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Angiotensin II-Induced Effects on Adipose and Skeletal Muscle Tissue Blood Flow and Lipolysis in Normal-Weight and Obese Subjects

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The present study was designed to investigate the effects of angiotensin II (Ang II) on adipose and skeletal muscle tissue blood flow and lipolysis in normal-weight and obese subjects using the microdialysis technique. Microdialysis probes were placed in the abdominal sc adipose tissue left and right from the umbilicus and in the gastrocnemius muscle of both legs in eight normal-weight and eight obese men. Probes were consecutively perfused with 1.0 nM Ang II, 1.0 μ M Ang II, and 1.0 μ M Ang II + 48 μ M hydralazine or with Ringer solution (control). Ethanol and glycerol concentrations in the dialysate were measured as an indicator of local blood flow and lipolysis, respectively. Ang II caused an increase in ethanol outflow/inflow ratio, compared with baseline values both in adipose tissue (average of both groups, Ang 1.0 nM: 0.03 ± 0.01 ,

$P = 0.02$; Ang 1.0 μ M: 0.05 ± 0.01 , $P < 0.01$) and muscle (average of both groups, Ang 1.0 nM: 0.02 ± 0.01 , $P = 0.09$; Ang 1.0 μ M: 0.04 ± 0.01 , $P = 0.01$), indicating a decrease in local blood flow. These effects were not significantly different in obese and normal-weight subjects. The decrease in local blood flow was accompanied by unchanged interstitial glycerol concentrations in adipose tissue (except during the supraphysiological dose) and skeletal muscle, suggesting that Ang II inhibits lipolysis in both tissues. Thus, the present data suggest that Ang II decreases local blood flow in a dose-dependent manner and inhibits lipolysis both in adipose and skeletal muscle tissue. These effects were not significantly different in obese and normal-weight subjects in both tissues. (*J Clin Endocrinol Metab* 89: 2690–2696, 2004)

THE OCTAPEPTIDE ANGIOTENSIN II (Ang II) is the active component of the renin-angiotensin system (RAS). In addition to Ang II synthesis in the circulation, several components of the RAS, like angiotensinogen, renin, and angiotensin-converting enzyme, have been identified in a variety of tissues (1, 2), implying that these tissues have the ability to synthesize Ang II independently of circulating RAS components. There is accumulating evidence to suggest that a local RAS is present both in adipose and skeletal muscle tissue. However, the physiological role of Ang II in these tissues remains to be established. There is evidence that the RAS in adipose tissue may affect adipocyte growth and differentiation, adipose tissue blood flow, and lipolysis and may be involved in the pathophysiology of obesity and obesity-related disorders, as has been reviewed recently (3).

Findings on the effects of Ang II on tissue blood flow and lipolysis are controversial. Boschmann *et al.* have shown that Ang II decreased blood flow and inhibited lipolysis in adipose tissue of normal-weight men (4) and women (5). However, they could not confirm these findings in subsequent studies, in which they showed that Ang II had no effect on adipose tissue blood flow and it stimulated rather than inhibited lipolysis in this tissue in normal-weight men (6). In another study in nonobese and obese subjects, Ang II had no effect on adipose and skeletal muscle tissue blood flow in

both groups and exerted a lipolytic effect in adipose tissue and an antilipolytic effect in skeletal muscle tissue in nonobese, but not in obese men (7). Finally, it has been demonstrated that iv infusion of Ang II reduces forearm blood flow, and locally produced Ang II seemed to be more important for vasoconstriction in the forearm than Ang II delivered via the circulation (8).

Thus, the effects of Ang II on blood flow and lipolysis in adipose and skeletal muscle tissue are not yet fully understood and need to be studied in further detail. In addition, with regard to the possible involvement of Ang II in the pathophysiology of obesity, it is of particular interest to find out whether Ang II exerts different effects on tissue blood flow and lipolysis in normal-weight and obese subjects because it has been shown before that adipose tissue blood flow and lipolysis are disturbed in obese individuals during catecholamine stimulation (9, 10). Therefore, the aim of this study was to examine the effects of Ang II on adipose and skeletal muscle tissue blood flow and lipolysis in the fasting state in normal-weight and obese subjects using the microdialysis technique.

Subjects and Methods

Subjects

Eight normal-weight (body mass index < 25 kg/m²) and eight obese (body mass index > 30 kg/m²) male subjects participated in this study. Physical characteristics of the subjects are summarized in Table 1. Body density was determined by underwater weighing after an overnight fast. Lung volume was measured simultaneously with the helium dilution technique using a spirometer (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands). Body weight was measured with a digital balance, accurate to 0.001 kg (type E1200; August Sauter GmbH, Albstadt, Germany).

Abbreviations: Ang II, Angiotensin II; ANP, atrial natriuretic peptide; RAS, renin-angiotensin system.

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Body fat percentage was calculated using the equation of Siri (11). All subjects were in good health as assessed by medical history and spent no more than 2 h/wk in organized sports activities. The Medical-Ethical Committee of Maastricht University approved the study protocol, and all subjects gave their written informed consent before participating in the study.

Protocol

All subjects were asked to refrain from drinking alcohol, smoking, and doing strenuous exercise for a period of 24 h before the study. Subjects came to the laboratory by car or bus in the morning after an overnight fast. On arrival, four microdialysis catheters (CMA 60, CMA Microdialysis AB, Stockholm, Sweden) were inserted before the start of the experiment. Microdialysis probes were placed in the abdominal sc adipose tissue under sterile conditions 6–8 cm left and right from the umbilicus. One hour before insertion of these probes, the skin was anesthetized by means of a cream containing lidocaine (25 mg/g) and prilocaine (25 mg/g) (EMLA, AstraZeneca BV, Zoetermeer, The Netherlands). Furthermore, microdialysis probes were inserted in the medial portion of the gastrocnemius muscle of both legs after anesthesia (xylocaine 2% without adrenalin, AstraZeneca). Thereafter, 90 min was allowed for recovery of the skeletal muscle and adipose tissue from the insertion trauma.

The probes ($30 \times 0.6 \text{ mm}^2$, molecular weight cut-off of 20 000) consisted of a dialysis tubing glued to the end of a double-lumen polyurethane cannula. The perfusion solvent enters the probe through the inner cannula, passes down to the tip of the probe, streams upward in the space between the inner cannula and the outer dialysis membrane and leaves the probe through the outer cannula via a side arm from which it is collected in a capped microvial.

After insertion, all probes were perfused with Ringer solution (147 mM sodium, 4 mM potassium, 2.25 mM calcium, and 156 mM chloride, Baxter BV, Utrecht, The Netherlands), supplemented with 50 mM ethanol, at a flow rate of $0.5 \mu\text{l}/\text{min}$ (Harvard microinfusion pump, Plato BV, Diemen, The Netherlands). After 90 min recovery, the real interstitial glycerol concentration was determined individually by means of the zero flow method (12, 13) (see *Zero flow method*). Microdialysate was collected in two 20-min fractions at a flow rate of $0.5 \mu\text{l}/\text{min}$ and in three 10-min fractions at flow rates of 1.0, 2.5, and $5.0 \mu\text{l}/\text{min}$. The calibration period with a flow rate of $5.0 \mu\text{l}/\text{min}$ was used as baseline measurement for the second part of the experiment.

During the second part of the experiment, the experimental probes in adipose and skeletal muscle tissue were consecutively perfused with 1.0 nM Ang II (Ang 1.0 nM), $1.0 \mu\text{M}$ Ang II (Ang $1.0 \mu\text{M}$), and $1.0 \mu\text{M}$ Ang

II + $48 \mu\text{M}$ hydralazine (Ang $1.0 \mu\text{M}$ + H), each dose for 60 min at a flow rate of $5.0 \mu\text{l}/\text{min}$, to examine the effect of Ang II on local blood flow and lipolysis (ethanol and glycerol concentrations in the dialysate, respectively). The highest perfusion dose of Ang II was also administered in combination with the vasodilator hydralazine to counteract the possible Ang II-induced vasoconstriction and, therefore, to distinguish between the possible direct and blood flow-mediated effects of Ang II on the interstitial glycerol concentration. The other probes in adipose and skeletal muscle tissue served as control probes and were perfused with Ringer solution (+50 mM ethanol) at a similar perfusion rate during the whole experiment. There was a wash-out period of 30 min perfusion with Ringer solution (+50 mM ethanol) between each perfusion step (Fig. 1).

Microdialysate was collected in fractions corresponding to 10 min during the second part of the experiment. In all samples collected at flow rates of 0.5, 1.0, and $2.5 \mu\text{l}/\text{min}$, dialysate glycerol concentration was measured. Both dialysate glycerol and ethanol concentrations were determined in all other samples. Furthermore, ethanol concentration was determined both in the ingoing and outgoing perfusion solvent to assess the ethanol outflow/inflow (out/in) ratio as indicator for local nutritive blood flow (ethanol dilution technique) (14, 15). The interstitial glycerol concentration was used to assess changes in lipolysis. Ethanol concentrations were determined the same day, whereas the samples for measurement of the interstitial glycerol concentration were immediately frozen in liquid nitrogen and stored at -80 C until analysis. All changes in ethanol out/in ratios and glycerol concentrations observed in the different experimental conditions were adjusted for corresponding changes in the control probes to take into account changes over time in tissue blood flow and lipolysis that were not due to the intervention. These adjustments were made by subtracting changes in the experimental probes by changes in the control probes. Adjusted values are presented as changes from baseline values because of the interindividual variations in baseline concentrations.

Zero flow method

During the first part of the experiment, the real interstitial glycerol concentration was determined by means of the zero flow method (12, 13). Therefore, the microdialysis probes were perfused with Ringer solution at a flow rate of $0.5 \mu\text{l}/\text{min}$ for 40 min and at consecutive flow rates of 1.0, 2.5, and $5.0 \mu\text{l}/\text{min}$ for 30 min. Dialysate glycerol concentrations were log transformed and plotted against perfusion rates. Linear regression analysis was used to calculate the glycerol concentration at zero flow rate, corresponding to the real interstitial glycerol concentration. The ratio between the dialysate glycerol concentration at $5.0 \mu\text{l}/\text{min}$ and the calculated interstitial glycerol concentration represents the *in vivo* recovery of the probe, and all dialysate glycerol samples collected during the second part of the experiment were adjusted for the *in vivo* recovery of the corresponding probe.

Analyses

Glycerol and ethanol concentrations were determined on a Cobas Fara centrifugal analyzer (Roche Diagnostica, Basel, Switzerland). Glycerol concentration was measured fluorometrically using a standard glycerol kit (Boehringer, Mannheim, Germany), but with adjusted concentrations of NADH, enzymes and buffer, to achieve accurate fluorometric detection. Ethanol concentration was measured spectrophotometrically

TABLE 1. Physical characteristics of subjects

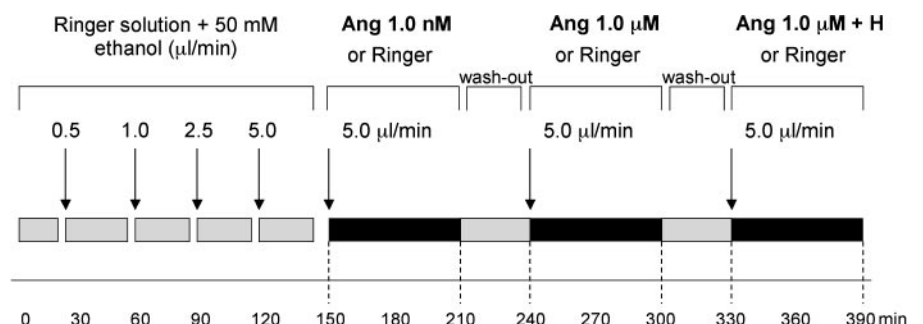
Characteristic	Normal weight (n = 8)	Obese (n = 8)
Age (yr)	52 ± 2	49 ± 3
Weight (kg)	76.6 ± 2.1^a	112.8 ± 6.4
Height (m)	1.78 ± 0.03	1.78 ± 0.02
BMI (kg/m^2)	24.3 ± 0.5^a	35.5 ± 1.8
Body fat (%)	22.8 ± 1.5^b	34.6 ± 2.7

Values are means \pm SEM. Unpaired *t* test.

^a $P < 0.001$.

^b $P < 0.01$.

FIG. 1. Experimental design.



at a wavelength of 340 nm using a standard ethanol kit (176290, Boehringer).

Statistical analysis

Data are presented as mean \pm SEM. To compare the effects of the different perfusion solvents within and between groups, a repeated-measures ANOVA was performed. *Post hoc* testing was performed using the Student's paired or unpaired *t* tests. Calculations were done using Statview 5.0 for iMac (SAS Institute, Cary, NC). $P < 0.05$ was considered to be statistically significant.

Results

By definition, obese men had a significantly higher body weight, body mass index, and body fat percentage, compared with normal-weight men. Age and height of both groups were similar (Table 1).

Ethanol out/in ratio

At baseline, ethanol out/in ratios were not significantly different in obese subjects, compared with normal-weight

subjects, both in adipose tissue [average of experimental and control probe: 0.84 ± 0.07 (obese) vs. 0.79 ± 0.10 (normal-weight), $P = 0.17$] and muscle [average of experimental and control probe: 0.51 ± 0.08 (obese) vs. 0.46 ± 0.06 (normal-weight), $P = 0.10$] (Fig. 2, A and B). As expected, baseline ethanol out/in ratios were higher in adipose tissue than in skeletal muscle in both groups, reflecting a lower blood flow in abdominal sc adipose tissue, compared with skeletal muscle.

During perfusion with Ang II, there was a significant dose-dependent increase in ethanol out/in ratios, compared with baseline values both in adipose tissue (average of both groups, Ang 1.0 nM: 0.03 ± 0.01 , $P = 0.02$; Ang 1.0 μ M: 0.05 ± 0.01 , $P < 0.01$) and muscle (average of both groups, Ang 1.0 nM: 0.02 ± 0.01 , $P = 0.09$; Ang 1.0 μ M: 0.04 ± 0.01 , $P = 0.01$) (Fig. 2, C and D), indicating a decrease in local nutritive blood flow in both tissues. At the lowest (physiological) dose of Ang II, the increase in ethanol out/in ratios from baseline was 0.04 ± 0.01 ($P =$

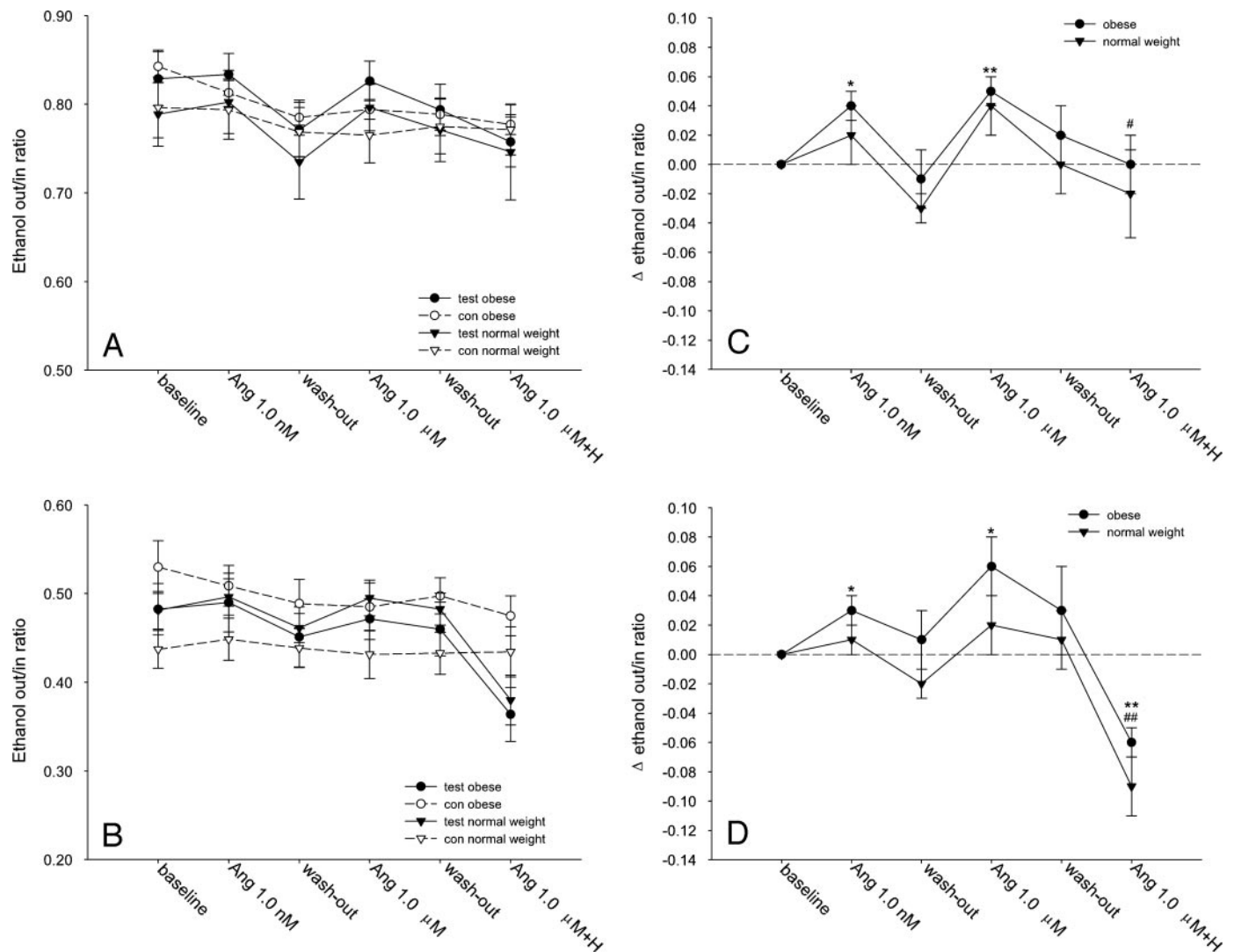


FIG. 2. Effects of Ang II on the ethanol out/in ratio in abdominal sc adipose tissue (A) and skeletal muscle (B) in eight normal-weight and eight obese subjects. Data are adjusted for corresponding changes in the control probes and presented as changes from baseline values in adipose tissue (C) and skeletal muscle (D). Values are means \pm SEM. *, $P < 0.05$ vs. baseline; **, $P < 0.01$ vs. baseline; #, $P < 0.05$ vs. Ang 1.0 μ M; ##, $P < 0.001$ vs. Ang 1.0 μ M.

0.01) and 0.02 ± 0.02 (NS) in adipose tissue and 0.03 ± 0.01 ($P = 0.08$) and 0.01 ± 0.01 (NS) in skeletal muscle in obese and normal-weight subjects, respectively. However, the differences in increase from baseline between groups did not reach statistical significance. When expressed as percentage increase in ethanol out/in ratio, similar results were obtained (data not shown).

In adipose as well as skeletal muscle tissue, perfusion with $1.0 \mu\text{M}$ Ang II + $48 \mu\text{M}$ hydralazine (Ang $1.0 \mu\text{M}$ + H) significantly lowered ethanol out/in ratios, compared with perfusion with the highest dose of Ang II alone (average of both groups: -0.05 ± 0.02 , $P = 0.01$ vs. -0.12 ± 0.01 , $P < 0.001$, respectively), indicating an increase in local blood flow. Compared with baseline values, perfusion with Ang $1.0 \mu\text{M}$ + H decreased ethanol out/in ratios in muscle (average of both groups: -0.08 ± 0.01 , $P < 0.001$) but did not significantly change ethanol out/in ratios in adipose tissue of both groups.

Interstitial glycerol concentration

Baseline interstitial glycerol concentrations in adipose tissue were not significantly different in obese compared with normal weight subjects (average of experimental and control probe: 197 ± 108 (obese) vs. $148 \pm 65 \mu\text{M}$ (normal-weight), $P = 0.14$) (Fig. 3A). Interstitial glycerol concentration in skeletal muscle of obese subjects was significantly higher than that of normal-weight subjects under baseline conditions (average of experimental and control probe: 103 ± 28 (obese) vs. $84 \pm 19 \mu\text{M}$ (normal-weight), $P = 0.04$) (Fig. 3B). As expected, baseline interstitial glycerol concentrations were higher in adipose tissue than in skeletal muscle in both groups. In adipose tissue, the only significant change in interstitial glycerol concentration from baseline was found during perfusion with Ang $1.0 \mu\text{M}$ (average of both groups: $51.3 \pm 20.2 \mu\text{M}$, $P = 0.02$) (Fig. 3C). This increase from baseline was $42.1 \pm 30.9 \mu\text{M}$ (NS) in obese and $59.4 \pm 28.2 \mu\text{M}$ ($P =$

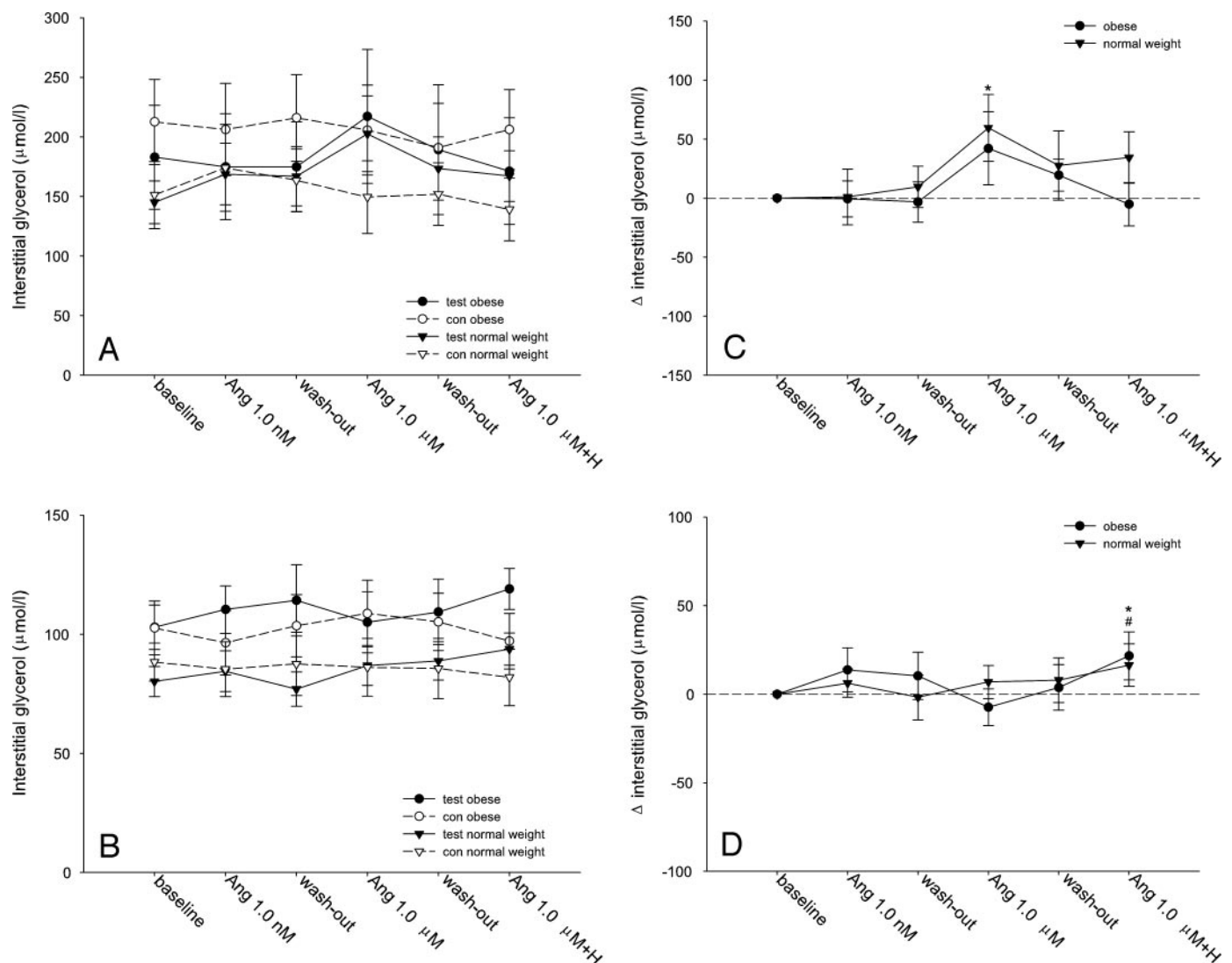


FIG. 3. Effects of Ang II on the interstitial glycerol concentration in abdominal sc adipose tissue (A) and skeletal muscle (B) in eight normal-weight and eight obese subjects. Data are adjusted for corresponding changes in the control probes and presented as changes from baseline values in adipose tissue (C) and skeletal muscle (D). Values are means \pm SEM. *, $P < 0.05$ vs. baseline; #, $P < 0.05$ vs. Ang $1.0 \mu\text{M}$.

0.07) in normal-weight subjects. However, the differences in increase from baseline between groups did not reach statistical significance. In skeletal muscle, the only significant change in interstitial glycerol concentration from baseline was observed during perfusion with Ang 1.0 μM + H (average of both groups: $19.1 \pm 8.8 \mu\text{M}$, $P < 0.05$) (Fig. 3D). When expressed as percentage increase in interstitial glycerol concentration, similar results were obtained (data not shown).

Discussion

It is tempting to postulate that in addition to Ang II produced in the circulation, locally produced Ang II may affect local nutritive blood flow and lipolysis in adipose and skeletal muscle tissue, and may thereby affect fat accumulation in these tissues. However, the physiological significance of Ang II in these processes remains to be established.

Local blood flow and lipolysis

The present data show that Ang II stimulation *in situ* causes an increase in ethanol out/in ratios both in adipose and skeletal muscle tissue, indicating a decrease in local blood flow. This finding is in agreement with the well-known vasoconstrictive effect of Ang II in the systemic circulation and its role in elevating blood pressure (16). The decrease in blood flow in both tissues was not significantly different in obese and normal-weight subjects. Inhibition of local blood flow will retain glycerol in the tissue (17), thereby increasing the interstitial glycerol concentration due to product accumulation. However, the Ang II-induced reduction in blood flow was accompanied by interstitial glycerol concentrations that were not different from baseline values in adipose tissue and skeletal muscle during stimulation with the lower dose of Ang II (1.0 nM), suggesting that Ang II inhibits lipolysis in both tissues. The elevation in glycerol concentration in the dialysate observed when adipose tissue was stimulated with the highest (supraphysiological) dose of Ang II, despite an Ang II-induced inhibition of lipolysis, might be explained by the extra vasoconstriction that occurred. However, we cannot exclude that Ang II stimulation *in situ* may cause a dose-dependent biphasic response, with an antilipolytic effect of Ang II at the physiological concentration, and no effect or a lipolytic effect at supraphysiological concentrations.

The normal concentration of Ang II in plasma is about 10 pM (18). However, it should be mentioned that local Ang II production might result in higher Ang II concentrations in the interstitial space than in the circulation. For example, Ang II concentration in the interstitial space of the rat kidney has been shown to be about 30-fold higher than the circulating Ang II concentration (19). In addition, with the present perfusion rate only a small fraction (probably less than 20%) of Ang II in the perfusate will diffuse into the interstitial space. Therefore, the lowest dose of Ang II used in the present study (1.0 nM) most likely corresponds to an interstitial Ang II concentration that is within the physiological range.

In line with our findings, others have found that Ang II reduces blood flow and inhibits lipolysis in adipose tissue of normal-weight men (4) and women (5). However, they could not confirm these findings in subsequent studies, in which they showed that Ang II had no effect on adipose and skeletal

muscle tissue blood flow, and it stimulated rather than inhibited lipolysis in adipose tissue, whereas it inhibited lipolysis in muscle in normal-weight men (6). In a recent study, Ang II had no effect on adipose and skeletal muscle tissue blood flow in nonobese and obese subjects and exerted a lipolytic effect in adipose tissue and an antilipolytic effect in skeletal muscle in nonobese but not in obese men (7). In contrary to the above-mentioned studies, control probes were used in the present study to take into account changes over time in tissue blood flow and lipolysis that were not due to the intervention. Additionally, the nonobese and obese subjects in the present study were well matched for age, which was not the case in the study of Boschmann *et al.* (7). Furthermore, a wash-out period of 30-min perfusion with Ringer solution between each perfusion step was included in our design to prevent that Ang II applied in a previous perfusion step would affect the following condition.

During perfusion with the highest dose of Ang II in combination with the vasodilating agent hydralazine (Ang 1.0 μM + H) in muscle, local blood flow was tremendously increased, and the interstitial glycerol concentration was significantly elevated, compared with the highest dose of Ang II alone, indicating that hydralazine itself exerts a strong lipolytic effect in skeletal muscle. During perfusion with Ang 1.0 μM + H in adipose tissue, blood flow and interstitial glycerol concentration were not significantly different from baseline. Because of the antilipolytic effect of Ang II in adipose tissue, this suggests that hydralazine also stimulates lipolysis in adipose tissue, although this effect is less clear than in skeletal muscle. Indeed, there is some indirect evidence that strengthens our observations. In vascular smooth muscle cells, the main action of hydralazine is to inhibit the inositol 1,4,5-triphosphate-induced release of Ca^{2+} from the sarcoplasmic reticulum (20). Interestingly, increasing intracellular Ca^{2+} inhibits lipolysis in a dose-dependent manner in rat adipocytes (21). In line with this, the *agouti* gene product inhibits lipolysis in human adipocytes via an increase in intracellular Ca^{2+} (22). Thus, low concentrations of intracellular Ca^{2+} may stimulate lipolysis. Therefore, hydralazine may lead to an increased rate of lipolysis by inhibiting the inositol 1,4,5-triphosphate-induced release of Ca^{2+} into the cytosol *in vivo*.

Underlying mechanisms for the antilipolytic effect

Although less is known about the physiological effects of Ang II on adipose and skeletal muscle tissue lipolysis, there are mechanisms that may explain the antilipolytic effect of Ang II found in the present study. There is evidence that Ang II may act on the sympathetic nervous system. Under resting conditions, lipolysis is primarily regulated (inhibited) by the activity of α_2 -adrenergic receptors (23, 24), and it has been demonstrated that Ang II augments the release of noradrenaline from brown adipose tissue sympathetic nerve terminals (25). Thus, the antilipolytic effects of Ang II observed in the present study may be due to stimulation or potentiation of α_2 -adrenergic activity. In addition, it has been demonstrated that binding of Ang II to the angiotensin type 1 receptor inhibits adenylate cyclase in smooth muscle cells, the kidney, and hepatocytes (26, 27). Therefore, Ang II might inhibit

adenylate cyclase in adipose and skeletal muscle tissue as well, which in turn may cause an inhibition of lipolysis (28). In this respect, a novel pathway controlling human adipose tissue lipolysis has been discovered recently. It was demonstrated that atrial natriuretic peptide (ANP) exerted a lipolytic effect via a cGMP-dependent pathway in human adipocytes (29). Interestingly, Ang II has been shown to counteract the ANP-stimulated cGMP synthesis in cultured podocytes (30) and glomerular mesangial cells (31). Therefore, it may be that Ang II inhibits lipolysis in adipose and skeletal muscle tissue by inhibiting the ANP-stimulated cGMP synthesis. Furthermore, Ang II has been shown to increase the intracellular Ca^{2+} concentration in pancreatic (32) and vascular smooth muscle cells (33). Taking into account the finding that increasing intracellular Ca^{2+} inhibited lipolysis (21, 22), it may be that the Ang II-induced antilipolytic effect in adipose and skeletal muscle tissue observed in the present study was caused by an increase in the intracellular Ca^{2+} concentration in these tissues.

Role of Ang II in obesity

The Ang II-induced effects on adipose and skeletal muscle tissue blood flow and lipolysis are not significantly different in obese and normal-weight subjects. However, the tendency toward a small difference in tissue blood flow and lipolysis between groups warrants further research to substantiate these results. Because there is evidence for the existence of a local RAS in adipose tissue (3), it is tempting to speculate that a higher Ang II formation in adipose tissue of obese subjects may contribute to fat accumulation in adipose and skeletal muscle tissue in these individuals. Interestingly, fat accumulation in skeletal muscle is negatively associated with insulin sensitivity in obese and type 2 diabetic subjects (34, 35). However, further studies are necessary because even if Ang II production in adipose tissue is elevated in obese subjects, it may be that higher Ang II concentrations lead to down-regulation of Ang II responsiveness. Furthermore, Ang II may be, at least in part, responsible for the reported lower basal adipose tissue blood flow in obese compared with lean subjects (9, 36–38) and may explain the significantly increased basal adipose tissue blood flow observed after weight reduction (9).

Conclusion

The present data suggest that Ang II stimulation *in situ* decreases local blood flow in a dose-dependent manner and inhibits lipolysis in both adipose and skeletal muscle tissue. These effects were not significantly different in obese and normal-weight subjects both in tissues. Furthermore, our data suggest that the vasodilator hydralazine exerts a lipolytic effect in skeletal muscle and adipose tissue, although this effect is less clear in the latter tissue.

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