Angiotensin II: a hormone that affects lipid metabolism in adipose tissue.

International Journal of Obesity (2006) 1–3
 0307-0565/06 $30.00
www.nature.com/ijo

SHORT COMMUNICATION

Angiotensin II: a hormone that affects lipid metabolism in adipose tissue

GH Goossens[1], EE Blaak[1], P Arner[2], WHM Saris[1] and MA van Baak[1]

[1]*Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, Maastricht, The Netherlands and* [2]*Department of Medicine, Huddinge University Hospital, Karolinska Institute, Stockholm, Sweden*

Background: Alterations in adipose tissue lipolysis may contribute to the pathophysiology of obesity and insulin resistance. We examined the effects of angiotensin II (Ang II) on abdominal subcutaneous adipose tissue lipolysis in humans.
Methods and results: First, adipocytes obtained from nine normal weight and seven obese subjects were stimulated with Ang II (10^{-14}–10^{-6} M). Glycerol concentration in the medium, used as an indicator of adipocyte lipolysis, was significantly reduced ($\sim$20%) after Ang II stimulation in adipocytes from normal weight ($P=0.04$) and obese subjects ($P<0.001$). Based on these observations, adipocytes of seven additional obese subjects were stimulated with lower doses of Ang II (10^{-17}–10^{-6} M) in the presence and absence of Ang II type 1 (AT_1) receptor blockade. Lipolysis was dose dependently inhibited by $\sim$20 to 25% after Ang II stimulation ($P=0.001$). AT_1 receptor blockade completely abolished the Ang II-induced effects ($P=0.35$).
Conclusion: Ang II directly inhibits abdominal subcutaneous adipocyte lipolysis in normal weight and obese subjects via the AT_1 receptor.
International Journal of Obesity advance online publication, 16 May 2006; doi:10.1038/sj.ijo.0803388

Keywords: angiotensin II; Ang II type 1 receptor; lipolysis; adipose tissue

Introduction

Abdominal obesity is strongly associated with insulin resistance. Part of this association may be explained by adipose tissue products that exert autocrine, paracrine and/or endocrine effects that may affect metabolism.[1–3]

A functional renin–angiotensin system (RAS) is present in human adipose tissue,[4–6] and local generation of angiotensin II (Ang II), the effector molecule of the RAS, by human (pre)adipocytes has been demonstrated.[7] The adipose tissue RAS may be involved in obesity-related disorders, such as insulin resistance,[8,9] possibly through an effect on adipocyte differentiation.[10,11] Furthermore, direct lipogenic effects of Ang II in human adipocytes have been shown,[12] and recent data from our laboratory suggest that Ang II may inhibit lipolysis *in vivo* in human adipose tissue and skeletal muscle.[13] However, no conclusions about the magnitude of the observed antilipolytic effects of Ang II could be drawn from these findings, as the Ang II-induced decrease in tissue blood flow may have masked direct effects on lipolysis to some extent. The objective of the present study was to investigate for the first time the direct effects of Ang II on abdominal subcutaneous adipose tissue lipolysis *in vitro* in normal weight and obese subjects.

Correspondence: Dr GH Goossens, Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, PO Box 616, Universiteitssingel 50, 6200 MD Maastricht, The Netherlands.
E-mail: G.Goossens@hb.unimaas.nl
Received 18 November 2005; revised 5 April 2006; accepted 8 April 2006

Materials and methods

Nine normal weight and seven obese male subjects participated in the first study. Seven additional obese subjects participated in a second experiment. Characteristics of the subjects are summarized in Table 1. The Medical-Ethical Committee of Maastricht University approved the study protocol and all subjects gave written informed consent before participating in the study.

Protocol

Abdominal subcutaneous adipose tissue ($\sim$2 g) was obtained by needle aspiration under local anesthesia after an overnight fast. Adipocytes were isolated from the subcutaneous fat tissue specimen as described previously.[14] Firstly, diluted suspensions of adipocytes ($\sim$5000–10 000 cells/incubation) from normal weight and obese subjects were incubated with

Table 1 Subjects' characteristics

	Normal weight	*Obese (study 1)*	*Obese (study 2)*
Age (years)	54±2	51±2	52±4
Weight (kg)	76.5±1.9	112.8±6.4*	109.1±6.3#
Height (m)	1.78±0.03	1.78±0.02	1.81±0.02
BMI (kg/m^2)	24.3±0.4	35.5±1.8*	33.3±1.9#
Body fat (%)	22.9±1.3	34.6±2.7*	34.4±3.1#

Abbreviation: BMI, body mass index. Values are means±s.e.m. *$P=0.001$ vs normal weight by unpaired *t*-test; #$P<0.005$ vs normal weight by unpaired *t*-test.

Ang II (10^{-14}–10^{-6} M; Sigma-Aldrich, Zwijndrecht, The Netherlands) for 2 h at 37°C in Krebs–Ringer phosphate buffer. Thereafter, incubation medium was immediately frozen in liquid nitrogen and stored at −80°C until analysis. Glycerol concentration in the medium, determined using a sensitive automated bioluminescence method,[15] was used as an indicator of lipolysis.

Adipocytes of an additional group of obese subjects were stimulated with lower Ang II concentrations (10^{-17}–10^{-6} M) in the presence and absence of AT_1 receptor blockade (10^{-4} M losartan; MSD, Haarlem, The Netherlands). Furthermore, adipocytes were incubated with N^6-(2-phenylisopropyl)adenosine (PIA: 100 nM) (Sigma-Aldrich) as a control point for maximal inhibition of lipolysis in the incubation system.

Statistical analysis. Data are mean±standard error of the mean (s.e.m.). Lipolytic rates are presented as relative changes from baseline because of interindividual variation in baseline glycerol concentrations. Effects of Ang II were compared by two-way repeated measures analysis of variance (ANOVA), using dose as within-subject factor and group or treatment as between-subject factor. One-way repeated measures ANOVA was performed to identify dose effects. $P<0.05$ was considered to be statistically significant, using SPSS 10.1 (Chicago, IL, USA).

Results and discussion

Basal lipolytic rates were not significantly different between normal weight and obese subjects (4.8±1.1 vs 11.4±5.1 μmol × 10^7 cells^{-1} × 2 h incubation^{-1}, respectively, $P=0.24$). Ang II significantly inhibited adipocyte lipolysis in normal weight ($P=0.04$) and obese subjects ($P<0.001$) (Figure 1a), with no differences between groups ($P=0.40$). In the second experiment, basal lipolytic rate was 5.0±1.0 μmol/10^7 cells/2 h incubation. Ang II dose dependently inhibited lipolysis by ~20–25% ($P=0.001$), and this effect was completely abolished by AT_1 receptor blockade ($P=0.35$) (Figure 1b).

These findings demonstrate that Ang II inhibits lipolysis through the AT_1 receptor in abdominal subcutaneous adipocytes in normal weight and obese subjects. PIA, used as a control point for maximal inhibition of lipolysis in the incubation system,[16] reduced the lipolytic rate by ~40%

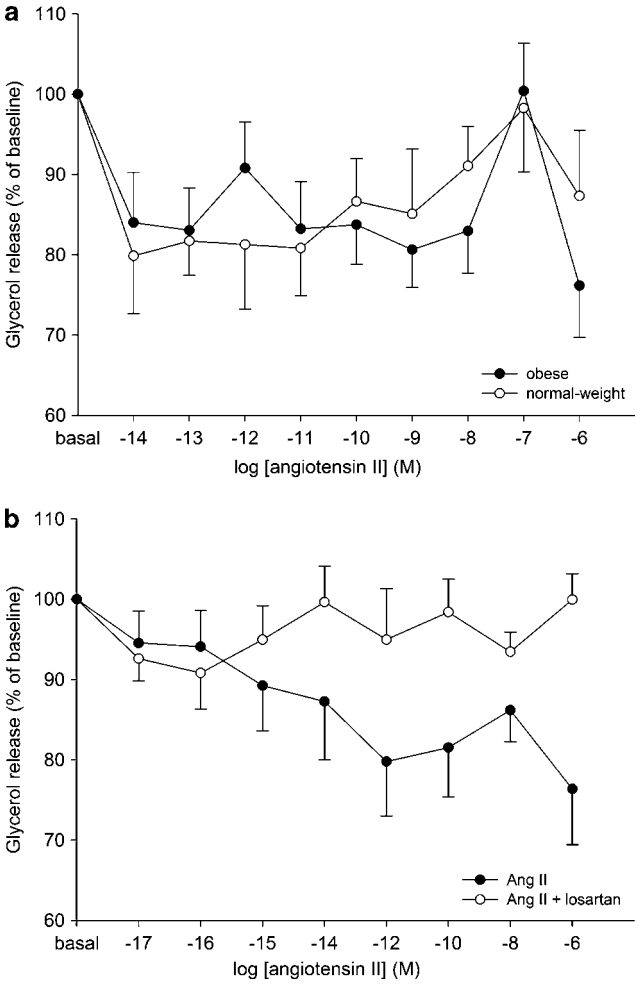

Figure 1 (**a**) Effects of Ang II stimulation on glycerol release from abdominal subcutaneous adipocytes from nine normal weight and seven obese subjects. Ang II reduced glycerol release both in normal weight ($P=0.04$ by one-way repeated-measures ANOVA) and obese subjects ($P<0.001$ by one-way repeated-measures ANOVA), with no differences between groups ($P=0.40$ by two-way repeated-measures ANOVA). Values are means±s.e.m. (**b**) Effects of Ang II stimulation on glycerol release from abdominal subcutaneous adipocytes from seven obese subjects in the presence and absence of AT_1 receptor blockade (10^{-4} M). Glycerol release was dose dependently reduced during stimulation with Ang II ($P=0.001$ by one-way repeated-measures ANOVA). Ang II had no significant effects on lipolysis in the presence of AT_1 receptor blockade ($P=0.35$ by one-way repeated-measures ANOVA). Values are means±s.e.m.

($P<0.01$) (data not shown). Therefore, the observed ~20–25% reduction of glycerol release after Ang II stimulation reflects a substantial inhibition of lipolysis. The normal circulating Ang II concentration is 10 pM.[17] It has previously been shown that Ang II concentrations are ~2–3-fold higher in the incubation medium of (pre)adipocytes than in the circulation.[4,7] Because stimulation of adipocytes with physiological concentrations of Ang II evoked near-maximal inhibition of lipolysis in the present experiments, Ang II may not play an important role in the regulation of adipocyte lipolysis. Instead, Ang II may exert a tonic suppression of adipocyte lipolysis.

The present findings are in line with previous observations suggesting that Ang II inhibits adipose tissue lipolysis in humans.[13] In contrast, no effect of Ang II on insulin-induced suppression of lipolysis was observed in obese women.[18] In addition to methodological differences compared with the present study, insulin may have masked a less pronounced antilipolytic effect of Ang II. In a rat model for increased RAS activity in cachectic patients with advanced heart failure, lipolysis was increased in some but not all fat depots.[19] Because expression of (anti)lipolytic receptors differs between species,[19] and the high Ang II concentrations activated the sympathetic nervous system, it is difficult to extrapolate these findings to Ang II effects on adipocyte lipolysis in humans.

It has been shown that Ang II exerts lipogenic effects in human adipocytes[12] and inhibits adipocyte differentiation.[11] Although Ang II effects in adipose tissue may not be involved in the expansion of fat mass, they may contribute to a reduced buffering capacity for lipid storage in adipose tissue in the long term, leading to an excessive influx of lipids to other tissues.[10]

In conclusion, Ang II inhibits lipolysis via the AT_1 receptor in abdominal subcutaneous adipocytes from normal weight and obese subjects. The present findings support the concept that the RAS in adipose tissue participates directly in fat metabolism and may contribute to the metabolic disturbances seen in obesity. This study was not designed to examine underlying signalling pathways, but focused on lipolysis as cellular end point. Identification of the underlying mechanisms for the antilipolytic effect of Ang II in human adipocytes may provide better insight into possible interactions between (anti)lipolytic pathways.

Acknowledgements

We thank Kerstin Wåhlén, Eva Sjölin and Freek Bouwman for excellent technical assistance. Losartan was provided by Merck Sharp and Dohme (Haarlem, The Netherlands).

References

1 Lafontan M. Fat cells: afferent and efferent messages define new approaches to treat obesity. *Annu Rev Pharmacol Toxicol* 2005; **45**: 119–146.
2 Arner P. Insulin resistance in type 2 diabetes – role of the adipokines. *Curr Mol Med* 2005; **5**: 333–339.
3 Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; **89**: 2548–2556.
4 Schling P, Schafer T. Human adipose tissue cells keep tight control on the angiotensin II levels in their vicinity. *J Biol Chem* 2002; **277**: 48066–48075.
5 Karlsson C, Lindell K, Ottosson M, Sjostrom L, Carlsson B, Carlsson LM. Human adipose tissue expresses angiotensinogen and enzymes required for its conversion to angiotensin II. *J Clin Endocrinol Metab* 1998; **83**: 3925–3929.
6 Crandall DL, Herzlinger HE, Saunders BD, Armellino DC, Kral JG. Distribution of angiotensin II receptors in rat and human adipocytes. *J Lipid Res* 1994; **35**: 1378–1385.
7 Schling P, Mallow H, Trindl A, Loffler G. Evidence for a local renin angiotensin system in primary cultured human preadipocytes. *Int J Obes Relat Metab Disord* 1999; **23**: 336–341.
8 Goossens GH, Blaak EE, van Baak MA. Possible involvement of the adipose tissue renin–angiotensin system in the pathophysiology of obesity and obesity-related disorders. *Obes Rev* 2003; **4**: 43–55.
9 Strazzullo P, Galletti F. Impact of the renin–angiotensin system on lipid and carbohydrate metabolism. *Curr Opin Nephrol Hypertens* 2004; **13**: 325–332.
10 Sharma AM, Janke J, Gorzelniak K, Engeli S, Luft FC. Angiotensin blockade prevents type 2 diabetes by formation of fat cells. *Hypertension* 2002; **40**: 609–611.
11 Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Mature adipocytes inhibit *in vitro* differentiation of human preadipocytes via angiotensin type 1 receptors. *Diabetes* 2002; **51**: 1699–1707.
12 Jones BH, Standridge MK, Moustaid N. Angiotensin II increases lipogenesis in 3T3-L1 and human adipose cells. *Endocrinology* 1997; **138**: 1512–1519.
13 Goossens GH, Blaak EE, Saris WH, van Baak MA. Angiotensin II-induced effects on adipose and skeletal muscle tissue blood flow and lipolysis in normal-weight and obese subjects. *J Clin Endocrinol Metab* 2004; **89**: 2690–2696.
14 Rodbell M. Metabolism of isolated fat cells: effects of hormones on glucose metabolism and lipolysis. *J Biol Chem* 1964; **239**: 375–380.
15 Hellmer J, Arner P, Lundin A. Automatic luminometric kinetic assay of glycerol for lipolysis studies. *Anal Biochem* 1989; **177**: 132–137.
16 Johnson JA, Fried SK, Pi-Sunyer FX, Albu JB. Impaired insulin action in subcutaneous adipocytes from women with visceral obesity. *Am J Physiol Endocrinol Metab* 2001; **280**: E40–E49.
17 Campbell DJ, Kladis A. Simultaneous radioimmunoassay of six angiotensin peptides in arterial and venous plasma of man. *J Hypertens* 1990; **8**: 165–172.
18 Perry CG, Palmer T, Cleland SJ, Morton IJ, Salt IP, Petrie JR *et al*. Decreased insulin sensitivity during dietary sodium restriction is not mediated by effects of angiotensin II on insulin action. *Clin Sci (London)* 2003; **105**: 187–194.
19 Cabassi A, Coghi P, Govoni P, Barouhiel E, Speroni E, Cavazzini S *et al*. Sympathetic modulation by carvedilol and losartan reduces angiotensin II-mediated lipolysis in subcutaneous and visceral fat. *J Clin Endocrinol Metab* 2005; **90**: 2888–2897.