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Progesterone-induced down-regulation of hormone sensitive lipase (*Lipe*) and up-regulation of G0/G1 switch 2 (*G0s2*) genes expression in inguinal adipose tissue of female rats is reflected by diminished rate of lipolysis

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ABSTRACT

Decreased lipolytic activity in adipose tissue may be one of the reasons behind excess accumulation of body fat during pregnancy. The aim of this study was to analyze the effect of progesterone on the expression of: (a) *Lipe* (encoding hormone-sensitive lipase, HSL), (b) *Pnpla2* (encoding adipose triglyceride lipase, ATGL), (c) abhydrolase domain containing 5 (*Abhd5*), and (d) G0/G1 switch 2 (*G0s2*) genes in white adipose tissue (WAT), as potential targets for progesterone action during the course of pregnancy. Administration of progesterone to female rats, which was reflected by approximately 2.5-fold increase in circulating progesterone concentration, is associated with a decrease in *Lipe* gene expression in the inguinal WAT. The expression of *Pnpla2* gene in all main fat depots of females and males remained unchanged after progesterone administration. Administration of progesterone resulted in an increase in the expression of *Abhd5* gene (whose product increases ATGL activity) and *G0s2* gene (whose product decreases ATGL activity) in the inguinal WAT of female rats. Mifepristone, a selective antagonist of progesterone receptor, abolished the effect of progesterone on *Lipe*, *Abhd5* and *G0s2* genes expression in the inguinal WAT. The decrease in *Lipe* and the increase in *Abhd5* and *G0s2* genes expression was associated with lower rate of stimulated lipolysis. Administration of progesterone exerted no effect on *Lipe*, *Abhd5* and *G0s2* genes expression and stimulated lipolysis in the retroperitoneal WAT of females, as well as in the inguinal, epididymal and retroperitoneal WAT of males. In conclusion, our findings suggest that progesterone decreases the rate of lipolysis in the inguinal WAT of female rats, inhibiting the activity of both ATGL (by stimulating synthesis of G0S2 – specific inhibitor of the enzyme) and HSL (due to inhibition of *Lipe* gene expression).

1. Introduction

Human gestation is usually associated with an increase in maternal body weight and fat mass [1]. Excessive fat accumulation is one of many factors contributing to this phenomenon. Some data suggest that progesterone may contribute to the increase in maternal body weight and fat mass during pregnancy. For instance, fat mass increases in pregnancy, when concentration of circulating progesterone is elevated [2]. The possible contribution of progesterone to the increase in body weight and fat mass of pregnant women was confirmed by the administration of this hormone to non-pregnant laboratory animals [3–5]. However, the mechanism of the progesterone-induced increase in body weight is still not fully understood. Quite recently, administration of progesterone to females was shown to cause an increase in orexigenic and a decrease in anorexigenic neuropeptide genes expression [6]. This may, at least in part, explain the increase in food intake, and resultant body weight and fat mass gain [6]. Some authors suggested that the anabolic-like effect of progesterone may be related to increased secretion of insulin [7]. Insulin signaling is known to enhance triacylglycerol (TAG) storage in adipocytes, both via increase in TAG

Abbreviations: ATGL, adipose triglyceride lipase; *Pnpla2*, patatin-like phospholipase domain containing 2-gene encoding ATGL; HSL, hormone-sensitive lipase; *Lipe*, gene encoding HSL; ABHD5 (CGI-58), abhydrolase domain containing 5; G0S2, G0/G1 switch 2; CIDEC (FSP27), cell death-inducing DFFA-like effector c; PLIN-1, perilipin-1; WAT, white adipose tissue; RU 486, mifepristone; LPL, lipoprotein lipase; FFA, free fatty acids; TAGs, triacylglycerols; DAGs, diacylglycerols; MAGs, monoacylglycerols; PPARG, peroxisome proliferator-activated receptor gamma.

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synthesis and due to decrease in TAG degradation [8]. Several studies showed that progesterone may increase (directly or indirectly – stimulating insulin secretion) accumulation of fat, enhancing lipogenesis [4,9–11], post-heparin lipoprotein lipase (LPL) activity [4] and differentiation of preadipocytes [9,12,13]. However, the results of the abovementioned studies are inconclusive. For example, Hamosh and Hamosh [14] showed that progesterone administration did not affect LPL activity in rat adipose tissue. This is consistent with results reported by Toth et al. [15]. Also the data on the effect of progesterone on adipocyte differentiation are conflicting; while no effect of progesterone on the differentiation of cultured human preadipocytes was found in one study [16], according to other authors, this hormone stimulated adipogenesis in 3T3-L1 cell line [12]. Theoretically, the progesterone-induced increase in fat accumulation may result from an inhibition of lipolysis by this hormone. Thus, decreased rate of lipolysis would be an important factor leading to an increase in fat accumulation in the adipocytes during the course of pregnancy. Accordingly, reduced rate of lipolysis, associated with a decrease in hormone-sensitive lipase (HSL, encoded by *Lipe*) mRNA levels, has been observed in obesity [17]. However, prior studies showed that neither unstimulated (basal) nor adrenaline-stimulated lipolysis in adipocytes was modulated by progesterone [4]. Lipolysis is a biochemical process during which TAGs stored in adipocytes are released into circulation as free fatty acids (FFA) and glycerol. Under physiological conditions, this process involves three different lipases that directly contribute to the TAG breakdown, as well as several regulatory proteins. The enzymatic breakdown of TAGs is believed to be initiated by adipose triglyceride lipase (ATGL, encoded by *Pnpla2*). The activity of this enzyme results in formation of FFA and diacylglycerols (DAGs). DAGs, in turn, are hydrolyzed by HSL to FFA and monoacylglycerols (MAGs). Finally, MAGs are hydrolyzed by monoglyceride lipase to FFA and glycerol [18,19]. The results of several studies suggest that both ATGL and HSL play key roles in the regulation of lipolysis in rodent adipocytes [18–20]. Since ATGL generates DAGs, a substrate for HSL, some authors consider ATGL as a rate-limiting enzyme in hormone-stimulated lipolysis [21]. ATGL and HSL are regulated both at transcriptional and post-translational levels (with the latter being the most important regulatory mechanism of lipolysis) and share some regulatory similarities [18,19,22]. For example, insulin-mediated decrease in lipolysis rate is associated with phosphorylation and activation of phosphodiesterase (hydrolyzing cAMP) and down-regulation of *Pnpla2* and *Lipe* genes expression [23,24]. The rate of lipolysis is modulated due to complex interactions between lipases and other proteins, such as perilipin-1 (PLIN-1), abhydrolase domain containing 5 (ABHD5, a co-activator of ATGL activity) and G0/G1 switch 2 (G0S2, an inhibitor of ATGL activity) [18,19,21]. Recent findings suggest that G0S2 exerts strong effect on lipolysis, TAGs deposition and energy metabolism [25,26]. Recently, we showed that progesterone selectively regulates expression of genes encoding some adipokines and lipogenic enzymes in the inguinal WAT of females, acting via progesterone receptor [5,11]. Therefore, it can be hypothesized that this hormone may be responsible for fat accumulation, both stimulating lipogenesis and decreasing the rate of lipolysis in the inguinal WAT of females due to inhibition of *Lipe* and *Pnpla2* genes expression and/or up-regulation of *G0s2* gene expression whose product decreases ATGL activity.

In this study, we analyzed: (a) expression of *Lipe* and *Pnpla2* genes, (b) expression of *Abhd5*- and *G0s2* genes – encoding proteins involved in regulation of ATGL in WAT of control and progesterone-treated female and male rats. Moreover, we studied the rate of lipolysis in WAT explants obtained from control and progesterone-treated rats. Our findings suggest that progesterone, acting in a progesterone receptor-dependent manner, down-regulates *Lipe* gene expression but exerts no effect on *Pnpla2* gene expression in the inguinal WAT of females. Moreover, administration of progesterone resulted in enhanced expressions of *Abhd5* and *G0s2* genes in the inguinal WAT of female rats. These changes were associated with lower rate of stimulated lipolysis in the inguinal WAT explants from female rats.

2. Materials and methods

2.1. Animals and treatment; tissue and blood collection

The experiment was performed on 10-week-old female and male Wistar rats. The animals were treated with progesterone and mifepristone (RU 486), as described recently [5]. Males and females were housed at 22 °C in individual wire-mesh cages under a light to dark (12:12 h) cycle with lights on at 7:00 a.m. The animals were allowed free access to tap water and food (commercial diet composition as described in [27]) and killed one month after the progesterone treatment (or 14 days after RU 486 implantation [5]). Inguinal and retroperitoneal WAT of females and inguinal, epididymal and retroperitoneal WAT of males were collected. The adipose tissues were weighed, rapidly frozen in liquid nitrogen and stored at −80 °C until analyses of mRNA and protein levels. Blood was collected from the neck artery, centrifuged (10 min at 1500 × *g*), and the separated serum was stored at −80 °C until progesterone and free fatty acid concentrations were measured. All procedures were approved by the Animal Experimentation Ethics Committee of the Medical University of Gdansk and were performed in accordance with the guidelines of the Medical University of Gdansk on the care and use of laboratory animals. Each experimental replication involved tissue from different female and male rats ($n = 10$ per group).

2.2. Determination of serum progesterone concentration

Serum progesterone concentration was measured by radioimmunoassay using a commercial immunoassay kit in accordance with the manufacturer's instruction (Institute of Atomic Energy POLATOM, Radioisotope Center, Poland).

2.3. RNA isolation

Total cellular RNA was extracted from frozen tissue by a guanidinium isothiocyanate/phenol/chloroform method [28]. The RNA concentration was determined from the absorbance at 260 nm. All samples had 260/280 nm absorbance ratio of approximately 2.0.

2.4. cDNA synthesis

First strand cDNA was synthesized from 4 μg of total RNA (RevertAid™ First Strand cDNA Synthesis Kit – Fermentas UAB, Lithuania). Prior to amplification of cDNA, each RNA sample was treated with RNase-free DNase I (Fermentas UAB, Lithuania) at 37 °C for 30 min.

2.5. Determination of HSL, ATGL, PLIN-1, ABHD5, G0S2, CIDEC, LPL, PPARG mRNA levels by real-time RT-PCR

HSL, ATGL, PLIN-1, ABHD5, G0S2, CIDEC, LPL and PPARG mRNA levels were quantified by real-time RT-PCR using Chromo4 Real Time Detection System (Bio-Rad Laboratories, Inc., USA). The primers were designed with sequence analysis software package (Informagen, Newington, USA) from gene sequence obtained from Ensembl Genome Browser (www.ensembl.org). The forward and

Table 1
Oligonucleotide primers sequences.

Gene	Primer sequence (5′-3′) forward and reverse
Actb (*beta-actin*)	TGT CAC CAA CTG ACG ATA
	GGG GTG TTG AAG GTC TCA AA
cyclophilin-A	CTG AGC ACT GGG GAG AAA GGA
	GAA GTC ACC ACC ACT GCA CA
Lipe	AAT GAC ACA GTC GCT GGT GGC G
	TGC CAC ACC CAA GAG CTG ACC T
Pnpla2	CCC TGA CTC GAG TTT CGG AT
	CAC ATA GCG CAC CCC TTG AA
Plin1	CGG ATT ATG CTG CCA ACA CC
	CTG AAG AAG GGG CTG ACT CC
Abhd5	AAC CCC AAG TGG TGA GAC AG
	GCG CCG AAG ATG ACT GAA AC
G0s2	TGA CCT CCT TCA GCG AGT G
	TCG GGA CTT CTG CGT CAT C
Cidec	GTG AAG GAA ATG CTC CGC TG
	TGT AAC TGG AGG TGC CAA GC
Lpl	AGT CCT CTC TCT GCA ATC AC
	ATC CAG CTG GGC CTA ACT TT
Pparg	CCA GAG TCT GCT GAT CTG CG
	GCC ACC TCT TTG CTC TGC TC

reverse sequences for real-time RT-PCR primers are presented in Table 1.

Real-time RT-PCR amplification was performed in a 20 μL volume using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). Each reaction included cDNA and 0.3 μM of each primer. Samples were incubated at 95 °C for 5 min for an initial denaturation and polymerase activation, followed by 35 PCR cycles of amplification (92 °C for 20 s, 57 °C for 20 s, and 72 °C for 40 s). Control reactions, with omission of the RT step or with no template cDNA added, were performed during each assay. All samples were run in triplicate. To compensate for variation in the amount of added RNA, and for the efficiency of the reverse transcription, β-actin mRNA was quantified in corresponding samples and the results were normalized to these values. It should be noted that similar results using β-actin and cyclophilin as housekeeping genes were found (not shown). Relative quantities of transcripts were calculated using the $2^{-\Delta\Delta CT}$ formula [29]. The results are expressed in arbitrary units, with one unit being the mean mRNA level determined in the control group. Amplification of specific transcripts was further confirmed by obtaining the melting curve profiles and subjecting the amplification products to agarose gel electrophoresis.

2.6. Western blot analysis

The levels of HSL, ATGL, phospho-HSL (pHSL), PLIN-1, ABHD5 and G0S2 protein were evaluated by Western blot procedure. Frozen adipose tissue was homogenized in 20 mM Tris–HCl buffer (pH 7.8) containing 0.2% Triton X-100 and protease inhibitor cocktail (Sigma, USA), and centrifuged (30,000 × *g* for 20 min). Aliquots of the supernatants containing 20 μg protein were separated by 10% SDS-PAGE and electroblotted to Immun-BlotTM PVDF Membrane (Bio-RAD Laboratories, Hercules CA, USA). The membranes were blocked with 5% albumin in phosphate-buffered saline (PBS) with 0.05% Tween 20 (Sigma–Aldrich, USA). Subsequently, the membranes were incubated with antibodies diluted in blocking buffer. Polyclonal rabbit antibodies against HSL (SAB4501763), phospho-HSL (pSer552 in human/Ser563 in rat; SAB4503879), ABHD5 (AV42455) and actin (A 5060), as well as HRP-conjugated secondary antibodies (A0545) were obtained from Sigma–Aldrich (USA). Polyclonal rabbit antibodies against ATGL (sc-67355) and G0S2 (sc-133424) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Polyclonal rabbit antibodies against PLIN-1 (bs-3789R) were obtained from Bioss Inc. (Woburn, Massachusetts, USA). The bands (visualized on the film after chemiluminescent detection) were compared with the molecular mass protein markers (SM26634) obtained from Fermentas (UAB, Lithuania), visualized on the membrane after electroblotting.

2.7. In vitro lipolysis assay

Explants were obtained immediately after dissection of adipose tissue from control and progesterone treated rats. Inguinal or other main fat depots (500 mg) were cut with scissors into small pieces (5–20 mg). The explants of WAT were incubated in 50-ml polypropylene tubes containing 5 mL of incubation buffer in gyratory water bath shaker (in an upright position) for 6 h (100 rpm). The buffer (pH 7.4) for incubation of adipose tissue was Dulbecco's modified Eagle's medium with 5.5 mM glucose and 25 mM HEPES supplemented with 1% bovine serum albumin fatty acid free (all ingredients from Sigma–Aldrich, St. Louis, MO, USA). The explants of WAT were incubated under sterile conditions with air as the gas phase. Lipolysis was stimulated by forskolin (final concentration 10 μM) or dibutyryl-cAMP (final concentration 0.2 mM), and its intensity was determined by analysis of FFA and glycerol release into the medium. Glycerol and FFA release could be detected within 30 min of incubation under these conditions and were linear with incubation time for at least 2 h. The aliquots of medium (50 μL) were collected after 30 min, 1 h, 2 h and 4 h from the start of incubation. Simultaneously, the explants of adipose tissue were incubated without forskolin or dibutyryl-cAMP. The results were expressed as μmoles of FFA or glycerol released per gram tissue per hour.

2.8. Determination of serum and medium concentrations of free fatty acids

Serum FFA concentration and the amount of fatty acids released by the explants of adipose tissue were measured by enzymatic colorimetric method according to the protocol supplied by Wako Chemicals GMBH (Germany).

2.9. Determination of glycerol concentration

Glycerol concentration was measured by commercial assay (enzymatic colorimetric method; free glycerol assay kit, K630-100) according to the protocol supplied by BioVission (Milpitas, CA 95035, USA).

2.10. Database sequence analysis

The putative progesterone receptor binding sites were sought in TRANSFAC database (BIOBASE, Beverly, MA) using AliBaba 2.1 software (BIOBASE). The sequences covering 2000 bp upstream and 300 bp downstream the transcription start site of rat *Lipe*, *Pnpla2*, *G0s2* and *Abhd5* genes were analyzed.

2.11. Statistics

The statistical significance of differences between the groups was assessed by one-way analysis of variance (ANOVA), and Tukey's post-hoc test was used for further determination of significance of differences. All the calculations were carried out with Sigma Stat software (Sigma Stat Inc.). The intergroup differences were considered significant at $p < 0.05$. All data are presented as mean ($n = 10$) ± standard error of mean (±S.E.M.).

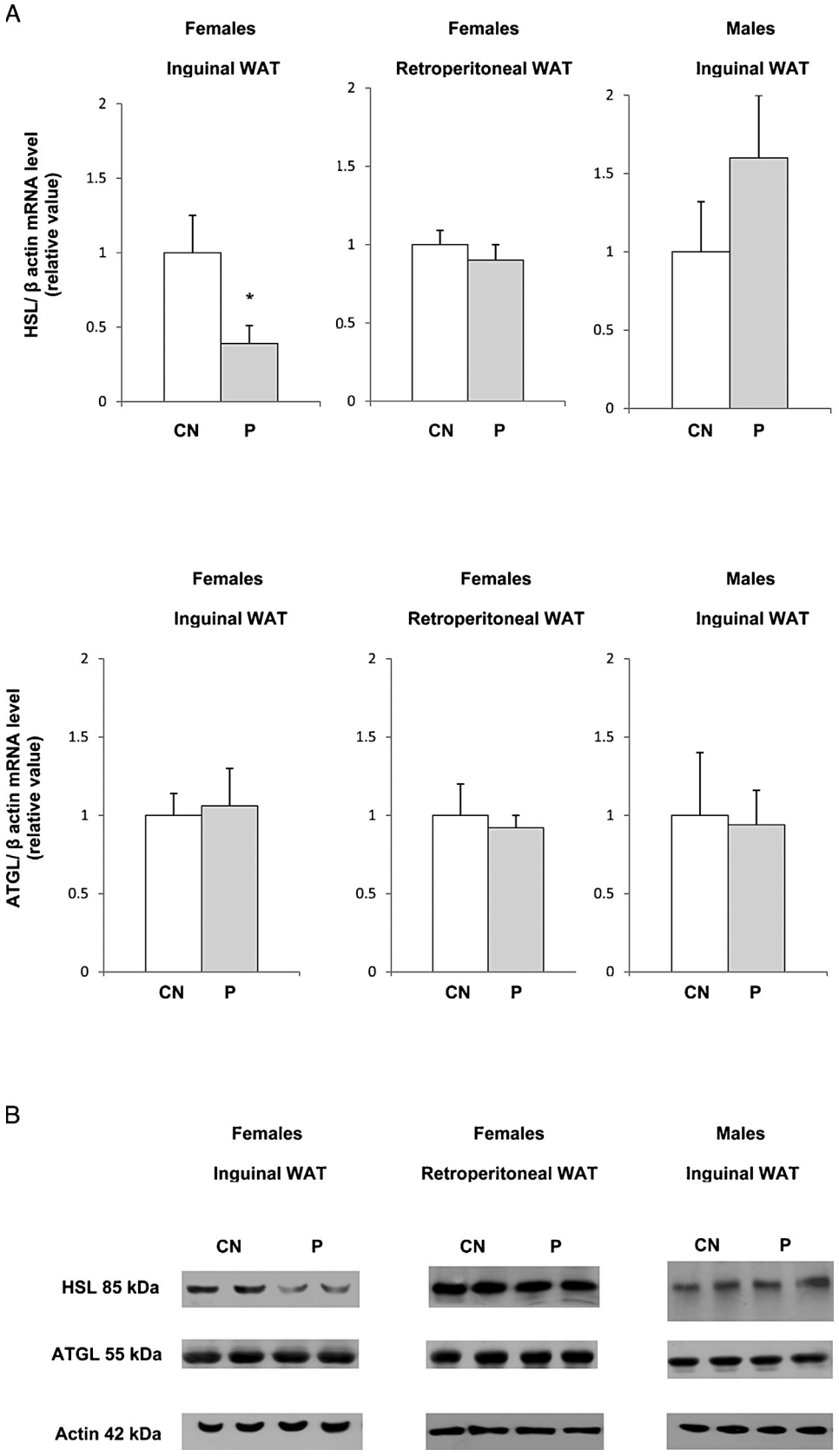

Fig. 1. The effect of progesterone administration on *Lipe* and *Pnpla2* genes expression in adipose tissue of rats. (A) The effect of progesterone administration on HSL and ATGL mRNA levels in WAT of rats. (Upper panel) HSL mRNA level relative to β-actin expression in WAT of control (CN) and progesterone-treated (P) females and males is shown on the graph (mean ± SEM, $n = 10$). $^{*}p < 0.05$ control females vs. progesterone-treated females. (Bottom panel) ATGL mRNA level relative to β-actin expression in WAT of control (CN) and progesterone-treated (P) females and males is shown on the graph (mean ± SEM, $n = 10$). (B) The effect of progesterone administration on HSL and ATGL protein levels in WAT of rats. CN: control rats, P: progesterone-treated rats. HSL and ATGL protein levels were assessed by Western blotting (two representative samples from each group are shown). 20 μg of protein per lane was loaded. Protein levels were normalized to actin. The bands were compared to the molecular mass protein markers.

3. Results

As expected based on the previously reported data [5], administration of progesterone to female rats resulted in an increase in total body weight (229 ± 4.0 g in non-treated females vs. 246 ± 4.2 g in progesterone-treated females, $p = 0.02$), and inguinal fat mass (1.9 ± 0.1 g in non-treated females vs. 2.45 ± 0.15 g in progesterone-treated females, $p < 0.01$). The mass of other main fat depots of progesterone-treated females did not differ significantly when compared to the controls (not shown). Progesterone exerted no effect on total body weight (324 ± 5.1 g in non-treated males vs. 323 ± 5.8 g in progesterone-treated males, $p = 0.4$) and inguinal fat mass (2.45 ± 0.15 g in non-treated males vs. 2.46 ± 0.12 g in progesterone-treated males, $p = 0.5$) of male rats. Administration of progesterone to females, reflected by approximately 2.5-fold increase in the concentration of circulating progesterone (31.3 ± 6.7 ng/mL in control females vs. 76.8 ± 8.4 ng/mL in progesterone-treated females, $p < 0.01$) resulted in approximately 2-fold decrease in HSL mRNA level in the inguinal WAT (Fig. 1A). The differences in HSL mRNA level in inguinal WAT of control and progesterone-treated female rats corresponded to differences in HSL protein level determined by Western blot analysis (Fig. 1B). Administration of progesterone exerted no effect on HSL mRNA (Fig. 1A, upper panel) and HSL protein (Fig. 1B) levels in the retroperitoneal WAT of females. Administration of progesterone to males, resulting in several-fold increase in the concentration of circulating progesterone (1.8 ± 0.5 ng/mL in control males vs. 29.2 ± 7.3 ng/mL in progesterone-treated males, $p < 0.01$), exerted no effect on HSL mRNA (Fig. 1A, upper panel) and HSL protein (Fig. 1B) levels in the inguinal WAT. Moreover, administration of progesterone had no effect on *Lipe* gene expression (at either mRNA or protein level) in the epididymal and retroperitoneal WAT of male rats (not shown). Contrary to HSL, either the ATGL mRNA level (Fig. 1A; bottom panel) or ATGL protein level (Fig. 1B) in the inguinal WAT of females did not change significantly in response to progesterone administration. Furthermore, administration of progesterone exerted no effect on ATGL mRNA (Fig. 1A; bottom panel) and ATGL protein levels (Fig. 1B) in the retroperitoneal WAT of females and inguinal WAT of males. Additional experiments showed that progesterone had also no effect on ATGL mRNA and ATGL protein levels in the epididymal and retroperitoneal WAT of males (not shown).

The specificity of progesterone effect on *Lipe* gene expression was further analyzed using mifepristone (RU 486), a specific antagonist of progesterone receptors. Administration of RU 486 almost completely abolished the inhibitory effect of progesterone on HSL mRNA level in the inguinal WAT of progesterone-treated females (Fig. 2). In contrast, RU 486 exerted no effect on HSL mRNA level in the inguinal WAT of progesterone non-treated females (Fig. 2). As progesterone was shown to exert a significant effect on HSL mRNA level solely in the inguinal WAT of females, we did not examine the influence of RU 486 on HSL mRNA levels in the retroperitoneal WAT of progesterone-treated females, as well as in the main fat depots of progesterone-treated male rats. The data presented in Fig. 2 point to a progesterone receptor-mediated inhibitory effect on the *Lipe* gene (HSL mRNA levels) expression in female inguinal WAT. Moreover, the abovementioned findings suggest that progesterone administration may inhibit lipolysis in the inguinal WAT of female rats. Basal (unstimulated) lipolysis, measured as FFA (Fig. 3) or glycerol (Fig. 4) release from inguinal WAT explants obtained from progesterone-treated females, was essentially similar as in the control (non-treated) rats. Dibutyryl-cAMP (the phosphodiesterase-resistant cAMP analogue) and forskolin (which stimulates adenylyl cyclase activity and consequently increases intracellular cAMP concentration) significantly increased the rate of lipolysis in those fat depots. Administration of progesterone was reflected by approximately 50% decrease in the lipolysis rate, irrespective of the presence of dibutyryl-cAMP or forskolin. Importantly, progesterone exerted no effect on stimulated lipolysis in the inguinal, retroperitoneal and epididymal WAT explants obtained from males, as well as in the retroperitoneal WAT explants from females (not shown). Altogether, the abovementioned data suggest that the pattern of changes in stimulated lipolysis in the inguinal WAT of female rats resembled the pattern of changes in HSL mRNA and protein levels.

The lipolytic activity of triacylglycerol lipases is regulated not only at a gene expression level but also at the post-translational level, involving phosphorylation of the enzymes and activity of regulatory proteins, such as PLIN-1 (involved in the regulation of both HSL and ATGL), ABHD5 (also referred to as CGI-58 in humans, a co-activator of ATGL) and G0S2 (a negative regulator of ATGL)

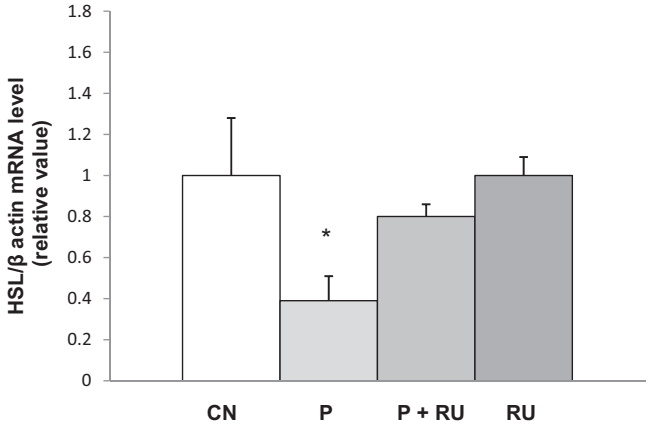

Fig. 2. The influence of mifepristone on HSL mRNA level in the inguinal adipose tissue of females. HSL mRNA level relative to β-actin expression in the inguinal adipose tissue of control (CN), progesterone-treated (P), progesterone and mifepristone-treated (P + RU) and mifepristone-treated (RU) females is shown on the graph (mean ± SEM, $n = 10$). *$p < 0.05$ progesterone-treated females vs. remaining groups.

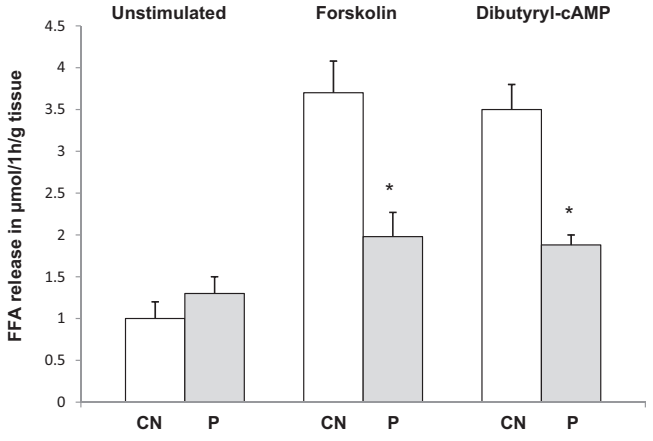

Fig. 3. The effect of progesterone administration on the rate of lipolysis in the inguinal adipose tissue explants from female rats. The unstimulated and stimulated FFA release (μmol/1 h/g tissue) from female inguinal WAT explants is shown on the graph. CN: control females, P: progesterone-treated females. Lipolysis was stimulated with forskolin (final concentration 10 μM) or dibutyryl-cAMP (final concentration 0.2 mM). The results are presented as mean ± SEM from 10 paired experimental replications. *$p < 0.01$ control females vs. progesterone-treated females.

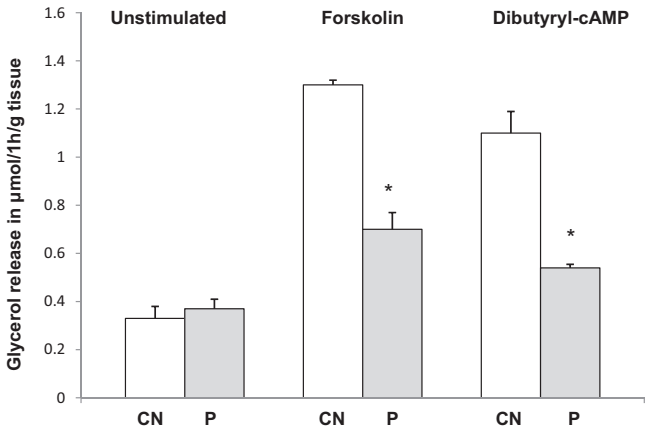

Fig. 4. The effect of progesterone administration on the rate of lipolysis in the inguinal adipose tissue explants from female rats. CN: control females, P: progesterone-treated females. The unstimulated and stimulated glycerol release (μmol/1 h/g tissue) from female inguinal WAT explants is shown on the graph. Lipolysis was stimulated with forskolin or dibutyryl-cAMP. The results are presented as mean ± SEM from 10 paired experimental replications. $^{*}p < 0.001$ control females vs. progesterone-treated females.

[19]. As shown in Fig. 5B, progesterone did not affect pHSL level in inguinal WAT of females. No effect of progesterone treatment on the HSL phosphorylation was also observed in the retroperitoneal WAT of females, as well in all main fat depots of males (not shown). Interestingly, administration of progesterone resulted in an increase in ABHD5 and G0S2 mRNA levels solely in the inguinal WAT of females (Fig. 5A). The differences in ABHD5 and G0S2 mRNA levels in the inguinal WAT of control, and progesterone-treated females were reflected by differences in ABHD5 and G0S2 protein levels (Fig. 5B). Administration of RU 486 almost completely abolished the stimulatory effect of progesterone on ABHD5 and G0S2 mRNA levels (not shown). No effect of progesterone administration on PLIN-1 mRNA (Fig. 5A) and PLIN-1 protein (Fig. 5B) levels in the inguinal WAT was observed. Similarly, administration of progesterone exerted no effect on the ABHD5, G0S2 and PLIN-1 mRNA levels, as well as on protein levels in the retroperitoneal WAT of females (not shown). Moreover, administration of progesterone had no effect on ABHD5, G0S2 and PLIN-1 mRNA as well as protein levels in all main fat depots of male rats (not shown).

We also analyzed the effect of progesterone on the expression of *Cidec* (*Fsp27*) gene (whose product is involved in lipid droplet formation [30]) in WAT of female and male rats. Administration of progesterone did not alter the CIDEC mRNA level in any of main fat depots of female and male rats (not shown).

Administration of progesterone did not affect serum concentration of FFA in female (0.217 ± 0.03 mmol/L in the controls vs. 0.226 ± 0.02 mmol/L in progesterone-treated rats, $p = 0.41$) and male rats (0.255 ± 0.03 mmol/L in the controls vs. 0.253 ± 0.03 mmol/L in progesterone-treated rats, $p = 0.48$).

Although analysis of extracellular lipolysis catalyzed by lipoprotein lipase (LPL) was out of the scope of this study, conflicting results regarding the effect of progesterone on LPL activity [4,14,15], as well as an inverse relationship between the levels of *Lipe* and *Lpl* genes expression (elevated level of LPL in HSL-deficient mice) [31], inclined us to examine the effect of progesterone administration on *Lpl* gene expression in main fat depots of females and males. Administration of progesterone exerted no effect on LPL mRNA level in the inguinal WAT of females, as well as in the other main fat depots of female and male rats (not shown).

As mentioned in Section 1, the results of previous studies dealing with the effect of progesterone on the differentiation of preadipocytes are inconclusive [9,12,13]. Although examining the effect of progesterone on adipose tissue differentiation was not an objective of this study, we decided to verify the effect of this hormone on PPARG (nuclear receptor prerequisite for the differentiation of adipocytes [32]) mRNA level in our experimental model. We revealed that progesterone had no effect on PPARG mRNA level in main fat depots of females and males (not shown).

4. Discussion

We showed for the first time that administration of progesterone causes marked decrease in dibutyryl-cAMP- or forskolin-stimulated lipolysis in the inguinal WAT explants, but not in the retroperitoneal fat depot of female rats. The decrease in the rate of lipolysis was associated with the decrease in *Lipe* gene expression. The expression of *Pnpla2* gene in the inguinal (and retroperitoneal) WAT of females was not affected by the progesterone treatment. More importantly, progesterone exerted no effect on lipolytic activity (and expressions of *Lipe* and *Pnpla2* genes) in main fat depots of males. These results point to gender- and tissue-specific effect of progesterone on the lipolytic activity of adipocytes.

Moreover, the data presented in this paper indicate for the first time that *Abhd5* and *G0s2* genes expression in female inguinal WAT are subjects to progesterone up-regulation. The fat depot specific stimulatory effect of progesterone on *Abhd5* and *G0s2* genes expression and recently published data indicating that G0S2 strongly affects lipolysis, TAGs deposition and energy metabolism [25,26], suggest that ATGL may be involved in the progesterone-mediated regulation of lipolysis in the inguinal WAT of females. Previous *in vitro* studies demonstrated that G0S2 is capable of suppressing ATGL activity, even in the presence of CGI-58 (ABHD5) [33]. Therefore, despite the lack of progesterone effect on *Pnpla2* gene expression, the influence of this hormone on G0S2 overproduction might lead to inhibition of ATGL activity. Thus, the progesterone-induced decrease in the rate of stimulated lipolysis in the inguinal WAT of female rats seems to be associated both with the decrease in *Lipe* gene expression and with the inhibition of ATGL activity, caused by elevated level of intracellular G0S2.

It is generally accepted that progesterone exerts its biological effect by binding to progesterone receptor, which mediates most of its physiological functions via transcriptional activation or inhibition of downstream genes. It is likely that binding to its receptor, progesterone is involved in transcriptional regulation of *Lipe*, *Abhd5* and *G0s2* genes expression. The effect of mifepristone, which abolishes the inhibitory effect of progesterone on HSL, ABHD5 and G0S2 mRNA levels, suggests that progesterone may inhibit *Lipe* and stimulate *Abhd5* and *G0s2* genes expression due to activation of inguinal adipocyte progesterone receptor. Moreover, bioinformatic analysis (AliBaba 2.1; www.gene-regulation.com) of the sequence covering 2000 bp upstream and 300 bp downstream transcription start site of *Lipe* gene predicted one progesterone receptor binding site (5′-tgtgtcctct-3′), localized between −916 and −925 upstream of the start of transcription. Putative progesterone binding sites were also found in *Abhd5* and *G0s2* genes (AliBaba 2.1; www.gene-regulation.com). Therefore, the interaction of progesterone–progesterone receptor complex with progesterone receptor binding sites may inhibit *Lipe*, and stimulate *Abhd5* and *G0s2* genes expression. Future studies should center around functional assessment of the in silico-identified putative progesterone receptor response elements of *Lipe*, *Abhd5* and *G0s2* genes and their contribution to the regulation of these genes by progesterone.

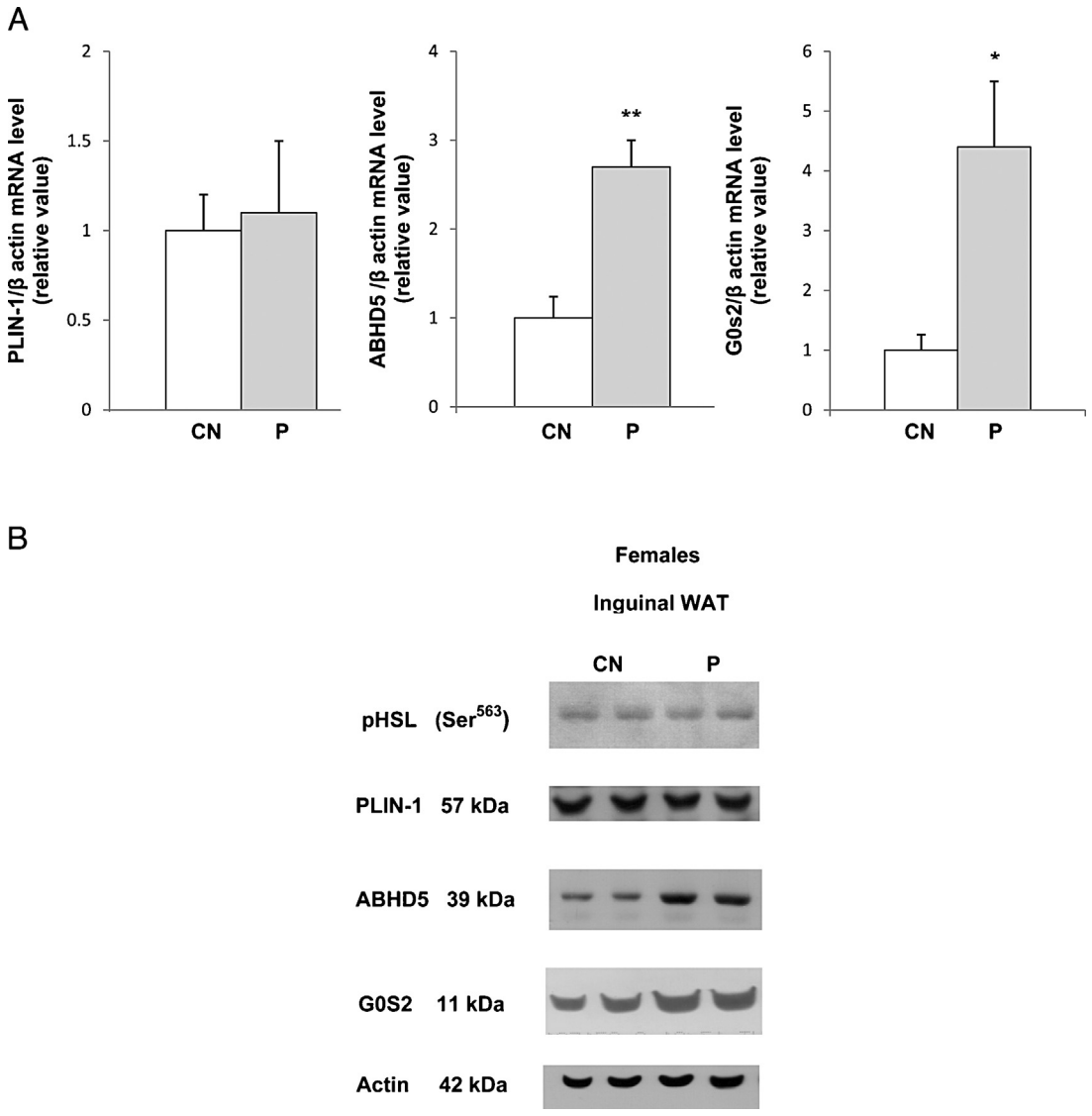

Fig. 5. The effect of progesterone administration on *Plin-1*,*Abhd5* and *G0s2* genes expression and phospho-HSL protein level in the inguinal adipose tissue of rats. (A) PLIN-1, ABHD5, G0S2 mRNA levels relative to β-actin expression in the inguinal WAT of control (CN) and progesterone-treated (P) females are shown on the graphs (mean ± SEM, $n = 10$). $^{*}p < 0.05$ control vs. progesterone-treated; $^{**}p < 0.01$ control vs. progesterone-treated. (B) The effect of progesterone administration on phospho-HSL, PLIN-1, ABHD5, G0S2 protein levels in the inguinal WAT of rats. Phospho-HSL (pHSL), PLIN-1, ABHD5, G0S2 protein levels were assessed by Western blotting. Two representative samples from control (CN) and progesterone-treated (P) females are shown. Twenty μg of protein per lane was loaded. Protein levels were normalized to actin. The bands were compared to the molecular mass protein markers.

Lack of putative progesterone binding site in *Pnpla2* gene (AliBaba 2.1; www.gene-regulation.com) may explain, at least in part, why progesterone exerted no effect on *Pnpla2* gene expression.

It is difficult to explain why lower levels of HSL and higher levels of ABHD5 and G0S2 mRNA and proteins, as well as inhibition of lipolysis, were observed in the inguinal, but not in the retroperitoneal WAT of the progesterone-treated females. One possible explanation for this phenomenon is relatively higher level of the progesterone receptor in female inguinal WAT [5]. The effect of mifepristone (Fig. 2), and very low density of progesterone receptor [5] as well as lack of progesterone effect on *Lipe*, *Abhd5* and *G0s2* genes expression in all main fat depots of males, provide strong evidence that the effect of progesterone on HSL, ABHD5 and G0S2 mRNA levels and lipolysis rate in female inguinal WAT is mediated by progesterone receptors. The significant differences between post-treatment concentrations of circulating progesterone in males and females may constitute an additional explanation for the lack of the effect of this hormone on HSL (as well as on ABHD5 and G0S2) mRNA levels in male inguinal WAT. As shown previously [5] and confirmed here, although the administration of progesterone to males results in several-fold increase in blood concentration of this hormone, it is still no greater than in control (i.e., non-treated) females. Therefore, blood concentration of progesterone in male rats seems to be too low to exert an inhibitory effect on *Lipe* and to stimulate *Abhd5* or *G0s2* genes expression. Altogether, these results suggest that both high concentration of circulating progesterone (higher than in control females) and high density of progesterone receptor in adipose tissue (similar to that observed in female inguinal WAT) are prerequisites of down-regulated expression of *Lipe* and up-regulation of *Abhd5* and *G0s2* genes in the inguinal WAT.

Inhibition of HSL activity by its selective inhibitor and low *Lipe* gene expression were previously shown to result in lower rate of lipolysis [34]. Moreover, contrary to the unstimulated process, stimulated lipolysis was proved to be abrogated in adipocytes

obtained from $HSL^{-/-}$ mice [35,36]. The results presented here are consistent with these findings.

The progesterone-induced inhibition of lipolysis in the inguinal WAT of female rats was not reflected by changes in circulating FFA concentration. There are at least three possible explanations for this phenomenon. Firstly, the effect of progesterone on the rate of lipolysis may be limited solely to the inguinal fat depots. Secondly, it should be remembered that we determined serum FFA concentrations in fed animals, and previous studies showed that fasting circulating FFA concentrations were lower in $HSL^{-/-}$ mice than in the controls [37]. Moreover, it should be noted that the progesterone-treated rats ate more food than the non-treated controls [6,38]. Thirdly, serum FFA concentrations are only partially modulated by WAT lipolysis [39].

Our results regarding the effect of progesterone on unstimulated (basal) lipolysis rate are consistent with previously reported findings [4]. However, our data on the rate of stimulated WAT lipolysis differ from those of prior studies in which adrenaline-stimulated lipolysis in adipocytes was not affected by progesterone [4]. These discrepancies can be associated with the fact that the previous studies included fat cells isolated from ovarian fat pads, and our findings suggest that the effect of progesterone on the expression of *Lipe*, *Abhd5* and *G0s2* genes is limited solely to the inguinal WAT of females.

One may speculate that the effect of progesterone on *Lipe*, *Abhd5* and *G0s2* genes expression is an example of long-term regulation of subcutaneous adipose tissue lipolysis, which may counteract catecholamine-stimulated lipolysis under pathophysiological conditions. Reduced ability of catecholamines to mobilize lipids from subcutaneous adipose tissue may be reflected by greater accumulation of fat in this particular fat depot. Recently published data suggest that partial inhibition of adipose tissue lipolysis may exert beneficial effect on glucose metabolism and insulin sensitivity, increasing glucose uptake and thus promoting lipogenesis [40]. It should not be excluded that the depot-specific inhibitory effect of progesterone on subcutaneous lipolysis (e.g., during pregnancy) improves glucose metabolism and insulin sensitivity.

Both the results of our recent studies and presented here findings suggest that the depot- and sex-specific enhanced synthesis of lipids [11] and inhibition of lipolysis (documented in this study) are major factors responsible for an increase in the inguinal fat mass, observed after progesterone administration. Previous studies showed that both metabolism and endocrine function of subcutaneous adipose tissue differ significantly from those of adipose tissue from other main depots [41–43]. Our findings add to this evidence, pointing to the presence of depot- and sex-specific differences in the adipose tissue characteristics. Moreover, our observations suggest that progesterone, as well as other steroid hormones, may contribute to differences in regional distribution of fat in males and females.

In conclusion, we showed that a chronic increase in serum progesterone concentration is associated with down-regulation of *Lipe* and up-regulation of *Abhd5* and *G0s2* genes expression in female inguinal WAT. The changes in genes expression are associated with diminished rate of stimulated lipolysis in the inguinal WAT explants. Although administration of progesterone exerted no effect on *Pnpla2* gene expression, our findings suggest that this hormone may decrease the rate of lipolysis in the inguinal WAT of females, inhibiting the activity of both ATGL (by stimulating synthesis of G0S2 – specific inhibitor of the enzyme) and HSL (due to inhibition of *Lipe* gene expression).

Therefore, the hereby presented findings suggest that accumulation of fat during pregnancy may at least in part result from progesterone-induced inhibition of stimulated lipolysis. These data adds to our previous findings on progesterone-mediated gender- and fat depot-specific regulation of lipogenic genes expression [5,11], providing additional information on the regulatory role of this hormone in lipid metabolism.

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