Larger Anti-adipogenic Effect of Angiotensin II on Omental Preadipose Cells of Obese Humans

Rodrigo Brücher, Mariana Cifuentes, María José Acuña, Cecilia Albala, and Cecilia V. Rojas

Abstract

BRÜCHER, RODRIGO, MARIANA CIFUENTES, MARÍA JOSÉ ACUÑA, CECILIA ALBALA, AND CECILIA V. ROJAS. Larger anti-adipogenic effect of angiotensin II on omental preadipose cells of obese humans.

Objective: The ability to form new adipose cells is important to adipose tissue physiology; however, the mechanisms controlling the recruitment of adipocyte progenitors are poorly understood. A role for locally generated angiotensin II in this process is currently proposed. Given that visceral adipose tissue reportedly expresses higher levels of angiotensinogen compared with other depots and the strong association of augmented visceral fat mass with the adverse consequences of obesity, we studied the role of angiotensin II in regulating adipogenic differentiation in omental fat of obese and non-obese humans.

Research Methods and Procedures: The angiotensin II effect on adipose cell formation was evaluated in human omental adipocyte progenitor cells that were stimulated to adipogenic differentiation in vitro. The adipogenic response was measured by the activity of the differentiation marker glycerol-3-phosphate dehydrogenase.

Results: Angiotensin II reduced the adipogenic response of adipocyte progenitor cells, and the extent of the decrease correlated directly with the subjects' BMI ($p = 0.01$, $R^2 = 0.30$). A 56.3 ± 3.4% and 44.5 ± 2.7% reduction of adipogenesis was found in obese and non-obese donors' cells, respectively ($p < 0.01$). The effect of angiotensin II was reversed by type 1 angiotensin receptor antagonist losartan.

Discussion: A greater anti-adipogenic response to angiotensin II in omental adipose progenitor cells from obese subjects opens a venue to understand the deregulation of visceral fat tissue cellularity that has been associated with severe functional abnormalities of the obese condition.

Key words: angiotensin II, adipogenesis, visceral fat, human adipose tissue

Introduction

Reported data point to a role for angiotensin II in the control of the formation of new adipose cells in subcutaneous adipose tissue (1–3). Interestingly, the gene for the angiotensin II precursor angiotensinogen is abundantly expressed in human visceral fat (4), where the augmented mass associates with the greatest risk for obesity-linked health problems (5). However, the function of the renin-angiotensin system in human visceral fat has not been addressed. This study investigated the effect of angiotensin II on the adipogenic differentiation of preadipose cells obtained from human omental adipose tissue from subjects with a broad range of BMIs.

Research Methods and Procedures

The effect of angiotensin II was tested on preadipose cells obtained from greater omental adipose tissue of 24 subjects (13 women and 11 men) who underwent elective abdominal surgery. No one was taking medications known to influence adipose tissue mass, distribution, or metabolism. The group included 15 subjects with BMI <30 kg/m^2 and 9 subjects with BMI ≥ 30 kg/m^2. The protocol and informed consent were approved by the Review Board at Instituto de Nutrición y Tecnología de los Alimentos (INTA) and the Health Service at Santiago.

Stroma-vascular cells were isolated (6), expanded in Dulbecco's modified Eagle's medium/Ham's F12 (1:1) supplemented with 10% fetal bovine serum, and at third passage were stimulated to differentiation by exposure to an adipogenic medium (6) containing 0.2 μM human insulin (Eli Lilly & Co., SA de CV, Mexico), 1 μM dexamethasone

Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, Chile.
Address correspondence to Cecilia V. Rojas, Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Casilla 138-11, Santiago, Chile.
E-mail: crojas@inta.cl

(Sigma, St. Louis, MO), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma), and 0.1 mM indomethacin (Sigma) in Dulbecco's modified Eagle's medium/Ham's F12 (1:1) with 1% fetal bovine serum, over a 10-day period. Media were replaced every 2 days. Adipogenic differentiation was assessed by the activity of the validated adipogenic marker glycerol-3-phosphate dehydrogenase (G3PDH)[1] in cell homogenates (7,8). The enzymatic reaction was linear with respect to time over the assay period. One unit of enzyme activity corresponds to the oxidation of 1 nmol of the reduced form of nicotinamide adenine dinucleotide per minute at 37 °C. Throughout the differentiation period, cells maintained under control conditions (culture medium with 1% fetal bovine serum) exhibited negligible G3PDH activity, comparable with baseline values in differentiation experiments (0.5 to 1 units/mg protein). G3PDH-specific activity in cells stimulated to adipogenic differentiation was normalized to the values measured in their respective controls.

Angiotensin II and its receptor antagonists (in aqueous solutions) were added simultaneously with the differentiation medium and maintained throughout the experiment. Supraphysiologic angiotensin II concentrations were used in experiments designed to evaluate its effect on adipogenic differentiation to compensate for the high angiotensin II metabolizing activity detected in cell cultures (data not shown). In the absence of exogenous angiotensin II, negligible concentrations of the hormone were detected in media conditioned by omental adipose tissue progenitor cells, regardless of the donor's BMI (data not shown).

Neither angiotensin II nor losartan modified G3PDH activity in progenitor cells that had not been induced to adipogenic differentiation. Any direct effect of angiotensin II or losartan on the enzyme assay was also ruled out (data not shown).

Data represent means ± standard error, and differences were analyzed by Student's *t* test. One-way ANOVA was used for multiple treatment analysis, followed by Tukey's post hoc comparison test. Pearson's correlation coefficient was used to evaluate the association between BMI and the effect of angiotensin II. $p < 0.05$ was considered significant.

Results

Progenitor cells obtained from omental adipose tissue stimulated with the adipogenic medium underwent differentiation, as evidenced by the occurrence of cytoplasmic lipid droplets, change in morphology, and a rise in the activity of the adipogenic marker G3PDH. The specific activity of G3PDH increased 7.8 ± 1.2-fold ($n = 24$) after a 10-day exposure to the adipogenic condition, and did not differ according to the subjects' sex or BMI. In line with reported intrinsic differences in the adipogenic potential of preadipose cells from various fat depots (8), the response of omental adipocyte progenitors to adipogenic stimulus was 10.4 ± 2.5-fold lower than that of precursor cells from a subset of five abdominal subcutaneous samples. The latter was included to corroborate their high adipogenic capacity in our experimental conditions.

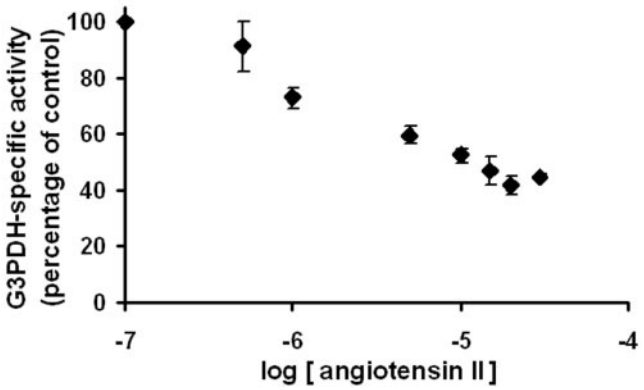

Figure 1: Dose-response relationship for the reduction of adipogenesis by angiotensin II. Progenitor cells were cultured for 10 days in adipogenic medium supplemented with different concentrations of angiotensin II (1×10^{-7} to 3×10^{-5} M). G3PDH-specific activity is expressed relative to the condition without angiotensin II. Each point corresponds to the mean ± standard error of 2 to 10 determinations in cells from different subjects.

Angiotensin II diminished the response of omental fat preadipose cells to the adipogenic stimulus in a dose-dependent manner (Figure 1), and at concentrations >10 μM, the maximal reduction was 47.6 ± 2.7% ($n = 24$). Inhibition of adipogenic differentiation by angiotensin II correlated directly with subject's BMI (Figure 2). Moreover, the effect of angiotensin II was significantly larger in omental adipose progenitor cells from obese donors compared with those from subjects with BMI <30 kg/m^2 (Table 1). The inhibition of adipogenesis by angiotensin II was overturned by the angiotensin II type 1 receptor antagonist losartan. As shown in Figure 3, angiotensin II alone reduced G3PDH-specific activity to 52.1 ± 1.9% of the controls without hormone, but when losartan was also added, G3PDH activity was 77.8 ± 1.8% of the controls ($n = 10$). These agents exerted an analogous effect in a subset of abdominal subcutaneous preadipose cells included in this study (Figure 3, top inset). The type 2 receptor inhibitor CGP42112A did not reverse angiotensin II reduction of adipogenesis in omental preadipose cells (Figure 3, bottom inset).

Discussion

Recruitment of precursor cells to undergo adipogenic differentiation seems to be necessary to cope with excessive

[1] Nonstandard abbreviation: G3PDH, glycerol-3-phosphate dehydrogenase.

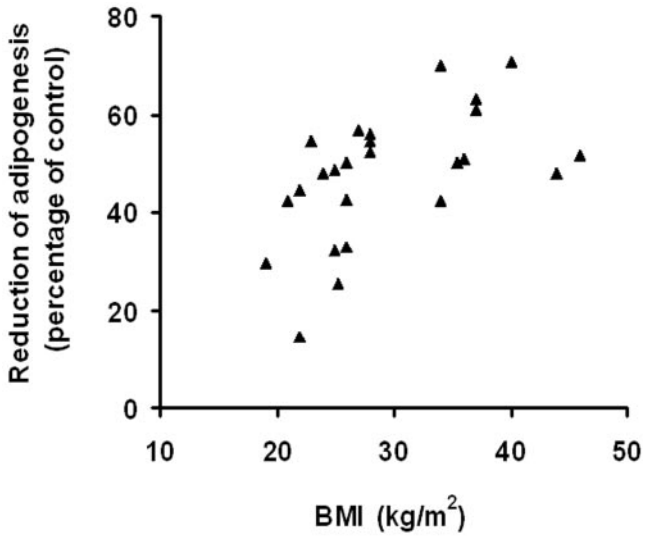

Figure 2: The extent of adipogenic inhibition by angiotensin II correlates with subject BMI. Reduction of G3PDH-specific activity in progenitor cells from omental adipose tissue induced to adipogenic differentiation in the presence of 15 μM angiotensin II vs. donors' BMI ($n = 24$). Correlation analysis shows $R^2 = 0.30$, $p = 0.01$.

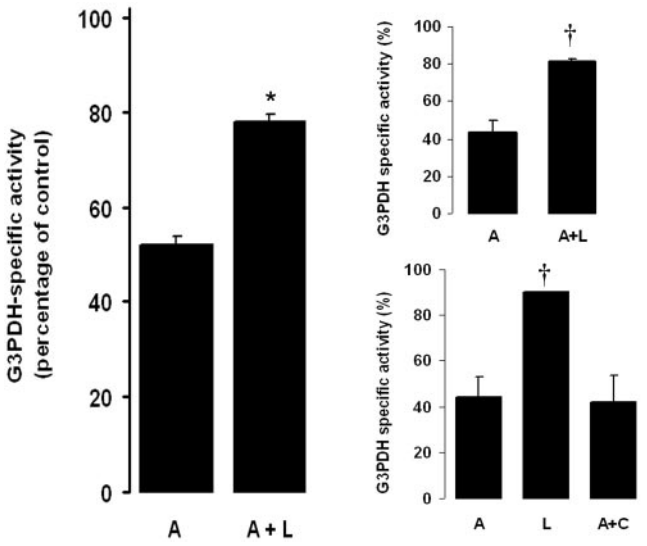

Figure 3: Reversal of angiotensin II inhibition of adipogenic differentiation by losartan. G3PDH-specific activity was measured in progenitor cells stimulated to adipogenic differentiation in the presence of 15 μM angiotensin II (A) or angiotensin II plus 30 μM losartan (A+L; $n = 10$). (Top Inset) The adipogenic response of progenitor cells from subcutaneous adipose tissue of two donors was evaluated under the same conditions. (Bottom Inset) G3PDH-specific activity in two samples of omental fat precursor cells stimulated to adipogenic differentiation and treated with either angiotensin II or losartan (L) alone, at the concentrations indicated above, or angiotensin II plus 10 μM CGP42112A (A+C). G3PDH-specific activity was expressed as a percentage of controls maintained only with adipogenic medium. * $p < 0.01$; † $p < 0.05$.

caloric intake, as suggested by the association between the impaired capacity to expand the adipocyte number with the development of insulin resistance and diabetes (9,10). Thus, the formation of new adipocytes is likely a tightly regulated process.

As communicated here, angiotensin II reduces the recruitment of undifferentiated cells from omental adipose tissue to undergo adipogenic differentiation in vitro. In agreement with previously reported results in cells from human subcutaneous fat (2,3), blockade of type 1 receptor prevented the anti-adipogenic effect of angiotensin II in cells from human omental fat, whereas inhibition of type 2 angiotensin receptor showed no effect.

The finding that human visceral fat shows significantly higher levels of angiotensinogen transcripts compared with subcutaneous fat (4,11,12) suggests a physiologic role for locally generated angiotensin II in the modulation of omental fat cellularity. Interestingly, we found that the effect of angiotensin II is larger in omental fat cells from subjects with BMI $\geq$30 kg/m^2. This result, together with the reported augmented angiotensinogen expression in obese subjects (11), prompted speculation on a connection between local angiotensin II production and omental fat dysfunction. As previously proposed (13), high local levels of angiotensin II would eventually result in a reduced number of newly differentiated adipocytes in visceral fat, a condition that is predicted to favor the predominance of hypertrophic fat cells and, thus, contribute to the adverse consequences of obesity. It is apparent that plasma and adipose tissue interstitial angiotensin II concentrations are independently regulated (14); therefore, further studies are needed to unravel the control of local angiotensin II generation/degradation in omental adipose tissue and fully comprehend its role in the cellularity and physiology of this depot. Likewise, prospective clinical trials are necessary to address the potential effect of pharmacologic angiotensin II blockers on adipose tissue cellularity and/or mass.

A deeper understanding of the mechanisms underlying the greater inhibition of adipogenesis in omental preadipose

Table 1. Inhibition of adipogenic differentiation was larger in preadipose cells from obese subjects

Group	BMI (kg/m^2)	Reduction (%)
Obese	38.2 ± 1.4	56.3 ± 3.4*
Non-obese	24.5 ± 0.7	44.5 ± 2.7

* $p < 0.01$.

cells from obese subjects, here reported, is expected to help in the search for novel targets for prevention and/or treatment of the harmful consequences of excessive visceral fat accumulation.

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