX activity was studied in order to confirm the existence of great differences in the metabolism of both WAT depots. In both genders, a greater COX specific activity was found in gonadal than in inguinal WAT, in agreement with previous results [48], suggesting a more active metabolism of visceral fat depots compared to the subcutaneous ones. Futhermore, UCP2 protein levels in both genders were also analyzed, obtaining, in parallel with the greater COX activity, much higher UCP2 levels in gonadal than in inguinal WAT. These results support the possible role of UCP2 in the prevention of mitochondrial production of reactive oxygen species [46, 47, 49].

Gender dimorphism in the lipolytic capacity of rat white adipocytes

In the present study, a gender dimorphism was found in both tissues in the activity and/or expression of the different elements of the adrenergic pathway. In both gonadal and inguinal WAT, females showed an amount and a balance of adrenoceptors which was more focused toward preventing lipid mobilization, with higher levels of α_{2A}-AR, but mainly a higher α_{2A}/β_3-AR ratio, compared to males. However, surprisingly, the incubation of isolated adipocytes from both tissues with the physiological agonist, noradrenaline, brought about a similar glycerol release in both genders. These results, although at first glance contradictory, could be explained by taking into account, not only the amount of the different ARs, but also other elements of the lipolytic pathway at the postreceptor level, which may also be differentially regulated in male and female rats. In this respect, when isolated adipocytes from both tissues were incubated with β-AR agonists, isoprenaline and BRL37344 (leaving aside the inhibition mediated by α_2-AR), the lipolytic response was greater in females than in males, although β_3-AR levels were not significantly different between genders. In addition, when increasing doses of noradrenaline were tested in the presence of RS79948 (α_2-AR antagonist) (data not shown), the stimulation obtained, similarly to isoprenaline stimulation, was higher in females compared to their male counterparts, which would support a greater inhibitory effect via α_2-AR in females.

Moreover, 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, but with variations between depots. Thus, in inguinal WAT, the lipolysis induced by forskolin was 2.5-fold higher in females, but was similar in both genders after stimulation with Bt_2cAMP, which would point to a higher adenylate cyclase capacity in females. However, in gonadal WAT, the stimulation both with forskolin and Bt_2cAMP brought about a higher glycerol release in females (2.7- and 2.8-fold higher in females than in males, respectively), which would show that the higher activity at the postreceptor step in females was not due to changes in adenylate cyclase capacity, but to differences in the capacity at the PKA or subsequent steps.

On the other hand, considering the relationship between the amount of HSL protein and its activity, in both tissues – in a basal situation without adrenergic stimulation – females would have a more active HSL protein than males. These differences could be due to gender-related variations in the post-transcriptional control of HSL, such as a different oligomerization [50] or variations in protein phosphorylation [51, 52]. One must take into account that, although HSL is the rate-limiting enzyme catalyzing lipolysis, and its activation is mainly mediated through phosphorylation by PKA, recent studies have documented that a portion of β adrenergic-stimulated lipolysis is mediated via the MAPK pathway and that HSL is a target of extracellular signal-regulated kinase [52]. Moreover, the phosphorylation of perilipins by PKA also plays an important role in lipolysis regulation [53, 54]. Therefore, lipolysis regulation is a complex process, which involves several interconnected signal transduction pathways and multiple regulatory steps, in which sex hormones have also been found to have a direct effect. In this sense, several studies suggest a direct effect of sex hormones on the metabolic control of WAT, for example affecting lipolysis through the modification of the sensitivity and/or density of α_2- and β_3-AR, as well as affecting other steps of the lipolytic pathway modulating the activity or expression of adenylate cyclase, PKA, or HSL [32–35, 55].

In summary, we found that lipolytic capacity was clearly higher in a visceral fat depot, such as gonadal, compared to a subcutaneous one, such as inguinal WAT, at the different steps of the adrenergic pathway studied. On the

other hand, in both tissues, females were able to counteract the greater inhibition of lipolysis via α_2-AR, by a higher lipolytic capacity at the postreceptor level. These gender-dependent differences in lipolytic capacity could be due, at least in part, to the action of sex hormones, which may act at different steps of the lipolytic pathway. To determine gender- or site-related changes in lipolytic capacity, not only the amount of ARs, but also the remaining elements of the adrenergic pathway must be taken into account. Furthermore, other pathways may be able to activate lipolysis, bringing about, overall, a complex regulation of the lipolytic process, with multiple interactions between different pathways, in which the hormonal environment would also play a role. The different behaviour between visceral and subcutaneous depots, together with gender-related differences, could be very important for the metabolic disorders associated with obesity, although a more detailed study of the direct effect of sex hormones is required.

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