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Angiotensin II directly impairs adipogenic differentiation of human preadipose cells

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Abstract Angiotensin II reduces adipogenic differentiation of preadipose cells present in the stroma-vascular fraction of human adipose tissue, which also includes several cell types. Because of the ability of non-adipose lineage cells in the stroma-vascular fraction to respond to angiotensin II, it is not possible to unequivocally ascribe the anti-adipogenic response to a direct effect of this hormone on preadipose cells. Therefore, we used the human Simpson–Golabi–Behmel syndrome (SGBS) preadipocyte cell strain to investigate the consequences of angiotensin II treatment on adipogenic differentiation under serum-free conditions, by assessing expression of typical adipocyte markers perilipin and fatty acid-binding protein 4 (FABP4), at the transcript and protein level. Reverse transcription-polymerase chain reaction showed that perilipin and FABP4 transcripts were, respectively, reduced to 0.33 ± 0.07 ($P < 0.05$) and 0.41 ± 0.19-fold ($P < 0.05$) in SGBS cells induced to adipogenic differentiation in the presence of angiotensin II. Western Blot analysis corroborated reduction of the corresponding proteins to 0.23 ± 0.21 ($P < 0.01$) and 0.46 ± 0.30-fold ($P < 0.01$) the respective controls without angiotensin II. Angiotensin II also impaired morphological changes associated with early adipogenesis. Hence, we demonstrated that angiotensin II is able to directly reduce adipogenic differentiation of SGBS preadipose cells.

Keywords Angiotensin II · Adipogenic differentiation · Anti-adipogenesis · Adipocyte number regulation · SGBS preadipose cells

Introduction

The renin–angiotensin system (RAS) is expressed in several tissues [1], though its function in specific cell types is not yet fully understood. Adipose tissue expresses all components of the RAS necessary to generate angiotensinogen-derived peptides and the machinery for cell signal transduction. Augmented angiotensinogen expression and enhanced RAS activity were found in adipose tissue from obese subjects with resistance to insulin [4]. A link between the RAS and the metabolic—inflammatory disorders commonly associated with obesity shortly emerged [2, 3], drawing attention to the effect of the RAS in adipose tissue. Most published data are consistent with the notion that locally generated angiotensin II regulates adipocyte number and triglyceride accumulation, thus affecting adipose tissue mass and function (Fig. 1).

Pharmacological and gene knockout approaches have supported a role for angiotensin II in regulating mammal adipose tissue mass and function. Angiotensin II promotes storage of triglycerides in response to mice nutritional status [5]. Moreover, angiotensin II function in the control of adipocyte number first arose from studies in transgenic mice. Reduced number of adipocytes was observed in the epididymal fat of angiotensinogen deficient mice that were genetically modified to over express this gene in adipose

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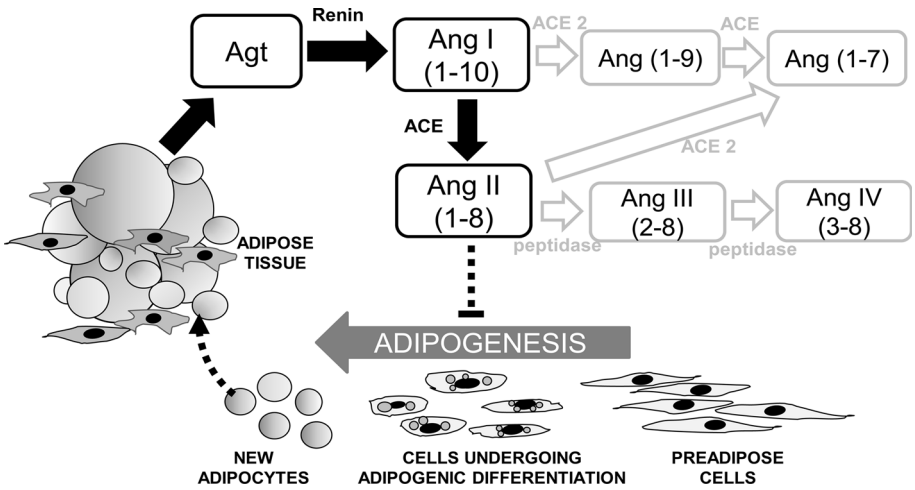


Fig. 1 Schematic representation of the RAS in adipose tissue and its inhibitory effect on adipogenesis. Increasing evidence supports participation of the RAS in modulating preadipose cell conversion into adipocytes; thus, influencing adipocyte number and adipose tissue functionality. Adipose tissue secretes angiotensinogen (Agt), which is sequentially converted to angiotensin I (Ang I) and angiotensin II (Ang II) by renin and angiotensin-converting enzyme (ACE), respectively. Shorter peptides (whose functions are not addressed here) are generated by ACE 2 and other peptidases. Angiotensin II inhibits differentiation of preadipose cells into adipocytes. Under conditions of energy surplus, triglycerides would accumulate in a limited number of adipocytes, leading to cell hypertrophy, which associates with adverse metabolic and inflammatory profiles typical of the obesity condition

tissue [6]. Further studies by several research groups have shown that angiotensin II is able to reduce in vitro adipogenic differentiation of preadipose cells present in the stroma-vascular fraction (SVF) of human subcutaneous and omental adipose tissue [7–11]. Besides preadipose cells, the SVF includes monocytes, macrophages, fibroblasts, leukocytes, endothelial cells, mesothelial cells, and other cell types, whose relative abundance is influenced by the topological origin of the fat tissue and the health condition of the donor. For example, mesothelial cells are a distinctive component of the omental fat SVF [12]. Likewise, monocytes and macrophages are numerous in the SVF of inflamed adipose tissue from obese subjects [13].

Because of the heterogeneous composition of the SVF, it is not possible to rule out that the anti-adipogenic effect of angiotensin II is mediated through non-adipose lineage cell types. Moreover, angiotensin II receptors are expressed in several cells comprised in the SVF, including preadipose cells, fibroblasts, monocytes, macrophages, and endothelial cells [1]. Angiotensin II stimulates these cells to secrete proteins such as transforming growth factor beta (TGF-β), endothelin, fibronectin, metalloproteinases, and collagen [14–16], which operate as negative effectors of adipogenesis [17, 18].

Consequently, this investigation was aimed to determine whether angiotensin II exerts a direct anti-adipogenic effect on human preadipose cells. We used the SGBS preadipocyte cell strain, which has been validated as a model for studies of human adipocyte biology [19]. This clonal cell strain maintains the capacity to undergo adipogenic differentiation over many generations, resulting in mature fat cells which are morphologically, biochemically, and functionally similar to human adipocytes from healthy subjects [20]. We had previously shown that angiotensin II activates a similar signal transduction response in SGBS cells and in primary preadipose cells from human SVF [21].

Once preadipose cells are stimulated to initiate the adipogenic program, activation of a transcriptional cascade drives the expression of genes encoding for proteins involved in mature adipocyte functions. Peroxisome proliferator-activated receptor gamma (PPARγ), which operates as "master transcriptional regulator of adipogenesis", regulates the expression of a number of adipocyte marker genes, such as perilipin 1 and FABP4 [18]. Perilipin is an abundant protein that coats lipid droplets in adipocytes and controls lipolysis restricting access to the hormone-sensitive lipase [22]. FABP4, also known as adipocyte protein 2 (aP2), is an intracellular lipid chaperone that participates in intracellular long-chain fatty acid and eicosanoid trafficking [23]. FABP4 is rich in adipocytes, though is also expressed in other cell types. In this study, expression of perilipin and FABP4 was used as indicator for terminal adipogenic differentiation in experiments designed to evaluate the anti-adipogenic effect of angiotensin II in SGBS cells. Treatment with angiotensin II impaired typical morphological and molecular changes associated with adipogenic differentiation of SGBS cells. Thus, showing that angiotensin II is able to directly impair adipogenesis in this model for human preadipose cells.

Materials and methods

Cell culture

Experiments were carried out with SGBS cells that were kindly provided by Dr. Martin Wabitsch (University of Ulm, Germany). Cells received at passage 28 were expanded in the laboratory upon arrival, and stored under liquid nitrogen until used. SGBS cells were cultured in basal medium containing Dulbecco's modified Eagle's medium (DMEM)/F-12 Ham 1:1 mixture (Sigma-Aldrich, St Louis, MO), 1.4×10^{-2} M $NaHCO_3$ (Sigma-Aldrich), 3.3×10^{-7} M biotin (Sigma-Aldrich), 1.7 10^{-7} M calcium pantothenate (Sigma-Aldrich), 100 U/ml penicillin, 0.1 mg/ml streptomycin (Hyclone/Thermo Scientific, South Logan, UT), and supplemented with 10 % fetal bovine serum (FBS; Hyclone/Thermo Scientific). Culture medium was routinely replaced every third day.

For comparison purposes, three SVF samples from human adipose tissue were included in this study. Human adipose tissue was obtained from non-obese healthy women that underwent elective abdominal surgery (gynecological procedures) at the Gynecology and Obstetrics Unit, Dr Luis Tisné Hospital. Donors signed informed consent. Fresh adipose tissue samples were immediately transported to the laboratory and the stromal vascular cells were obtained as described before [24]. Adherent cells were seeded on plastic culture dishes, and expanded at 37 °C in a humidified atmosphere with 5 % CO_2. Harvested cells were stored in liquid nitrogen for later use. Experiments were carried out with cells from the second or third passage. Cell count was determined under a light microscope by means of a hemocytometer.

Adipogenic differentiation

SGBS cells were induced to adipogenic differentiation in the conditions that we previously used to study the effect of angiotensin II on preadipose cells isolated from the SVF of human adipose tissue [24]. SGBS cell cultures that had reached 80–90 % confluence were serum deprived for 16 h and incubated for 4 days in SV induction medium, which consists of serum-free basal medium supplemented with 2.5×10^{-7} M dexamethasone (Sigma-Aldrich), 5×10^{-4} M 3-isobutyl-1-methylxanthine (IBMX; Calbiochem/Merck Millipore, Darmstadt, Germany), and 1×10^{-6} M human insulin (E. Lilly & Co, México DF, México). The response to the standard protocol for adipogenic differentiation of SGBS cells [19] was determined in parallel. To this purpose, adipogenesis was induced with SG medium, consisting of serum-free basal medium supplemented with 2.5×10^{-8} M dexamethasone (Sigma-Aldrich), 2×10^{-8} M human insulin (Sigma-Aldrich), 2.5×10^{-4} M IBMX (Sigma-Aldrich), 2×10^{-6} M Rosiglitazone (Cayman Chemical Co, Ann Arbor, MI), 2×10^{-10} M 3,5,3′-triiodothyronine (Sigma-Aldrich), 1×10^{-7} M hydrocortisone (Sigma-Aldrich), and 0.01 mg/ml apotransferrin (Sigma-Aldrich). Adipogenic differentiation of SVF preadipose cells from human adipose tissue was induced with SV medium. After a 4-day period in the corresponding induction medium, SGBS and SVF cells were maintained in basal medium supplemented with 2×10^{-8} M human insulin, 2×10^{-10} M triiodothyronine, 0.01 mg/ml apotransferrin, and 1×10^{-7} M hydrocortisone, until the end of the experiment.

When included, angiotensin II (aqueous solution) was added before induction of adipogenic differentiation, and maintained throughout the experiment. The dose response of human preadipose cells to angiotensin II had been previously determined [10]. In order to compensate for the high rate of angiotensin II withdrawal detected in SVF cell cultures, angiotensin was used at a 1.5×10^{-5} M supraphysiological concentration.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was extracted according to Chomczynski and Sacchi [25], and treated with RNase-free DNAase to remove any traces of genomic DNA. First-strand cDNA was synthesized with Superscript II reverse transcriptase (Invitrogen Corp., Carlsbad, CA), using 5×10^{-7} g oligo-dT_{15} primer (Promega Corp., Madison, WI). Semi-quantitative PCR was catalyzed by recombinant Taq DNA polymerase (Kapa Biosystems, Boston, MA) in the conditions recommended by the manufacturer. The sequence for PCR primers is included in Table 1. Perilipin primers were modified from Forcheron et al. [26], and those for FABP4 cDNA amplification were designed from mRNA sequence (GenBank accession number NM_001442). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA was amplified as internal reference for normalization of FABP4 and perilipin amplicons. GAPDH primers were complementary to all four mRNA variants (GenBank accession numbers: NM_001289746.1, NM_001289745.1, NM_001256799.2, and NM_002046.5). The number of PCR cycles was adjusted for individual reactions to ensure that the product was linear with respect to cDNA input. PCR products were separated by agarose gel electrophoresis and stained with gel red (Biotium Inc., Hayward, CA). The intensity of fluorescence signals was measured using the FOTO/Analyst™ Luminary/FX workstation (Fotodyne Inc., Hartland, WI). The relative abundance of cDNAs was expressed as the ratio perilipin/GAPDH and FABP4/GAPDH.

Western Blot analysis

Cell lysates for Western Blot analyses were prepared with RIPA buffer (consisting of 5×10^{-2} M Tris–HCl, 1.5×10^{-1}

Table 1 Sequence for PCR Primers

Target	Forward	Reverse
FABP4	tgc agc ttc ctt ctc acc ttg a	tcc tgg ccc agt atg aag gaa atc
Perilipin	aga aac agc atc agc gtt cc	tgg tcc tca tga tcc tcc tc
GAPDH	gtc aac gga ttg gtc gta ttg g	agt ctt ctg ggt ggc agt gat gg

M NaCl, 1 % Nonidet P-40, 0.5 % sodium deoxycholate, 0.2 % SDS, pH 8.0, supplemented with Complete™ protease inhibitors cocktail, Pepstatin A (Roche Applied Science, Mannheim, Germany), and Halt™ Phosphatase Inhibitor (Pierce/Thermo Scientific, Rockford, IL). 20 to 60 micrograms of protein per sample were separated by 10 or 20 % polyacrylamide gel electrophoresis under denaturing conditions, electrotransferred to 0.45 µm Immobilon-P polyvinylidene difluoride membranes (Merck Millipore), and probed with corresponding primary antibodies. The antibodies specific for perilipin, FABP4, and β-tubulin were purchased from Cell Signaling Technology Inc. (Danvers, MA). β-tubulin was used as internal reference for perilipin and FABP4 protein expression.

Immune complexes were revealed with appropriate horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch Laboratories, West Grove, PA), and enhanced chemiluminescence (Merck Millipore) was detected with the FOTO/Analyst™ Luminary/FX workstation. Perilipin and FABP4 to β-tubulin protein ratios were calculated from digital images analyzed with the Total Lab TL100 software (Fotodyne Inc.) and expressed relative to the corresponding control in each experiment.

Statistical analysis

All experiments were performed in triplicate. Means and standard deviation were determined, and pair-wise comparisons were validated by Student's t test. A probability (P) < 0.05 was considered significant.

Results

Differentiation of SGBS cells under the culture conditions used to induce adipogenesis of preadipose cells from the human adipose tissue SVF

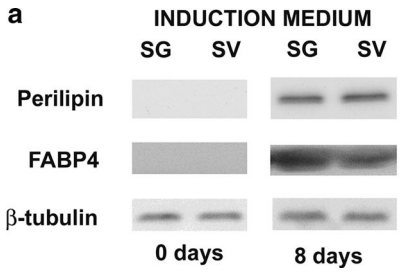
As mentioned above, the consequences of angiotensin II treatment on the conversion of SGBS cells into adipocytes were assessed under the experimental conditions previously used to investigate the effect of this hormone on the adipogenic differentiation of preadipose cells from the SVF of human adipose tissue. To this purpose, we first compared the adipogenic response of SGBS cells to induction medium for SVF preadipose cells (SV medium) with that for SGBS cells (SG medium). Western Blot analysis revealed that typical adipocyte marker proteins perilipin and FABP4 were undetectable at day 0, and reached similar level at the eighth day under adipogenic conditions, regardless of the composition of the induction medium (Fig. 2a). The perilipin/β-tubulin and FABP4/β-tubulin ratios determined in SGBS cells induced to differentiation with SV medium were comparable with those in SG medium (Fig. 2b). Thus, the use of SV medium to evaluate the effect of angiotensin II on the adipogenic differentiation of SGBS cells was validated.

Angiotensin II impairs typical morphological changes associated with the adipogenic differentiation of SGBS cells

Phase contrast images in Fig. 3 illustrate morphological features of confluent SGBS cell cultures before (a) and 8 days after induction of adipogenic differentiation in the absence (b) or presence (c) of angiotensin II. Multiple lipid droplets—showing a broad size range—were evident in the cytoplasm of SGBS cells maintained in adipogenic medium without angiotensin II. In contrast, SGBS cells treated with angiotensin II throughout the differentiation period displayed a reduced number of small lipid droplets. Moreover, SGBS cells under the latter condition exhibited the typical spindle-like shape of non-differentiated cells (a) in comparison with controls in the absence of angiotensin II, which displayed a rounded form. It is worth mentioning that differences in SGBS cell morphology were evident at day 4 after induction of adipogenesis (Fig. 4). At this time point, cells not exposed to angiotensin II showed a polygonal shape and tiny lipid droplets in their cytoplasm, compared with those induced to differentiation with angiotensin II. The morphology of the latter resembled that of non-differentiated SGBS cells, and showed scarce lipid droplets.

Reduced level of transcripts for typical adipocyte markers in SGBS cells induced to adipogenic differentiation in the presence of angiotensin II

Transcripts for typical adipocyte markers FABP4 and perilipin, which were not measurable in undifferentiated



b

ADIPOCYTE MARKER
(relative level)

Induction medium	Perilipin / β-tubulin	FABP4 / β-tubulin
SG	1.0 ± 0.2	1.0 ± 0.1
SV	1.1 ± 0.2	0.8 ± 0.1

Fig. 2 Adipogenic response of SGBS cells induced to differentiation with SGBS or SVF medium. **a** Representative Western Blot analysis of typical adipocyte markers perilipin and FABP4 in SGBS cells at 0 and 8 days after induction of adipogenic differentiation with SG or SV medium. **b** Perilipin and FABP4 to β-tubulin protein ratios were determined from three independent experiments in which SGBS cells were induced to adipogenic differentiation with SV medium, and expressed relative to the values obtained with SG medium

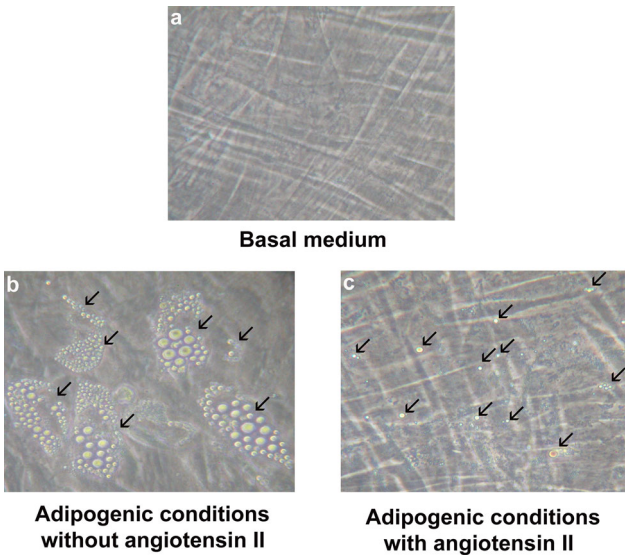


Fig. 3 Angiotensin II impairs morphological changes associated with adipose conversion of SGBS cells. Phase contrast images illustrate morphological features of SGBS cells maintained in basal medium (**a**) or adipogenic conditions during 8 days, in the absence (**b**) or presence of angiotensin II (**c**). *Arrows* point to cells bearing lipid droplets. Magnification ×400

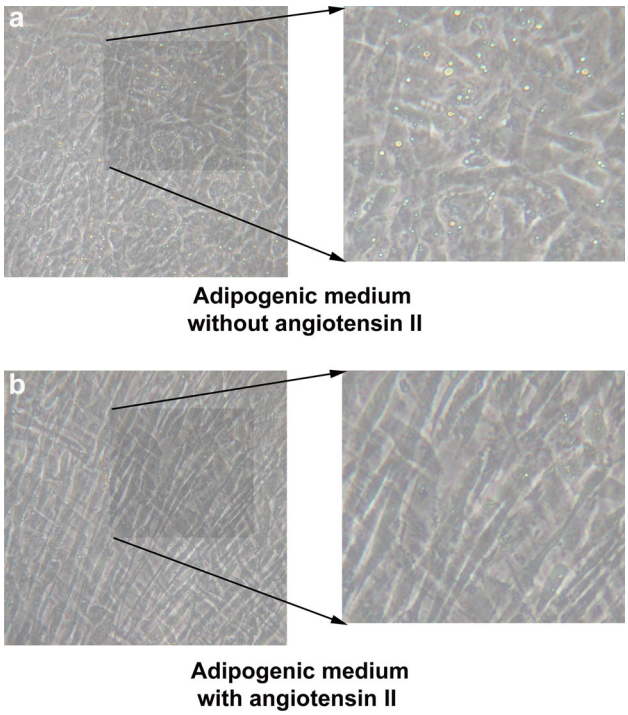
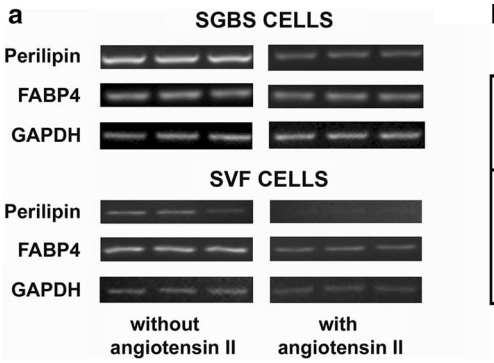


Fig. 4 Angiotensin II impairs early morphological changes in SGBS cells stimulated to adipogenic differentiation. Phase contrast images illustrate morphological features of SGBS cells maintained 4 days in adipogenic conditions without (**a**) or with angiotensin II (**b**). Magnification ×200

SGBS cells, became detectable 4 days after induction of adipogenic differentiation, and progressively increased thereafter (not shown). The effect of angiotensin II on the abundance of these transcripts was evaluated at day 8 under adipogenic conditions. Figure 5a shows decreased level of perilipin and FABP4 mRNA in SGBS cells treated with angiotensin II. The perilipin/GAPDH and FABP4/GAPDH ratios were 0.33 ± 0.07-fold ($P < 0.05$) and 0.41 ± 0.19-fold ($P < 0.05$) the corresponding values determined in cells differentiated in the absence of angiotensin II (Fig. 5b). It is worth noting that the relative level of perilipin and FABP4 transcripts was reduced to a similar extent in preadipose cells from the SVF that were treated with angiotensin II throughout the adipogenic differentiation period (Fig. 5b).

Angiotensin II reduces typical protein markers of adipogenic differentiation

In consonance with diminished perilipin and FABP4 transcripts, supplementation of the adipogenic medium with angiotensin II led to reduced abundance of the corresponding proteins (Fig. 6a, b). As depicted in Fig. 6, at the eighth day of differentiation the perilipin/β tubulin and FABP4/β tubulin ratios, respectively, were 0.23 ± 0.21 fold ($P < 0.01$) and 0.46 ± 0.30-fold ($P < 0.01$) the matching control without angiotensin II.



b

TRANSCRIPT LEVEL (fold change)

Preadipose cells	Perilipin / GAPDH	FABP4 / GAPDH
SGBS	0.33 ± 0.07*	0.41 ± 0.19*
SVF	0.26 ± 0.08*	0.43 ± 0.01*

Fig. 5 Reduced levels of transcripts for adipocyte markers in SGBS cells induced to adipogenic differentiation in the presence of angiotensin II. **a** Triplicate PCR amplifications of perilipin, FABP4, and GAPDH transcripts in SGBS and SVF cells at day 8 after induction of adipogenic differentiation without or with angiotensin II. **b** FABP4 and perilipin to GAPDH ratios determined in cells treated with angiotensin II were expressed as fold change versus the value in corresponding controls without this hormone; $*P < 0.05$, $n = 3$

Discussion

Adipose tissue is known to play a major role in energy homeostasis. This highly malleable tissue expands and contracts according with the energy balance. Adipose tissue mass is determined by adipocyte volume (predominance of triglyceride storage vs release) and cell number (prevalence of adipose cell formation vs death) [27]. In conditions of excessive food intake, both increased fat cell volume and number appear to be involved in the response to cope with the energy surplus. Hypertrophic or hyperplastic mechanisms prevail in different adipose tissue depots [28] and contexts, as evidenced by tracking adipocyte turnover in the AdipoChaser mouse [29]. When these mice are fed on a high-fat diet, adipocyte hypertrophy mainly contributes to early subcutaneous adipose tissue expansion; whereas, new adipocyte formation is detected in visceral fat after extended periods of high caloric intake [29]. Both intrinsic properties of adipose cells residing at specific anatomical sites and locally generated factors may account for distinct regional responses to overfeeding.

As mentioned before, angiotensin II is among locally secreted adipokines implicated in modulation of fat mass. The effect of angiotensin II was previously studied in human bone marrow-derived mesenchymal stem cells [30] and cell cultures of the SVF obtained from human adipose tissue [7–11]. However, a direct anti-adipogenic action of this hormone on human preadipose cells was not unequivocally demonstrated, because the SVF contains several cell types aside from preadipose cells, which can respond to angiotensin II and mediate the reported effect.

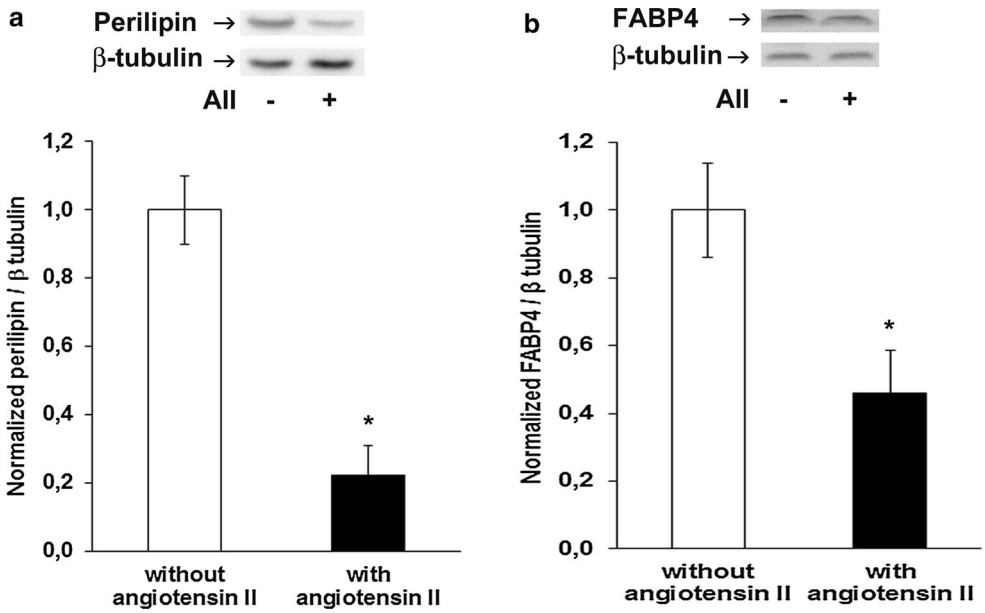


Fig. 6 Angiotensin II reduced protein markers for adipogenic differentiation in SGBS cells. *Upper panels* in **a** and **b** illustrate Western Blot analysis of perilipin, FABP4, and β-tubulin in SGBS cell lysates obtained after 8 days under adipogenic conditions with or without angiotensin II (AII). **a** perilipin/β-tubulin and **b** FABP4/β-tubulin ratios determined in cells treated with angiotensin II were normalized versus the corresponding values in controls without angiotensin II (the latter were 0.9 ± 0.23 and 1.3 ± 0.40, respectively). Data are mean ± SE, $n = 6$, $*P < 0.01$

Therefore, we sought to investigate angiotensin II effect on the SGBS cell model. Using this clonal human-derived cell strain, we avoided the potential influence of different angiotensin II-responsive cell types that are comprised in primary cultures of the SVF obtained from human adipose tissue. Here we report morphological and molecular evidence that angiotensin II directly impairs differentiation of human preadipose cells into adipocytes. Exposure to angiotensin II altered the distinctive morphological transition that precedes expression of distinct molecular markers of adipogenesis. Predominance of thin spindle-like cells (similar to undifferentiated cells) was found in SGBS cell cultures induced to adipogenic differentiation in the presence of angiotensin II, in contrast to the rounded shape of corresponding controls (Figs. 3, 4). In addition, SGBS cells treated with angiotensin II throughout the differentiation period showed scarce cytoplasmic lipid droplets, even 8 days after the induction of adipogenesis (Fig. 3). The corresponding controls displayed small cytoplasmic lipid droplets early after induction of adipogenic differentiation, which were larger and numerous at day 8. At a molecular level, treatment with angiotensin II decreased the expression of typical markers for adipogenesis. Transcripts for FABP4 and perilipin were reduced—to a similar extent—in SGBS and SVF cells stimulated to adipogenic differentiation in the presence of angiotensin II (Fig. 5). Diminished level of the corresponding proteins was also detected in SGBS cells exposed to angiotensin II throughout the differentiation period (Fig. 6). These results support a direct negative effect of angiotensin II on the conversion of human preadipose cells into adipocytes. The potential contribution of non-adipose lineage components of the SVF to the anti-adipogenic response remains to be investigated.

As discussed above, the capacity to form new adipocytes is required for maintenance of adipose tissue function. It is currently thought that impaired adipogenesis would favor adipocyte hypertrophy in conditions of energy surplus (Fig. 1), and predispose to the development of metabolic disturbances frequently associated with obesity. The importance of new adipocyte formation in adipose tissue physiology and whole-body metabolism is emphasized in lipoatrophy; an outmost condition in which the impaired capacity to form adipocytes associates with metabolic abnormalities, such as insulin resistance and diabetes. Conversely, predominance of small adipocytes in adipose tissue appears to protect against obesity-related diseases [31, 32], given that newly differentiated adipocytes have a normal secretory profile and full responsiveness to physiological signals [33]. A recent cross-sectional study underscores this relationship, showing that fat cell number positively correlates with insulin sensitivity, HDL cholesterol, and negatively associates with plasma insulin and triglyceride levels in obese subjects [34]. It appears that individuals with the capacity to increase their fat cell number can better cope with the adverse consequences of overfeeding, ameliorating the pernicious outcome of adipocyte hypertrophy, and ectopic lipid accumulation. In agreement with the emerging role for the RAS in adipose tissue physiology, rodents with genetic deficiency of components involved in angiotensin II production or intracellular signaling showed recovery of metabolic abnormalities commonly linked with obesity [3, 35]. Moreover, pharmacological inhibition of the RAS (after prolonged administration of angiotensin II receptor blockers or angiotensin-converting enzyme or renin inhibitors) in humans and murine models of obesity and diabetes also led to amelioration of metabolic alterations [35, 36]. Improvement of metabolic parameters associates with increased adipogenesis and abundance of small functional adipocytes in mouse adipose tissue [37].

These findings unraveled a significant role for the RAS in obesity-associated pathologies, which is beginning to be acknowledged. On this ground, it is of great interest to elucidate angiotensin II contribution to the mechanisms that control predominance of adipocyte hyperplasia or hypertrophic growth in human adipose tissue. Here we demonstrated that angiotensin II directly acts on a clonal strain of human preadipose cells to impair adipogenic differentiation. Identification of preadipose cells as direct targets for angiotensin II is important to better comprehend the consequences for pharmacological blockade of RAS activity. Additional studies will contribute to a better understanding of RAS effects in adipose cells and to development of nutritional or pharmacological approaches to prevention/treatment of obesity-associated comorbidities.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committees at University of Chile and the Metropolitan Health Service, Santiago, Chile, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all participants included in the study. This article does not contain any studies with animals performed by any of the authors.

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