Pregnancy Effects on Rat Adipose Tissue Lipolytic Capacity are Dependent on Anatomical Location

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Key Words

Adipose tissue • Pregnancy • Lipolytic pathway • Lipoprotein lipase • Adrenergic receptors

Abstract

Pregnancy is characterized by changes in maternal adiposity. The aim of this study was to carry out a detailed analysis of the different steps of the adrenergic pathway, lipoprotein lipase (LPL) levels and adipocyte size, in order to evaluate the response of white adipose tissue (WAT) to the metabolic changes during pregnancy depending on the anatomical location. In general, the levels of the proteins of the lipolytic pathway decreased with pregnancy. In retroperitoneal WAT adenylate cyclase (AC) levels decreased from 100% in controls to 44% by day 13 and 11% by day 20. In mesenteric WAT the α_{2A}/β_3 -adrenergic receptor balance seemed to be one of the main regulatory points of the lipolytic pathway and the reduction in the postreceptor element levels was clearly lower than for the other two depots (PKA levels reduced from 100% in controls to 72% by day 20, while in the other two depots it decreased to 30%, and AC and HSL levels did not show statistically significant changes in this depot). In contrast, the LPL-

to-HSL ratio may be a major regulatory point in gonadal WAT. In summary, we describe regional differences in the regulation of WAT metabolism throughout pregnancy, which may be of great importance to determine the role of the different fat depots during late pregnancy. Thus, gonadal and mesenteric WAT changed to a lipolytic state to sustain the rapid foetal growth, although with differences between them in the main regulatory points, while retroperitoneal WAT could have a role later on, during lactation.

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Introduction

Pregnancy is a physiological state characterized by changes in maternal adiposity, with a prevalence of lipid deposition in adipose tissue during the first phase of pregnancy (anabolic phase) and a predominance of lipid mobilization during the last third (catabolic phase) [1]. Insulin concentration [2, 3] and sensitivity [4, 5] are enhanced from early pregnancy, while both hyperinsulinemia and insulin resistance are consistently present during late pregnancy [6].

Apart from insulin, catecholamines are also of major importance for the endocrine control of lipid mobilization from white adipose tissue (WAT). Catecholamines, by the activation of β -adrenergic receptors (AR), stimulate lipid mobilization in WAT, mainly through the production of cyclic adenosine monophosphate (cAMP) by adenylate cyclase (AC), and the activation of cAMP-dependent protein kinase (PKA) which, in turn, activates hormone sensitive lipase (HSL), leading to lipolysis [7, 8]. In rodents, β_3 -AR is quantitatively the most abundant β -adrenergic receptor in white adipocytes [9, 10]. Catecholamines are also able to prevent lipid mobilization, acting through α_2 -AR and inhibiting cAMP production [8]. 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 [11]. However, the other elements of the adrenergic pathway, and not only the amount of ARs, must be taken into account in order to determine the lipolytic capacity [12].

The antilipolytic action of insulin is a multistep process, with both acute and long-term effects. Among its actions, insulin plays an important role on the regulation of fat accumulation. In this sense, insulin is an important regulator of lipoprotein lipase (LPL) [13], which catalyses the hydrolysis and uptake of circulating triglycerides [14], and its activity is especially high in adipose tissue, where it plays a key role in the regulation of triglyceride storage [14].

It is well known that adipose tissue is a heterogeneous organ with marked variations in fat cell metabolism depending on the anatomical location [15-17]. Thus, regional differences in lipolytic activity have been observed both in rats [18-20] and humans [21, 22]. Previous studies have reported changes with pregnancy at certain steps of the lipolytic pathway in WAT [23-25], but they have failed to assess differences between different fat depots with pregnancy, throughout the whole lipolytic pathway. Thus, the aim of this study was to carry out, in different fat depots, a detailed analysis of the different steps of the adrenergic pathway, LPL levels and adipocyte morphometric parameters, in order to evaluate the response of WAT to the two metabolic phases of pregnancy depending on the anatomical location.

Materials and Methods

Materials

Routine chemicals used were supplied by Amersham Pharmacia Biotech (Little Chalfont, UK), Panreac (Barcelona, Spain), Sigma-Aldrich (St. Louis, MO, USA) and Bio-Rad

Laboratories (Hercules, CA, USA).

Antibodies for β_3 -AR, α_2 -AR, PKA (α catalytic subunit) and AC were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Antibody for HSL was kindly supplied by Dr. F.B. Kraemer [26]. Antibody for LPL was previously developed in our laboratory [27]. Appropriate secondary antibodies were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals and tissue collection

Animal experiments were performed in accordance with general guidelines approved by our institutional ethics committee and EU (86/609/EEC) regulations. Female Wistar rats, three-months old, purchased from Criffa (Barcelona, Spain), were individually housed 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). Female rats were mated with adult males, and day 0 of pregnancy was determined by the presence of spermatozoa in the vaginal smears. The pregnant rats were studied on days 11 and 13 of pregnancy (the days around the formation of placenta) and on day 20 of pregnancy (one day before parturition). Age-matched unmated female rats (100 $\pm$ 10 days) were used as non-pregnant controls. All animals were killed by decapitation at the start of the light cycle. The gonadal, retroperitoneal and mesenteric WAT were rapidly dissected, weighed and frozen in N₂ liquid and stored at -75 °C until processed.

Histological analysis

Small pieces of gonadal, retroperitoneal and mesenteric WAT from three animals per group were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) and then embedded in paraffin by standard procedures. Blocks were sectioned and stained with hematoxylin and eosin. Morphometric parameters (area, perimeter and major axis) were determined in tissue sections by computer-assisted image analysis using Scion Image software (Frederick, MD, USA). For each sample, 100 adipocytes from different sections were analyzed.

Western-blot for α_2 -AR, β_3 -AR, AC, PKA, HSL and LPL

Small pieces (approx. 180 mg) of tissue were homogenized in HEPES/sucrose buffer (250 mM sucrose, 1 mM HEPES, 0.2 mM EDTA), with a teflon/glass homogenizer. The homogenates were centrifuged at 500 $\times$ g for 10 minutes at 4 °C. The pellets containing nuclei and membrane debris were removed and the fat-free infranatant was used for the Western-blot analysis. Protein concentration was measured using the method of Lowry et al [28]. Varying amounts of total protein from the homogenates (50 μ g for AC, and 25 μ g for the other proteins analysed) were fractionated by 10% sodium dodecyl sulfate polyacrilamide gel electrophoresis according to Laemmli [29] and electrotransferred onto a nitrocellulose filter as described elsewhere [30]. Staining with Ponceau S was used to provide visual evidence for correct loading and electrophoretic transfer of proteins to nitrocellulose filter. The membranes were blocked overnight with 5% powdered milk in PBS. Goat polyclonal antibodies against α_2 - and β_3 -AR, rabbit polyclonal antibody

Table 1. Effects of pregnancy on body and WAT depot weights. All data are expressed in grams. Data represent the mean $\pm$ sem of 7 animals per group. The percentage of weight increase with respect to control rats is showed in brackets. Significant differences were assessed using ANOVA ($p < 0.05$), with D as effect of the day of pregnancy. Post-hoc comparison: ^a different from control, ^b different from day 11 of pregnancy and ^c different from day 13.

	BODY WEIGHT	GONADAL	RETROPERITONEAL	MESENTERIC
Control	232 $\pm$ 4	3.83 $\pm$ 0.36	1.46 $\pm$ 0.09	1.81 $\pm$ 0.11
Day 11	287 $\pm$ 4 ^a	5.21 $\pm$ 0.37 ^a (+36%)	2.02 $\pm$ 0.22 ^a (+38%)	2.39 $\pm$ 0.29 (+32%)
Day 13	299 $\pm$ 6 ^a	6.05 $\pm$ 0.47 ^a (+58%)	2.10 $\pm$ 0.16 ^a (+44%)	2.91 $\pm$ 0.22 ^a (+61%)
Day 20	340 $\pm$ 10 ^{a,b,c}	5.47 $\pm$ 0.69 ^a (+43%)	2.35 $\pm$ 0.24 ^a (+61%)	2.34 $\pm$ 0.11 (+29%)
ANOVA	D	D	D	D

Table 2. Effects of pregnancy on the morphometric parameters (area, perimeter and major axis) of adipocytes from gonadal, retroperitoneal and mesenteric WAT. Data represent the mean $\pm$ sem of 300 adipocytes of 3 animals per group (100 adipocytes per animal). Significant differences were assessed using ANOVA ($p < 0.05$), with D as effect of the day of pregnancy. Post-hoc comparison: ^a different from control, ^b different from day 11 of pregnancy and ^c different from day 13.

Parameter		GONADAL	RETROPERITONEAL	MESENTERIC
Area (μm^2)	Control	3289 $\pm$ 43	3331 $\pm$ 11	1649 $\pm$ 18
	Day 11	3793 $\pm$ 44 ^a	4275 $\pm$ 10 ^a	1699 $\pm$ 4 ^a
	Day 13	3678 $\pm$ 9 ^{a,b}	4956 $\pm$ 12 ^{a,b}	1546 $\pm$ 7 ^{a,b}
	Day 20	4229 $\pm$ 42 ^{a,b,c}	5256 $\pm$ 50 ^{a,b,c}	1647 $\pm$ 9 ^{b,c}
	ANOVA	D	D	D
Perimeter (μm)	Control	216.7 $\pm$ 1.4	219.3 $\pm$ 0.4	156.3 $\pm$ 0.8
	Day 11	237.5 $\pm$ 1.4 ^a	250.7 $\pm$ 0.2 ^a	157.9 $\pm$ 0.2 ^a
	Day 13	232.0 $\pm$ 0.4 ^{a,b}	268.3 $\pm$ 0.3 ^{a,b}	151.5 $\pm$ 0.3 ^{a,b}
	Day 20	247.3 $\pm$ 1.4 ^{a,b,c}	275.3 $\pm$ 1.4 ^{a,b,c}	156.0 $\pm$ 0.4 ^{b,c}
	ANOVA	D	D	D
Major axis (μm)	Control	72.4 $\pm$ 0.5	73.9 $\pm$ 0.2	53.2 $\pm$ 0.2
	Day 11	82.3 $\pm$ 0.5 ^a	85.3 $\pm$ 0.1 ^a	54.0 $\pm$ 0.1 ^a
	Day 13	79.8 $\pm$ 0.1 ^{a,b}	90.4 $\pm$ 0.1 ^{a,b}	51.7 $\pm$ 0.1 ^{a,b}
	Day 20	83.6 $\pm$ 0.4 ^{a,b,c}	93.5 $\pm$ 0.5 ^{a,b,c}	52.8 $\pm$ 0.1 ^{a,b,c}
	ANOVA	D	D	D

against AC, PKA and HSL, and chicken polyclonal antibody against LPL were used as primary antibodies. Anti-goat, anti-rabbit and anti-chicken IgG-alkaline phosphatase antibodies were used as secondary antibodies, respectively. Development of the immunoblots was performed using an enhanced chemiluminescence Western blotting analysis system (Amersham). Bands in films were analysed by scanner photodensitometry and quantified using the Kodak 1D Image Analysis Software (New Haven, CT, USA).

Statistical analysis

All data are presented as group mean values $\pm$ sem of 7 animals per group, except for morphometric parameters that represent the mean values $\pm$ sem of 300 adipocytes of 3 animals per group (100 adipocytes per animal). Differences between

groups were assessed by one-way analysis of variance (ANOVA) and LSD *post hoc* comparison when an effect of the day of pregnancy was shown. The analysis was performed with SPSS 11.0 for Windows. The level of probability was set at $p < 0.05$ as statistically significant.

Results

Effects of pregnancy on body and WAT depot weights

Table 1 shows changes in body weight as well as gonadal, retroperitoneal and mesenteric WAT weight

throughout pregnancy. Body weight increased with pregnancy, being nearly 1.5-fold by day 20, as compared to control rats, due mainly to both fetal growth and maternal fat deposition. Retroperitoneal WAT, which is considered as a non-visceral, non-subcutaneous depot [21], showed an increase in its weight throughout pregnancy reaching a 1.6-fold increase by day 20.

Both gonadal and mesenteric WAT depots, which are visceral depots, showed an increased weight during the first two thirds of gestation, reaching an approximately 1.6-fold increase by day 13, as compared to control rats. This increase was followed by a decrease in their weight during late pregnancy, with a higher percentage of weight reduction from day 13 to 20 in the case of mesenteric (20%) than in gonadal (10%).

Histological analysis of gonadal, retroperitoneal and mesenteric WAT

The effects of pregnancy on the morphometric parameters (area, perimeter and major axis) of adipocytes from gonadal, retroperitoneal and mesenteric WATs are shown in Table 2. Following a similar profile as for tissue weight, retroperitoneal adipocytes underwent a progressive increase in their size, reaching a 1.6-fold increase in their area by day 20, as compared to control rats. The morphometric parameters of gonadal adipocytes did not show the same profile as the depot weight, but increased with pregnancy, reaching a 1.3-fold increase by day 20. Mesenteric adipocytes did not increase significantly their size throughout pregnancy.

Effects of pregnancy on the relative levels of α_{2A} - and β_3 -AR, AC, PKA, HSL and LPL protein in gonadal, retroperitoneal and mesenteric WAT

Table 3 shows specific α_{2A} - and β_3 -AR content in gonadal, retroperitoneal and mesenteric fat depots, from control and pregnant rats.

There was a clear reduction in both α_{2A} - and β_3 -AR content by day 20 of pregnancy in the three different WAT depots studied, as compared to control rats. This reduction was higher for retroperitoneal WAT, in which there was a statistically significant decrease already on day 13.

Table 4 shows the specific protein levels of AC, PKA, HSL and LPL, in gonadal, retroperitoneal and mesenteric fat depots, from control and pregnant rats.

AC levels decreased throughout pregnancy both in gonadal and retroperitoneal WAT. This decrease was higher in the case of retroperitoneal WAT, reaching a 56% decrease by day 13 and a 90% decrease by day 20, which

may be important to allow the increase in the size of this depot even during the last part of pregnancy. As for mesenteric WAT, there were no statistically significant changes in AC levels.

In the same way as adrenergic receptors, PKA and HSL showed a decrease in their specific protein content with pregnancy, in the three WAT depots, although the decrease in mesenteric HSL levels were not statistically significant.

The three different WAT depots suffered a continuous reduction in their LPL protein levels with pregnancy, and this reduction was similar for the three fat depots.

Discussion

In this study we have investigated the effects of pregnancy on the lipolytic capacity of rat WAT, depending on the anatomical location of the depot. Our results indicate that in general the lipolytic cascade in WAT underwent an inhibition as pregnancy went on, as reflected by the decrease in the levels of the different proteins of the lipolytic pathway. However, there were some differences between depots, which may be of great importance in order to determine their role during late pregnancy.

The three WAT depots studied showed an increase in their weight with pregnancy, but with some differences during the last third of pregnancy. The changes observed in the weight of gonadal and mesenteric fat depots makes us think that during late pregnancy these depots, which are closer to the foetuses, changed to a state in which the active lipid accumulation ceases and lipid mobilization begins, in order to contribute to the rapid foetal growth. On the other hand, retroperitoneal WAT weight did not follow the same profile as gonadal and mesenteric WAT, but showed a continuous increase in its weight with pregnancy, as the size of retroperitoneal adipocytes did. These results show that the retroperitoneal fat depot did not follow the two metabolic phases that occur during pregnancy, which is in accordance with previously reported results [24].

The adipocyte area data suggested that the increase in the weight of both gonadal and retroperitoneal fat pads would be mainly due to the hypertrophy of these tissues. However, the morphometric parameters of mesenteric adipocytes did not show major changes with pregnancy, despite the increase in weight of this fat depot, suggesting a higher effect of hyperplasia in this tissue, although further

Table 3. Effects of pregnancy on the relative levels of α_{2A} - and β_3 -AR protein in the gonadal, retroperitoneal and mesenteric WAT. The mean values of the different parameters of control rats were set at 100%. Data represent the mean $\pm$ sem of 7 animals per group. a.u. means arbitrary units. Significant differences were assessed using ANOVA ($p < 0.05$), with D as effect of the day of pregnancy. Post-hoc comparison: ^a different from control, ^b different from day 11 of pregnancy and ^c different from day 13.

Parameter		GONADAL	RETROPERITONEAL	MESENTERIC
α_{2A} -AR a.u. (% of control levels)	Control	100.0 $\pm$ 11.3	100.0 $\pm$ 8.2	100.0 $\pm$ 15.4
	Day 11	99.7 $\pm$ 15.2	110 $\pm$ 14	93.7 $\pm$ 16.8
	Day 13	88.4 $\pm$ 14.6	75.0 $\pm$ 13.4	99.0 $\pm$ 13.5
	Day 20	30.4 $\pm$ 4.7 ^{a,b,c}	17.3 $\pm$ 4.6 ^{a,b,c}	37.2 $\pm$ 4.1 ^{a,b,c}
	ANOVA	D	D	D
β_3 -AR a.u. (% of control levels)	Control	100.0 $\pm$ 12.4	100.0 $\pm$ 8.9	100.0 $\pm$ 14.4
	Day 11	115 $\pm$ 14	104 $\pm$ 9	67.4 $\pm$ 8.2
	Day 13	98.0 $\pm$ 11.0	75.7 $\pm$ 10.0 ^{a,b}	76.5 $\pm$ 14.8
	Day 20	27.6 $\pm$ 3.9 ^{a,b,c}	22.1 $\pm$ 3.6 ^{a,b,c}	34.2 $\pm$ 5.9 ^{a,b}
	ANOVA	D	D	D

Table 4. Effects of pregnancy on the relative levels of AC, PKA, HSL and LPL protein in the gonadal, retroperitoneal and mesenteric WAT. The mean values of the different parameters of control rats were set at 100%. Data represent the mean $\pm$ sem of 7 animals per group. a.u. means arbitrary units. Significant differences were assessed using ANOVA ($p < 0.05$), with D as effect of the day of pregnancy. Post-hoc comparison: ^a different from control, ^b different from day 11 of pregnancy and ^c different from day 13.

Parameter		GONADAL	RETROPERITONEAL	MESENTERIC
AC a.u. (% of control levels)	Control	100.0 $\pm$ 16.1	100.0 $\pm$ 13.8	100.0 $\pm$ 54.5
	Day 11	58.3 $\pm$ 12.2 ^a	87.9 $\pm$ 8.3	105 $\pm$ 20
	Day 13	74.4 $\pm$ 11.2	44.1 $\pm$ 10.4 ^{a,b}	66.1 $\pm$ 24.7
	Day 20	42.7 $\pm$ 12.1 ^a	11.2 $\pm$ 2.5 ^{a,b,c}	135 $\pm$ 58
	ANOVA	D	D	-
PKA a.u. (% of control levels)	Control	100.0 $\pm$ 8.1	100.0 $\pm$ 11.0	100.0 $\pm$ 14.5
	Day 11	87.4 $\pm$ 8.0	96.1 $\pm$ 3.7	121 $\pm$ 10
	Day 13	86.3 $\pm$ 4.5	93.5 $\pm$ 7.6	121 $\pm$ 14
	Day 20	34.6 $\pm$ 6.0 ^{a,b,c}	32.9 $\pm$ 5.3 ^{a,b,c}	72.0 $\pm$ 9.3 ^{b,c}
	ANOVA	D	D	D
HSL a.u. (% of control levels)	Control	100.0 $\pm$ 11.1	100.0 $\pm$ 6.9	100.0 $\pm$ 9.5
	Day 11	52.3 $\pm$ 11.8 ^a	81.8 $\pm$ 8.9	120 $\pm$ 16
	Day 13	63.8 $\pm$ 7.0 ^a	69.1 $\pm$ 6.0 ^a	109 $\pm$ 14
	Day 20	50.9 $\pm$ 4.8 ^a	52.1 $\pm$ 7.3 ^{a,b}	71.8 $\pm$ 6.6
	ANOVA	D	D	-
LPL a.u. (% of control levels)	Control	100.0 $\pm$ 15.7	100.0 $\pm$ 3.9	100.0 $\pm$ 11.2
	Day 11	86.4 $\pm$ 16.1	84.1 $\pm$ 5.6	85.0 $\pm$ 9.4
	Day 13	64.4 $\pm$ 6.0	82.8 $\pm$ 6.1	71.3 $\pm$ 8.3 ^a
	Day 20	45.0 $\pm$ 6.1 ^{a,b}	53.0 $\pm$ 10.0 ^{a,b,c}	60.4 $\pm$ 8.4 ^a
	ANOVA	D	D	D

studies should be done. Our results pointed to an inhibition of the lipolytic pathway in the three fat depots in pregnant rats, with decreased levels of the different elements of the pathway, mainly during late pregnancy.

In the mesenteric fat depot, the balance between α_{2A} - and β_3 -AR, which shows the level of inhibition vs. activation of the lipolytic pathway at the adrenergic receptor level [7, 31, 32], could be one of the main

regulatory points of the lipolytic pathway. In that sense, in this depot both inhibitory α_{2A} -AR and stimulatory β_3 -AR levels showed an important decrease with pregnancy, but if we take into account the α_{2A} - / β_3 -AR ratio, it followed the same profile as the depot weight (increasing from 1 to 1.3 by day 13 and decreasing again to 1.1 by day 20). However, this parallelism was not observed either in the gonadal or in the retroperitoneal WAT.

Mesenteric WAT did not show statistically significant changes in AC and HSL content, and the reduction in PKA levels was clearly lower than for the other two depots. All in all, these results would help explain the changes in the weight of mesenteric WAT. Thus, this depot is the one that showed a higher reduction in its weight during the last phase of pregnancy, since it was also the depot that maintained higher levels of the different elements of the lipolytic pathway during pregnancy. Therefore, it would contribute to the high growth of the foetuses, mainly during late pregnancy.

Unlike the mesenteric depot, gonadal and retroperitoneal WAT showed an important reduction not only in both adrenergic receptor levels, but also in the content of the other elements of the lipolytic pathway, AC, PKA and the rate-limiting enzyme of lipolysis, HSL.

Since insulin has been shown to decrease β_3 -AR levels [33-35], the reduction observed in this adrenoceptor, mainly in late pregnancy, could be explained by the hyperinsulinemia present in late pregnancy [6, 24], despite the insulin resistance in fat depots [6, 36], since the antilipolytic effect of insulin is considered the most sensitive of its metabolic actions (i.e. it requires a much lower insulin concentration) [37]. Sympathetic nervous activity has been described to diminished in retroperitoneal and gonadal WAT with pregnancy [38], in order to save energy for foetal growth and maternal fat storage. Since α_2 -AR has a higher affinity for norepinephrine than β_3 -AR [31, 39], in late pregnancy, with lower levels of β_3 -AR and norepinephrine, the decrease of α_{2A} -AR may be a feedback downregulation.

Moreover, a decreased response of retroperitoneal adipocytes to norepinephrine or isoproterenol during late pregnancy has been reported in the rat [24, 40]. Due to the highly sensitive antilipolytic effect of insulin [37], hyperinsulinemia would account for this reported decreased catecholamine-stimulated lipolysis, despite the insulin resistance in late pregnant rat WAT [36]. This decreased lipolysis would result in the maintenance or even the expansion of adipose stores. In this sense, the fact that all the elements of the lipolytic pathway decreased both in gonadal and retroperitoneal fat depots, but that their weight showed a different profile during late pregnancy, could be therefore explained by slight differences in the degree of insulin resistance between these two fat depots. Furthermore, differences in sympathetic nervous system and/or in the lipolytic response to norepinephrine between them could not be discarded. Thus, the differences observed between these two depots would point to a shift to a lipolytic state in the

gonadal depot during late pregnancy in order to allow the fast foetal growth. On the other hand, the fact that retroperitoneal WAT weight increased all through pregnancy would point to a possible role of this depot, not during the last part of pregnancy, but later on, during lactation. In this sense, the hydrolysis, and release to the bloodstream, of fat stores from the retroperitoneal WAT during lactation would allow a fatty acid supply to contribute to milk synthesis in the mammary gland.

Our results showed that adipocytes from the three fat depots reduced their LPL content in pregnancy, having higher levels at the beginning of pregnancy, in order to allow triglyceride deposition, followed by lower levels during the last third, when there is a prevalence of lipid mobilization, which is in accordance with reports by other authors [41, 42]. Thus, the reduced hydrolysis and uptake of circulating triglycerides by these depots in late pregnancy would allow the circulating triglycerides to be used for milk synthesis in the mammary gland, which increases its LPL levels with pregnancy [43]. Once again, insulin would play a key role, regulating LPL expression and activity [14]. It has been proposed that the decrease in LPL in WAT during late pregnancy is not only a consequence of the changes in circulating insulin levels, but also of the changes in tissue responsiveness to insulin [41].

In the gonadal WAT depot, the balance between LPL and HSL levels may be a major regulatory point for the accumulation and mobilization of fats. Thus, the LPL-to-HSL ratio, which could provide a measure of the relative triglyceride storage compared with triglyceride breakdown [43], showed a profile that followed the depot weight (rising from 1 to 1.65 by day 11 and decreasing again to 0.88 by day 20) in gonadal WAT, but not in the other two fat depots.

In summary, in this study we have described regional differences in the regulation of WAT metabolism throughout pregnancy. Thus, during the last third of pregnancy, gonadal and mesenteric WAT depots changed to a state with a prevalence of lipid mobilization, in order to sustain the rapid foetal growth in late pregnancy, although with differences between them in the main regulatory points. In contrast, retroperitoneal WAT showed an increase in its weight throughout pregnancy, which would point to a possible role of this depot later on, during lactation. All the elements of the lipolytic pathway, and not only the amount of adrenergic receptors, must be taken into account in order to determine site-related changes in the balance between the accumulation and mobilization of fats during pregnancy.

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