Disclosure of Financial Relationships

Judith A. Miller, MD, FRCP(C), MSc, MHSc

NO, neither I nor my spouse/partner have anything to disclose.

Sex Differences in Renin Angiotensin System Function in Humans

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Outline

- Introduction
- Impact of estrogen on the RAS
- Sex differences in RAS function
- Diabetes and RAS function
- OC impact on RAS function
- Summary and Conclusions

Renin Angiotensin System

The key system for controlling blood pressure and pressure-natriuresis

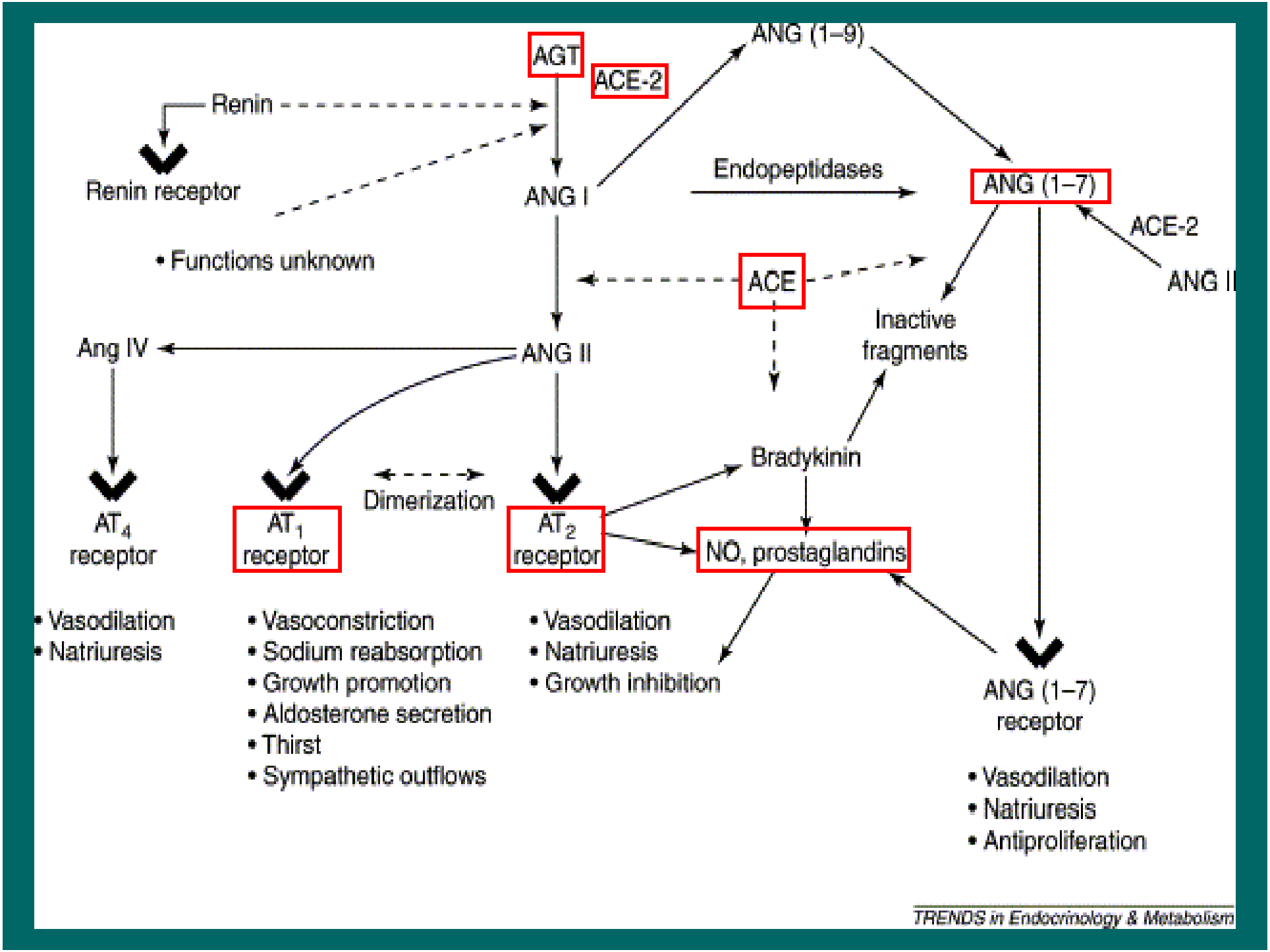
- Hemodynamic Effects
  - ↑ BP through systemic vasoconstriction
  - ↑ Pgc through $R_E > R_A$
- Mitogenic Effects
  - promotes mesangial matrix deposition
  - regulates TGF-$ß_1$ production

Sex-Differences in RAS Function

- A promoter region in the angiotensinogen gene is responsive to estrogen

  (Gordon MS et al: J Hypertens 10:361-366, 1992)

- Exogenous estrogen raises plasma, hepatic and renal concentrations of angiotensinogen


AGT
ACE-2
ANG (1–9)
Renin
Renin receptor
• Functions unknown
ANG I
Endopeptidases
ANG (1–7)
ACE-2
ANG II
ACE
Inactive fragments
Ang IV
ANG II
Bradykinin
Dimerization
AT4 receptor
AT1 receptor
AT2 receptor
NO, prostaglandins
• Vasodilation
• Natriuresis
• Vasoconstriction
• Sodium reabsorption
• Growth promotion
• Aldosterone secretion
• Thirst
• Sympathetic outflows
• Vasodilation
• Natriuresis
• Growth inhibition
ANG (1–7) receptor
• Vasodilation
• Natriuresis
• Antiproliferation
TRENDS in Endocrinology & Metabolism

Bi-directional effects of estrogen on RAS function

- ⇑ angiotensinogen expression in liver, kidney
- ⇑ renin activity
- ⇓ ACE-1 activity; ⇑ ACE-2 activity
- ⇓ Ang II-induced aldosterone release
- Downregulates Ang II type 1 receptor
- Upregulates Ang II type 2 receptor
- Induces synthesis of Ang 1-7
- ⇑ activity of vasodilator counter-regulatory pathways (NO, COX)

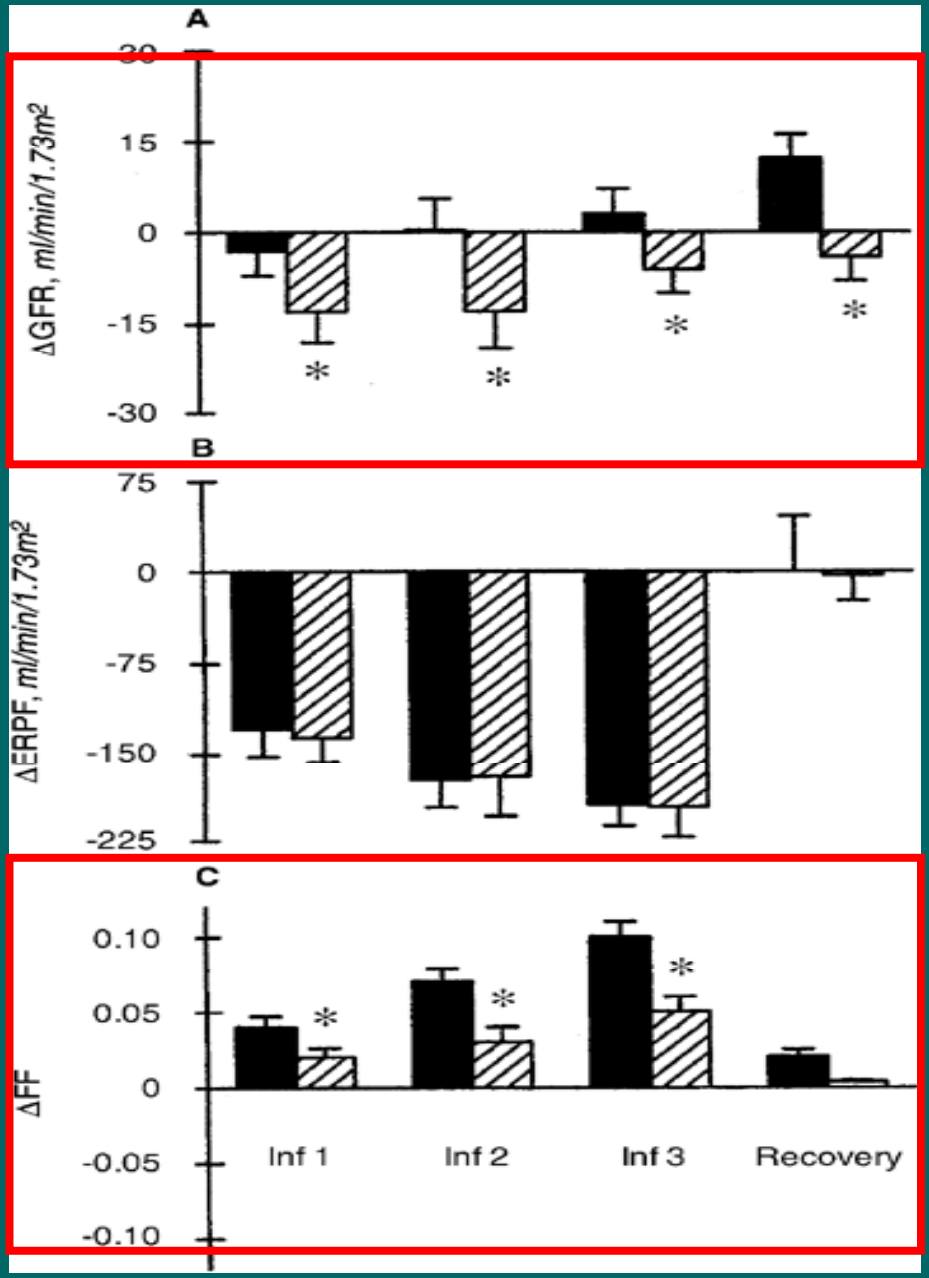


Fig. 1 . Change in the glomerular filtration rate (ΔGFR), renal plasma flow (ΔERPF) and filtration fraction (ΔFF) in response to Ang II infusion at 0.5 ng/kg/min (Inf 1), 1.5 ng/kg/min (Inf 2), 2.5 ng/kg/min (Inf 3), and at recovery in men (clear bars) and women (hatched bars)

Miller et al: Kidney Int 55:278-85, 1999

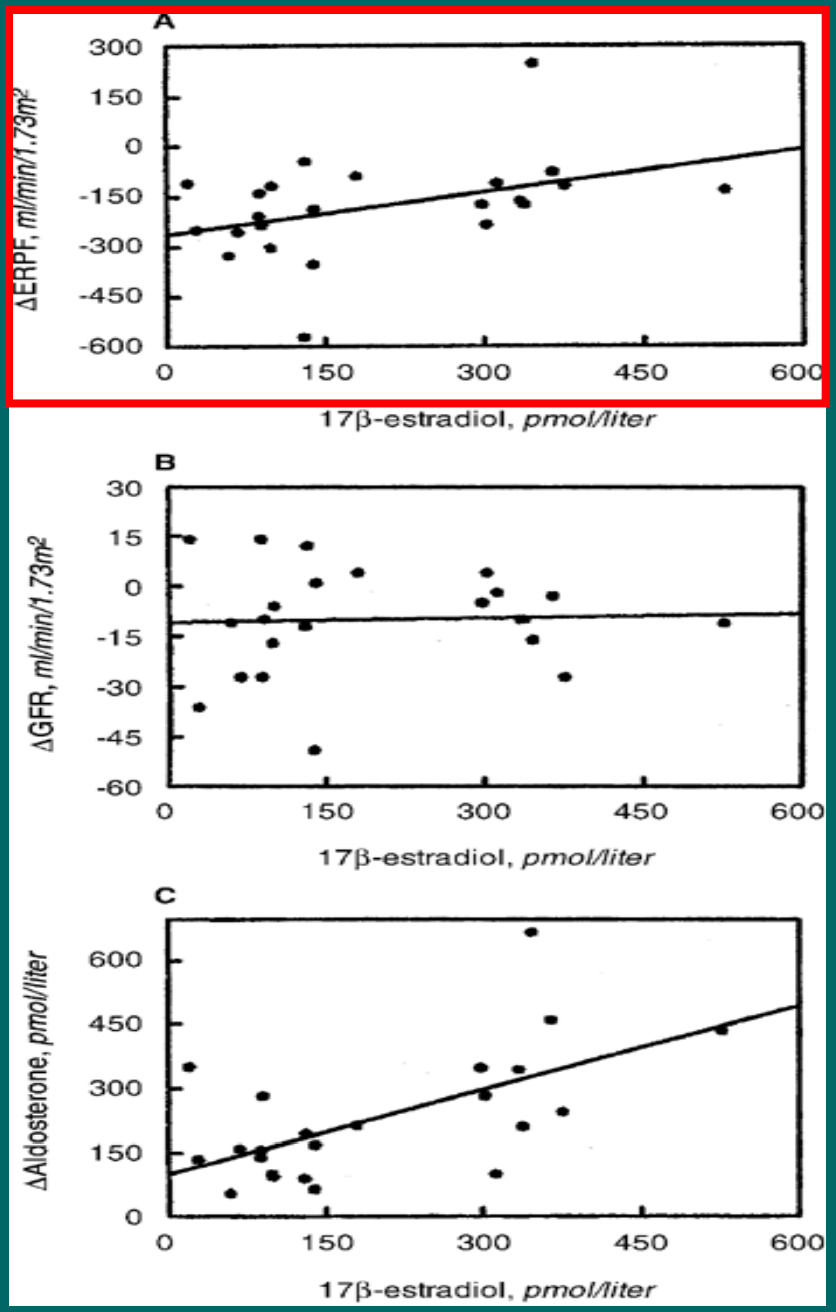


Fig.3. Relationship between 17ß -estradiol plasma concentrations and change in renal plasma flow (ΔERPF; *P* = 0.03, *r* = 0.39), glomerular filtration rate (ΔGFR; *P* = 0.77, *r* = 0.05), and aldosterone ΔAldosterone; *P* = .008, *r* = 0.49), in response to Ang II infusion (1.5 ng/kg/min).

Miller et al: Kidney Int 55:278-85, 1999

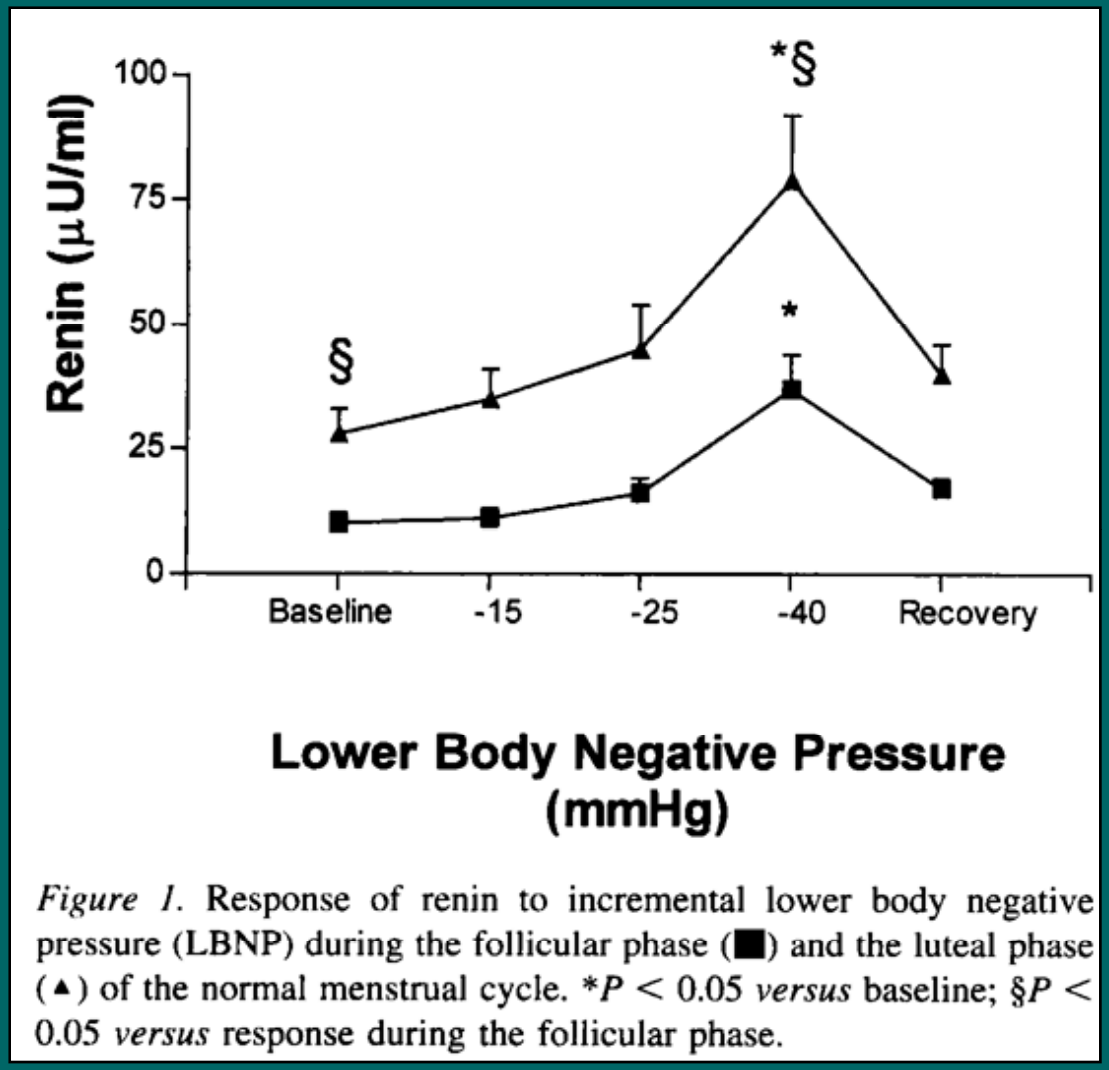


Figure 1. Response of renin to incremental lower body negative pressure (LBNP) during the follicular phase (■) and the luteal phase (▲) of the normal menstrual cycle. *$P < 0.05$ *versus* baseline; §$P < 0.05$ *versus* response during the follicular phase.

Response of Renin to incremental LBNP during the follicular phase compared to the luteal phase of the menstrual cycle in young healthy women

Demonstrates that the baseline level and the response are both augmented rather than blunted in the luteal phase.

Chidambaram M et al: J Am Soc Nephrol 13:446-452, 2002

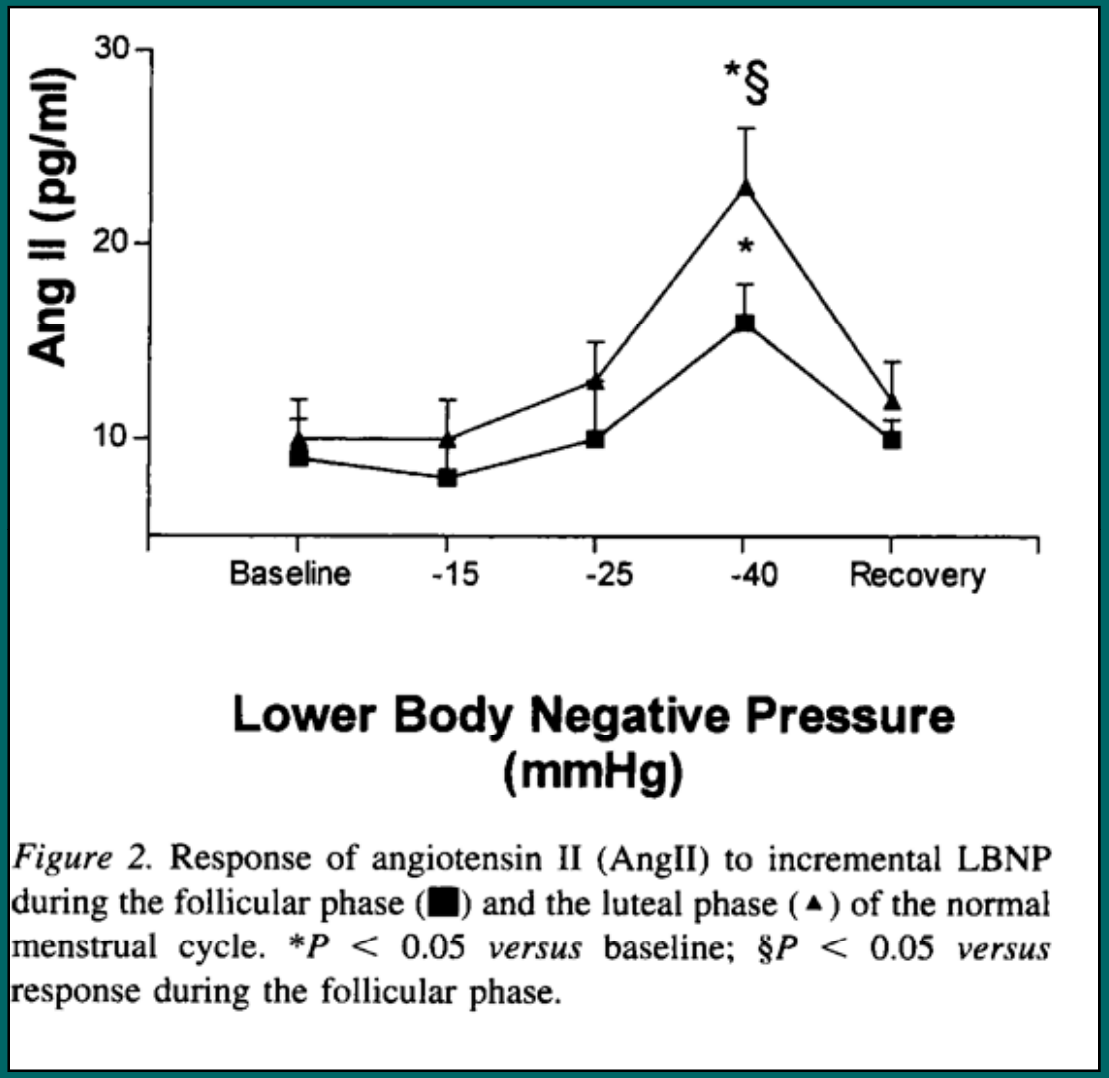


Figure 2. Response of angiotensin II (AngII) to incremental LBNP during the follicular phase (■) and the luteal phase (▲) of the normal menstrual cycle. *$P < 0.05$ *versus* baseline; §$P < 0.05$ *versus* response during the follicular phase.

Response of Ang II to incremental LBNP during the follicular phase compared to the luteal phase of the menstrual cycle in young healthy women.

Demonstrates an augmented response in the luteal phase.

Chidambaram M et al: J Am Soc Nephrol 13:446-452, 2002

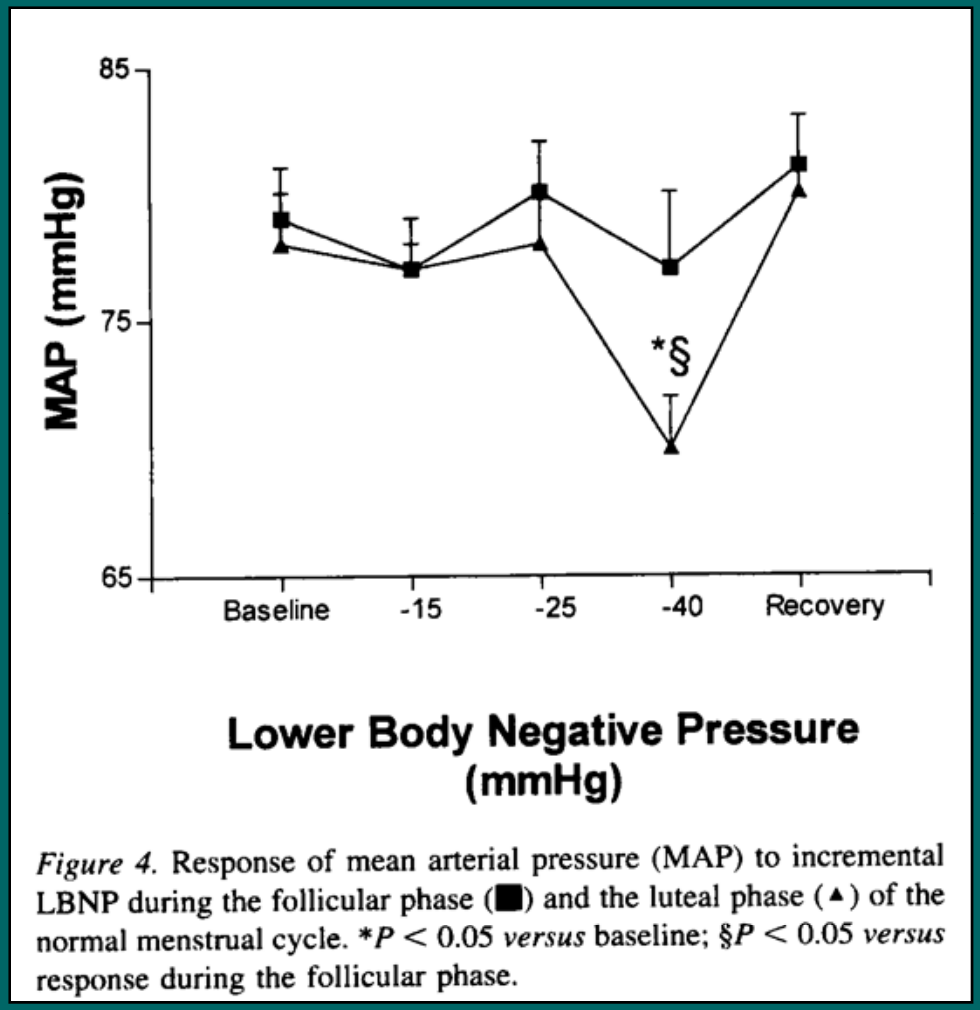


Figure 4. Response of mean arterial pressure (MAP) to incremental LBNP during the follicular phase (■) and the luteal phase (▲) of the normal menstrual cycle. $*P < 0.05$ *versus* baseline; $\S P < 0.05$ *versus* response during the follicular phase.

Response of Mean Arterial Pressure to incremental LBNP during the follicular phase compared to the luteal phase of the menstrual cycle in young healthy women

Demonstrates a depressor response in spite of augmented RAS components in the luteal phase.

Chidambaram M et al: J Am Soc Nephrol 13:446-452, 2002

Controlled diet X 7 days

⇓

Graded Ang II

⇓

Irbesartan 75 mg od X 2 weeks

⇓

Graded Ang II

⇓

Irbesartan 75 mg od X 2 weeks

⇓

Graded Ang II

⇓

Irbesartan 150 mg od X 2 weeks

⇓

Graded Ang II

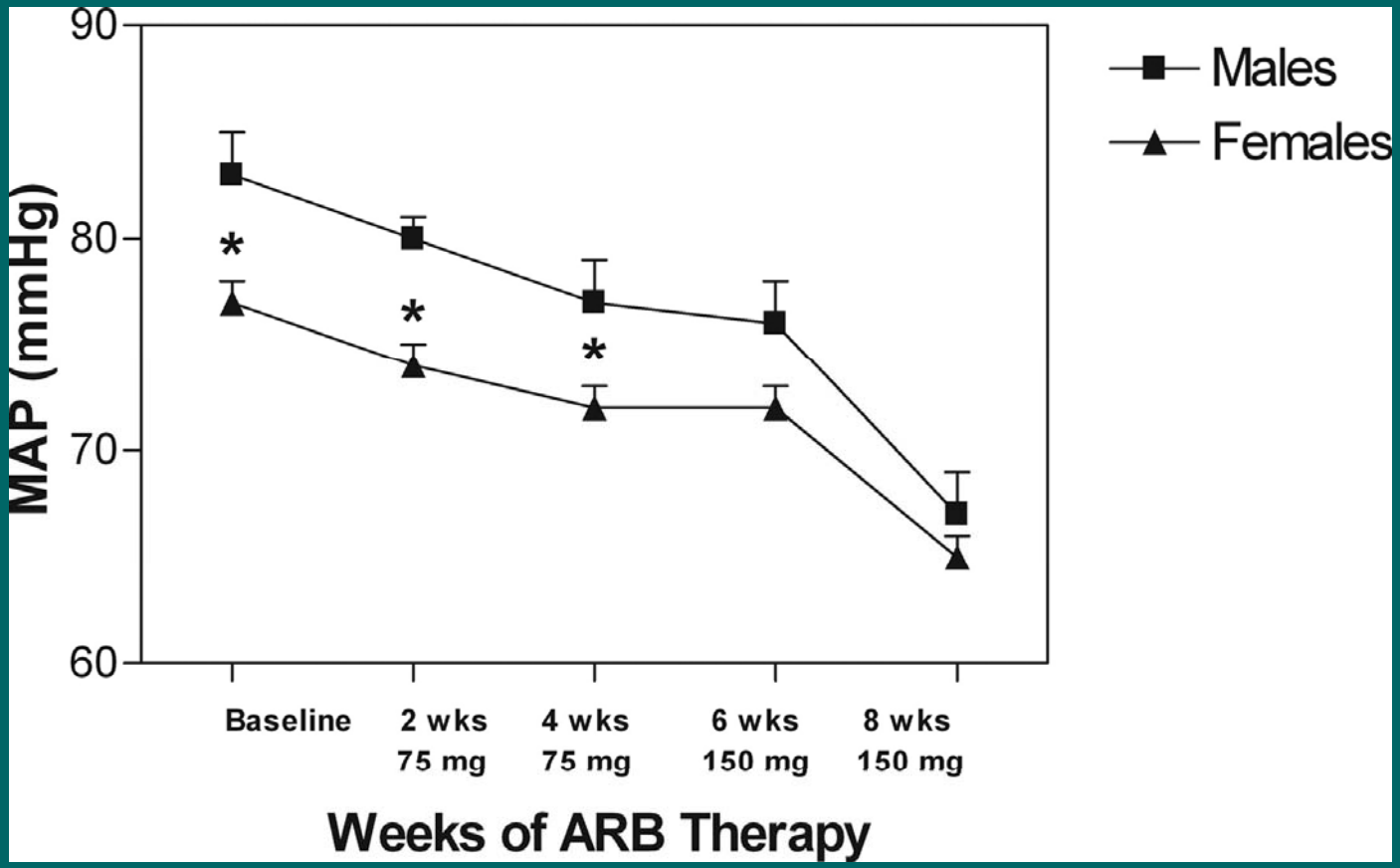
⇓

Irbesartan 150 mg od X 2 weeks

⇓

Graded Ang II

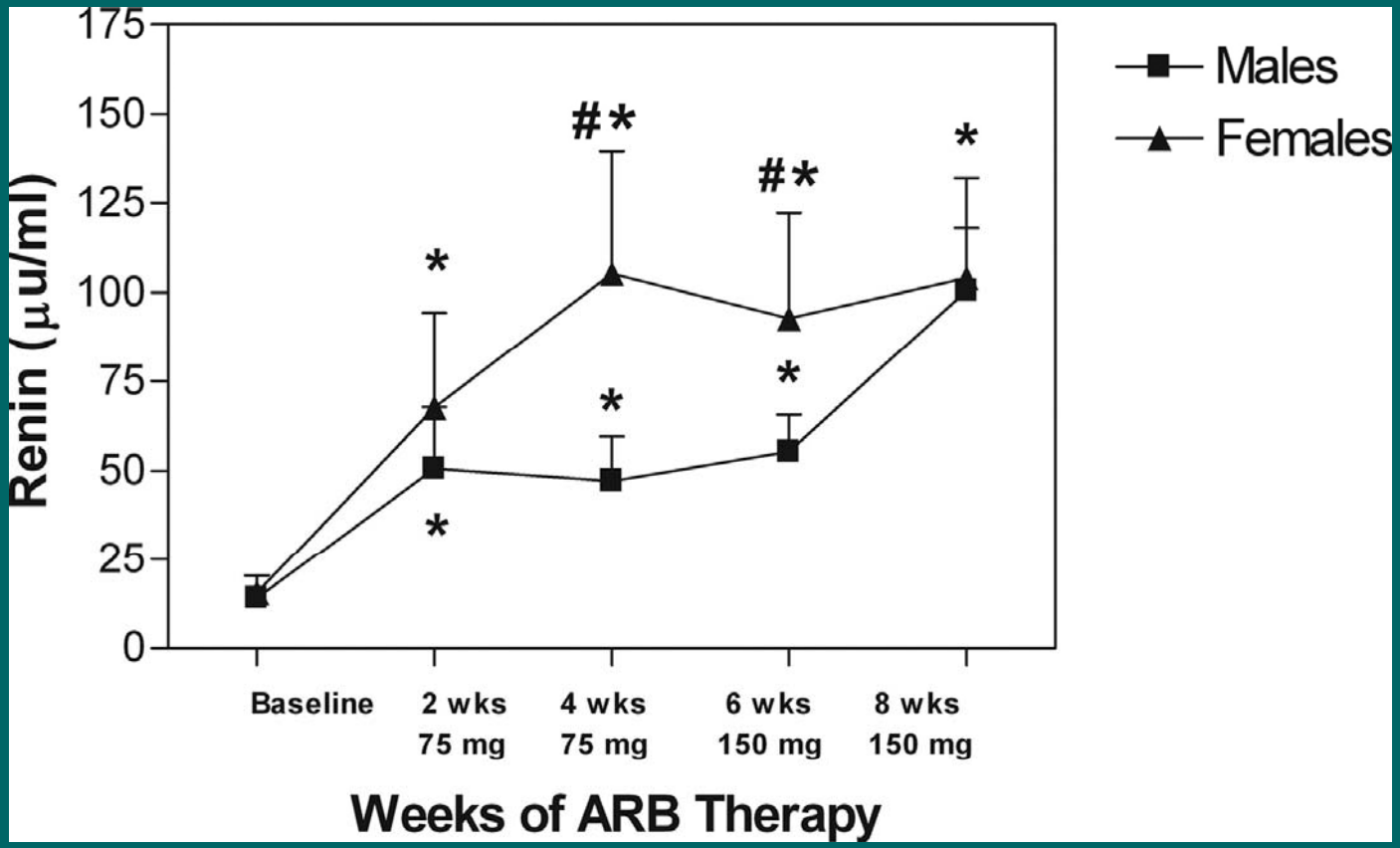
Figure 1. Mean arterial pressure at baseline and in response to incremental angiotensin II (AngII) receptor blockers (ARB) in men and women at 75 mg at 2 and 4 wk and at 150 mg at 2 and 4 wk



Miller, J. A. et al. J Am Soc Nephrol 2006;17:2554-2560

JASN

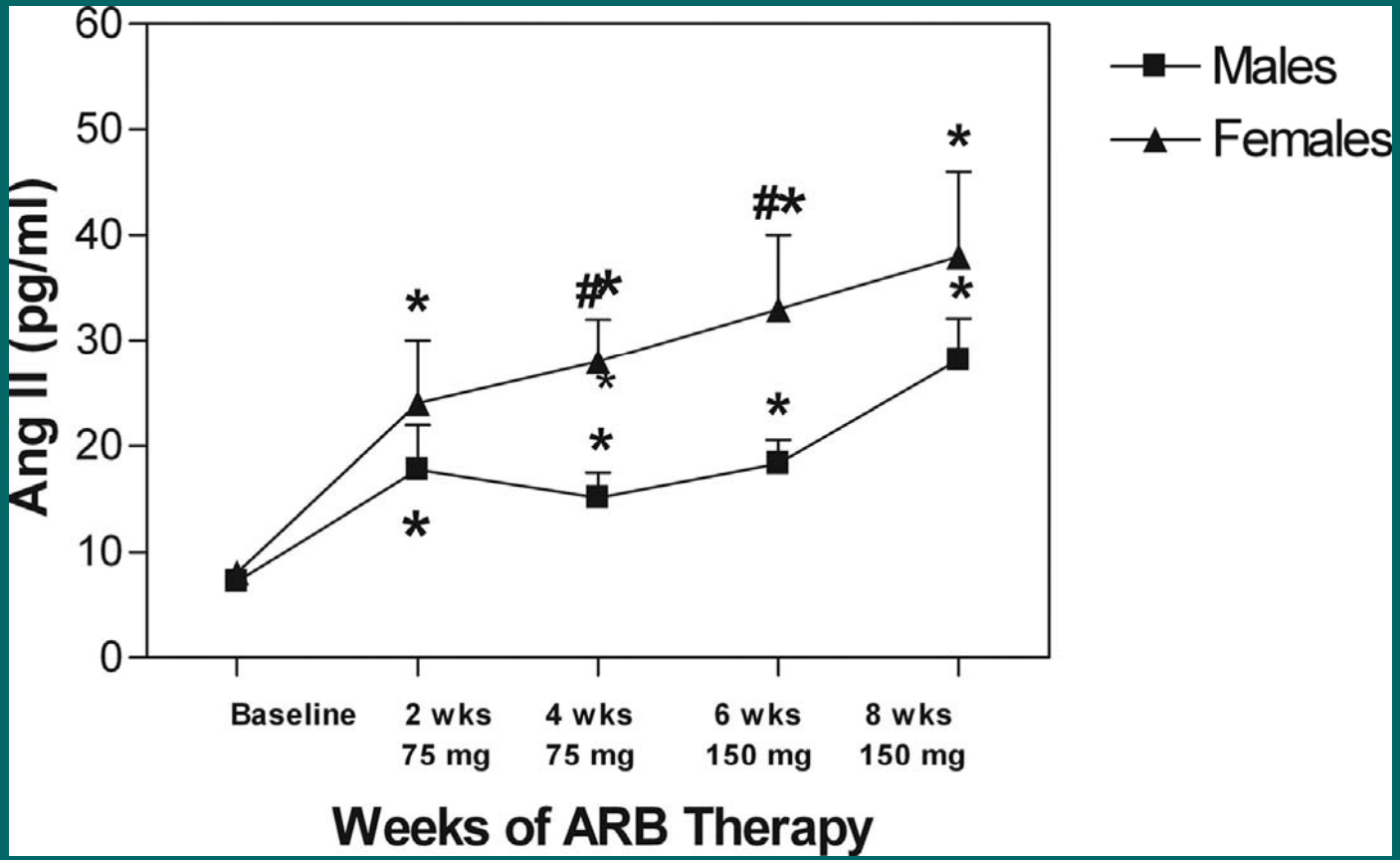
Figure 2. Plasma renin concentration at baseline and in response to incremental ARB in men and women at 75 mg at 2 and 4 wk and at 150 mg at 2 and 4 wk



Miller, J. A. et al. J Am Soc Nephrol 2006;17:2554-2560

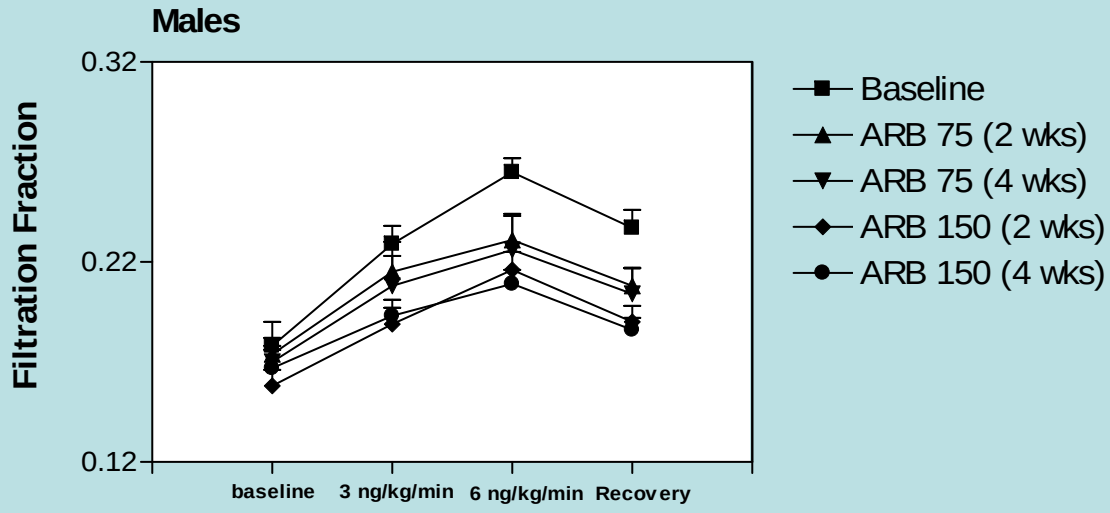
JASN

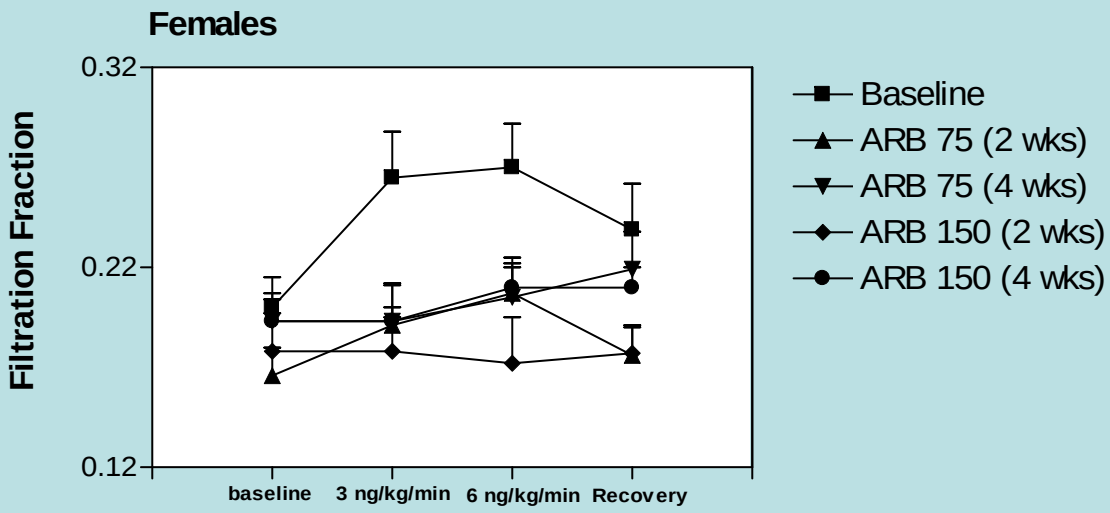
Figure 3. Plasma AngII at baseline and in response to incremental ARB in men and women at 75 mg at 2 and 4 wk and at 150 mg at 2 and 4 wk

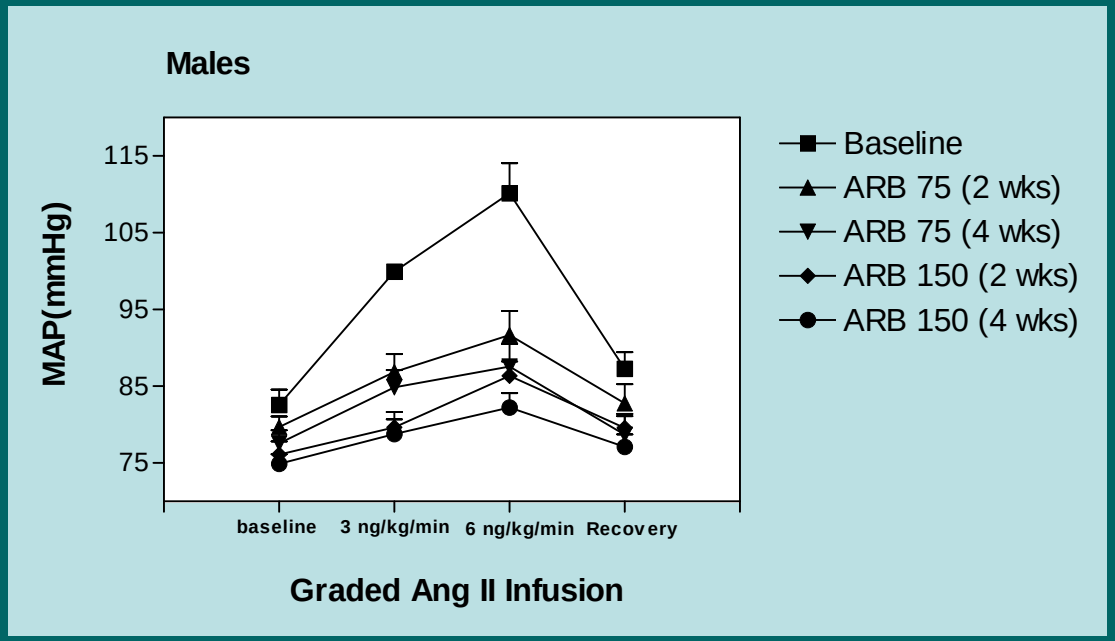


Miller, J. A. et al. J Am Soc Nephrol 2006;17:2554-2560

JASN


Males
Filtration Fraction
0.32
0.22
0.12
baseline
3 ng/kg/min
6 ng/kg/min
Recovery
Graded Ang II Infusion
Baseline
ARB 75 (2 wks)
ARB 75 (4 wks)
ARB 150 (2 wks)
ARB 150 (4 wks)


Females
Filtration Fraction
0.32
0.22
0.12
baseline
3 ng/kg/min
6 ng/kg/min
Recovery
Graded Ang II Infusion
Baseline
ARB 75 (2 wks)
ARB 75 (4 wks)
ARB 150 (2 wks)
ARB 150 (4 wks)


Males
MAP(mmHg)
115
105
95
85
75
baseline
3 ng/kg/min
6 ng/kg/min
Recovery
Graded Ang II Infusion
Baseline
ARB 75 (2 wks)
ARB 75 (4 wks)
ARB 150 (2 wks)
ARB 150 (4 wks)

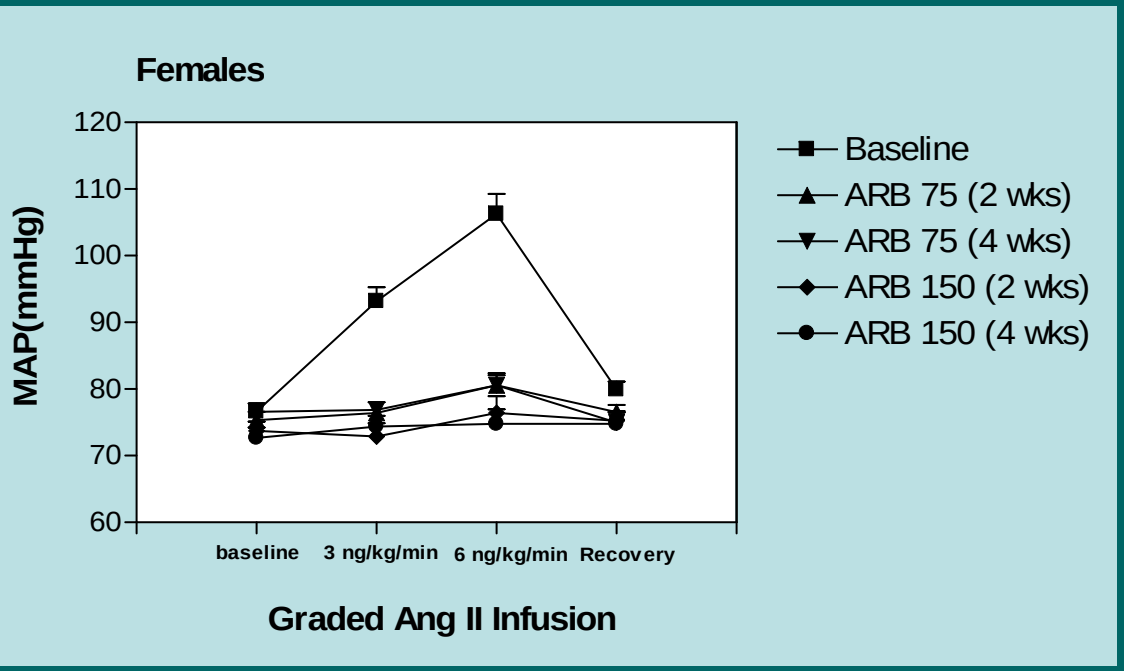
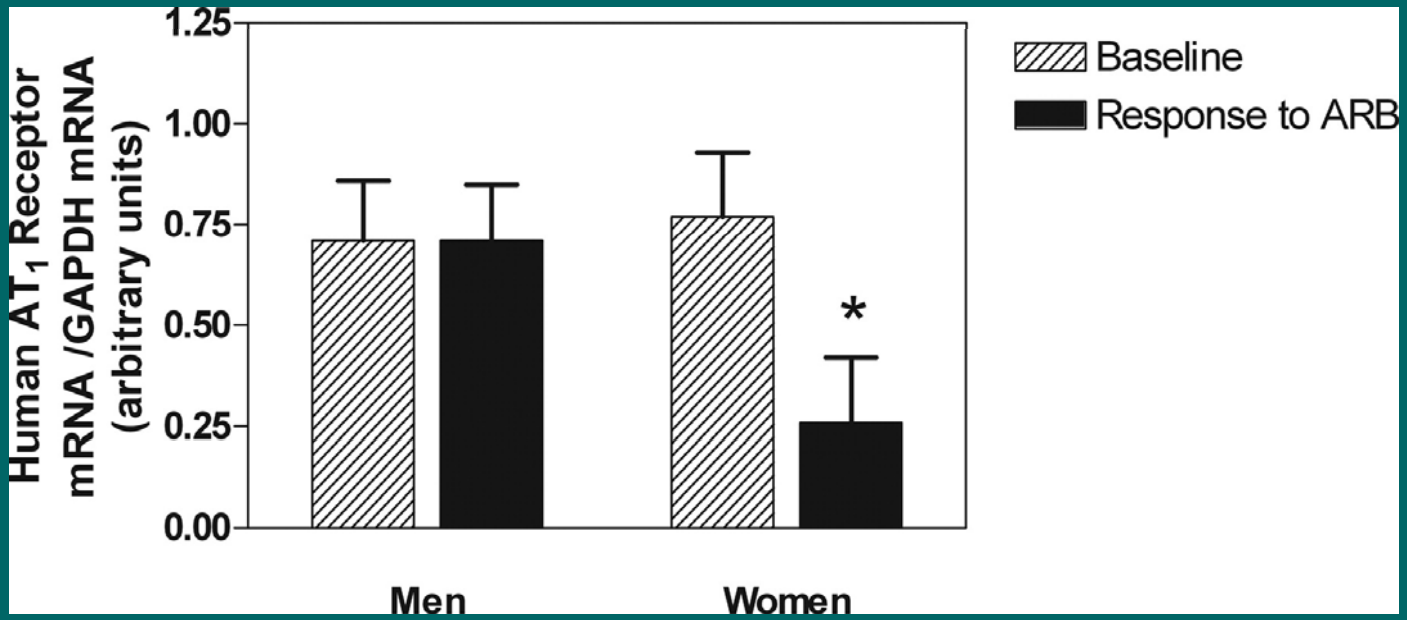

Females
MAP(mmHg)
120
110
100
90
80
70
60
baseline
3 ng/kg/min
6 ng/kg/min
Recovery
Graded Ang II Infusion
Baseline
ARB 75 (2 wks)
ARB 75 (4 wks)
ARB 150 (2 wks)
ARB 150 (4 wks)

Figure 4. AngII type 1 (AT1) receptor mRNA expression at baseline and after 8 wk of irbesartan in men and women



Miller, J. A. et al. J Am Soc Nephrol 2006;17:2554-2560

JASN

Summary of Results

- Sex-mediated differences in the renal response to Ang II exist, with high estrogen states resulting in blunting of vasoconstriction
- Circulating RAS components are elevated in the luteal high estrogen phase, yet there is discordance in the hemodynamic response, suggesting that high endogenous estrogen blunts the response to RAS activation
- There is sexual dimorphism in the response to RAS blockade, probably due to the estrogen effect.

Background

- Female sex confers protection against progression in non-diabetic proteinuric nephropathies
- Sex-mediated protection in diabetes mellitus is less certain

Impact of Sex on Progression of Diabetic Nephropathy

Reference	Method	Study population	Follow-up (years)	Population size	Outcome measurement	Worse outcome for males?[a]	Comments
Type I diabetes mellitus							
Mangili *et al.* [4]	X-sectional	SCr <1.5 Age 16–65	N/App	3636	Albuminuria	Yes	Multivariate analysis
Orchard *et al.* [5]	X-sectional	Mean age 25 years	N/App	657	Albuminuria	Yes	
Breyer *et al.* [6]	Prospective	SCr <2.5 mg/dl Upr >500 mg/day	3	409	Doubling of SCr or ESRD	No	Estimate of RR not reported
Coonrod *et al.* [7]	Prospective	Normal U_{alb}	2	256	Albuminuria	No	
Mulhauser *et al.* [8]	Prospective	Age 15–40 SCr <2.0	6	636	Albuminuria/SCr	No	Multivariate analysis
Jacobsen *et al.* [9•]	Prospective	Normotensive U_{alb} >200 μg/min	5.5	59	ΔGFR	Yes	Multivariate analysis
Type II diabetes							
Savage *et al.* [10]	X-sectional	Age 40–74	N/App	933	Albuminuria	Yes	Analysis of ABCD trial
Ravid *et al.* [11]	Prospective	Normotensive Norm. U_{alb}, SCr Age 40–60	7.8	574	Albuminuria/ΔSCr	Yes	
Ruggenenti *et al.* [12]	Prospective	Upr >500 mg/day Or SCr >1.4	1.8	65	Doubling of SCr or ESRD	No	$P = 0.15$

[a]Yes, males have higher prevalence/incidence of nephropathy or increased risk of progression of nephropathy.
ABCD, Appropriate Blood Pressure Control in Diabetes Trial; ESRD, end-stage renal disease; GFR, glomerular filtration rate; RR, relative risk; SCr, serum creatinine; Upr, urine protein excretion; U_{alb}, urine albumin excretion.

Seliger SL et al: Curr Opin Nephrol Hyper 10:219-225, 2001

Factors Associated with Development of Nephropathy

- Hyperfiltration
- Hyperglycemia $\rightarrow$ Hemodynamic Changes
- Hyperglycemia $\rightarrow$ Growth Factors
- Hypertension
- Genetic Predisposition
- Renin Angiotensin System
- Black, Aboriginal or Mexican-American

Factors Associated with Development of Nephropathy

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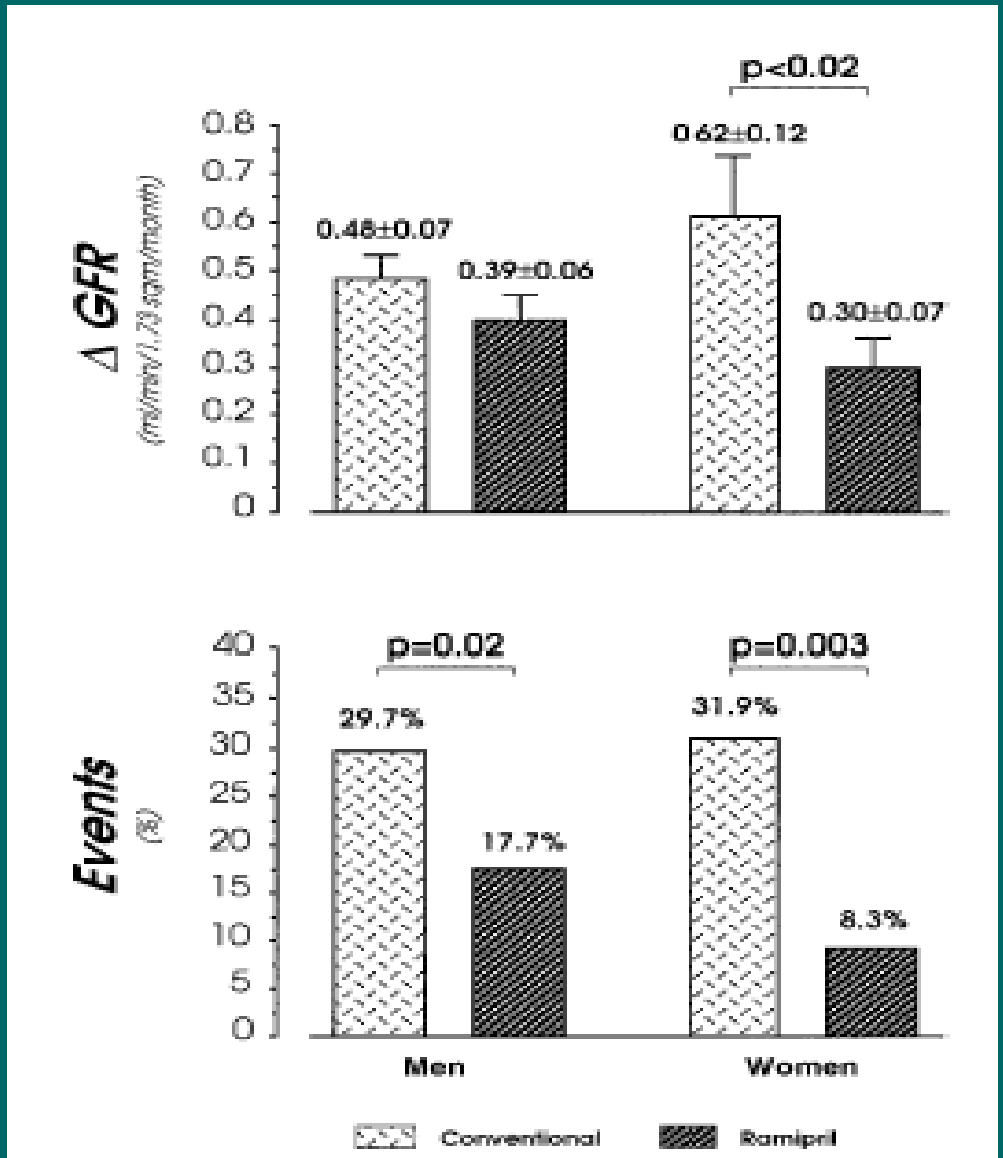
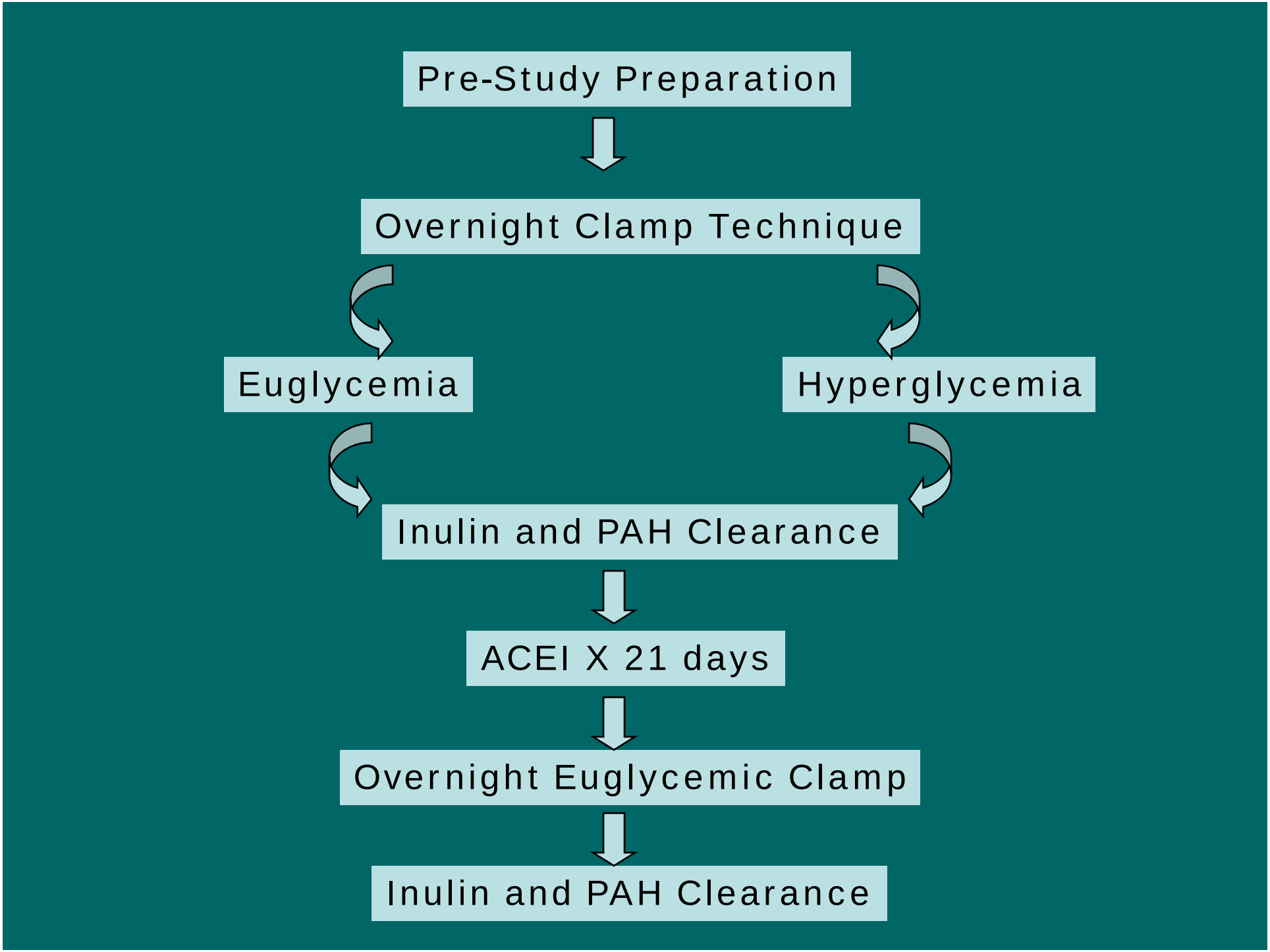


Figure 1. Rate of decline in GFR and incidence of end-stage renal disease (ESRD) (events) in the two treatment groups according to gender.

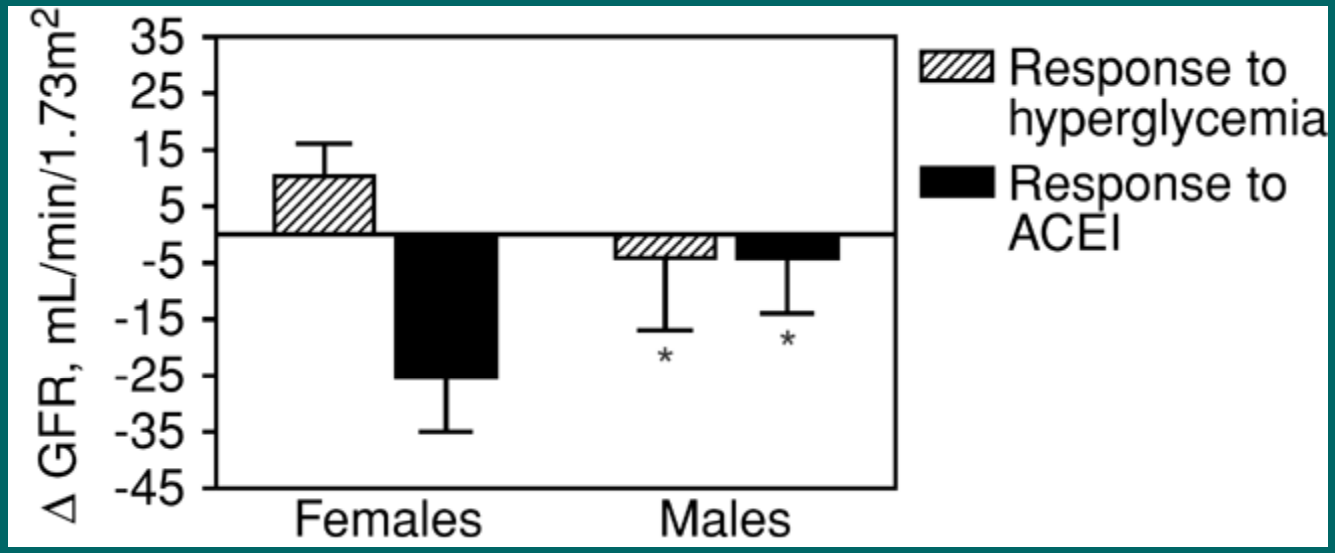
Ruggenenti P et al: J Am Soc Nephrol 11:88-96, 2000


Pre-Study Preparation
Overnight Clamp Technique
Euglycemia
Hyperglycemia
Inulin and PAH Clearance
ACEI X 21 days
Overnight Euglycemic Clamp
Inulin and PAH Clearance

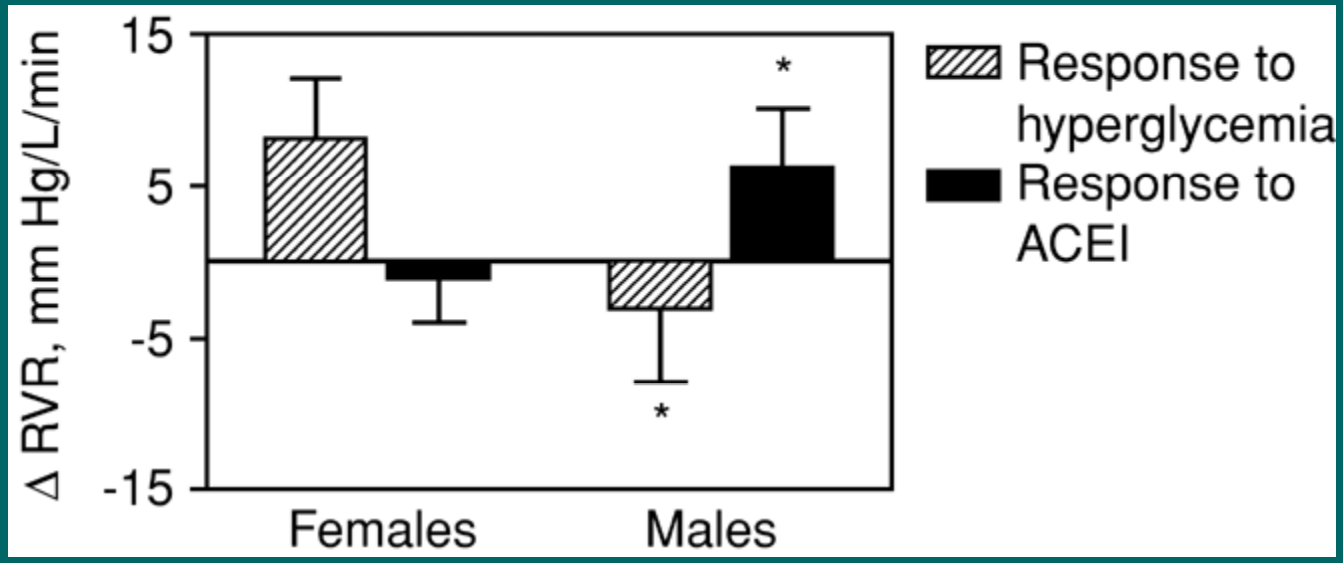
Hemodynamic Responses to ACEI

	Female			Male		
	Baseline	Post-ACEI	p	Baseline	Post-ACEI	p
MAP	76.9±7.5	71.4±6.4	0.0002	78.4±7.4	70.1±6.2	0.002
GFR	135.6±43.9	112.7±24.2	0.04	155.2±59.9	151.2±44.1	0.64
ERPF	647.6±122.7	639.2±134.5	0.55	796.7±206.2	777.7±150.6	0.58
FF	0.21±0.05	0.18±0.04	0.05	0.20±0.08	0.20±0.07	0.71
RBF	1039.9±171	1013.0±188	0.34	1339.6±373	1297.1±257	0.58
RVR	75.9±12.2	74.7±15.7	0.69	61.9±13.6	55.8±12.4	0.19

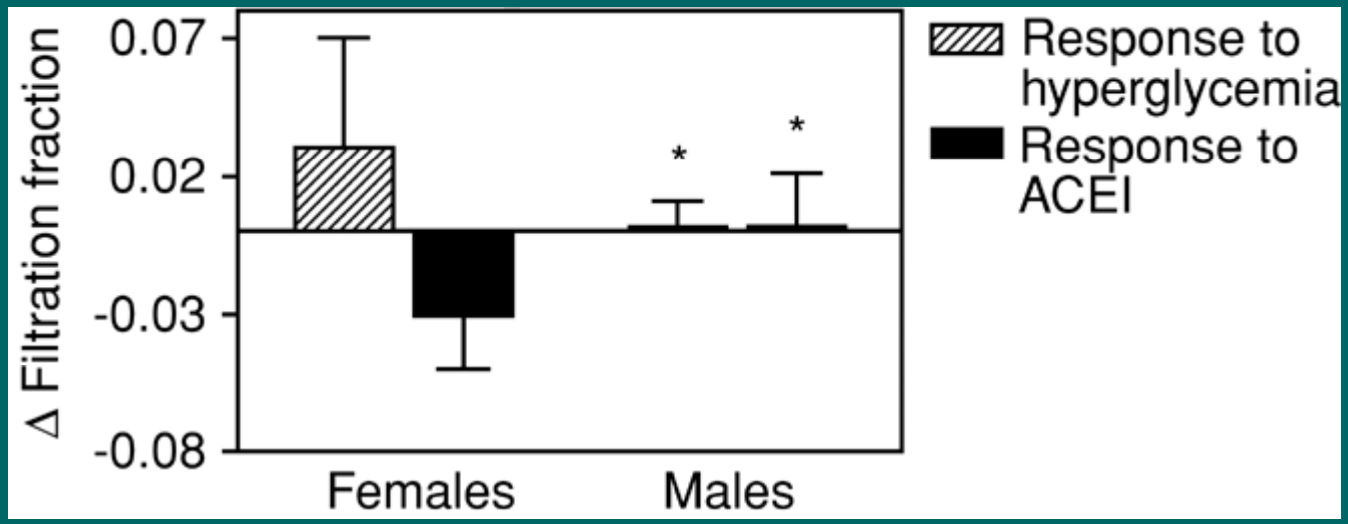
Sex Differences in Response to RAS Blockade



Sex Differences in Response to RAS Blockade



Sex Differences in Response to RAS Blockade

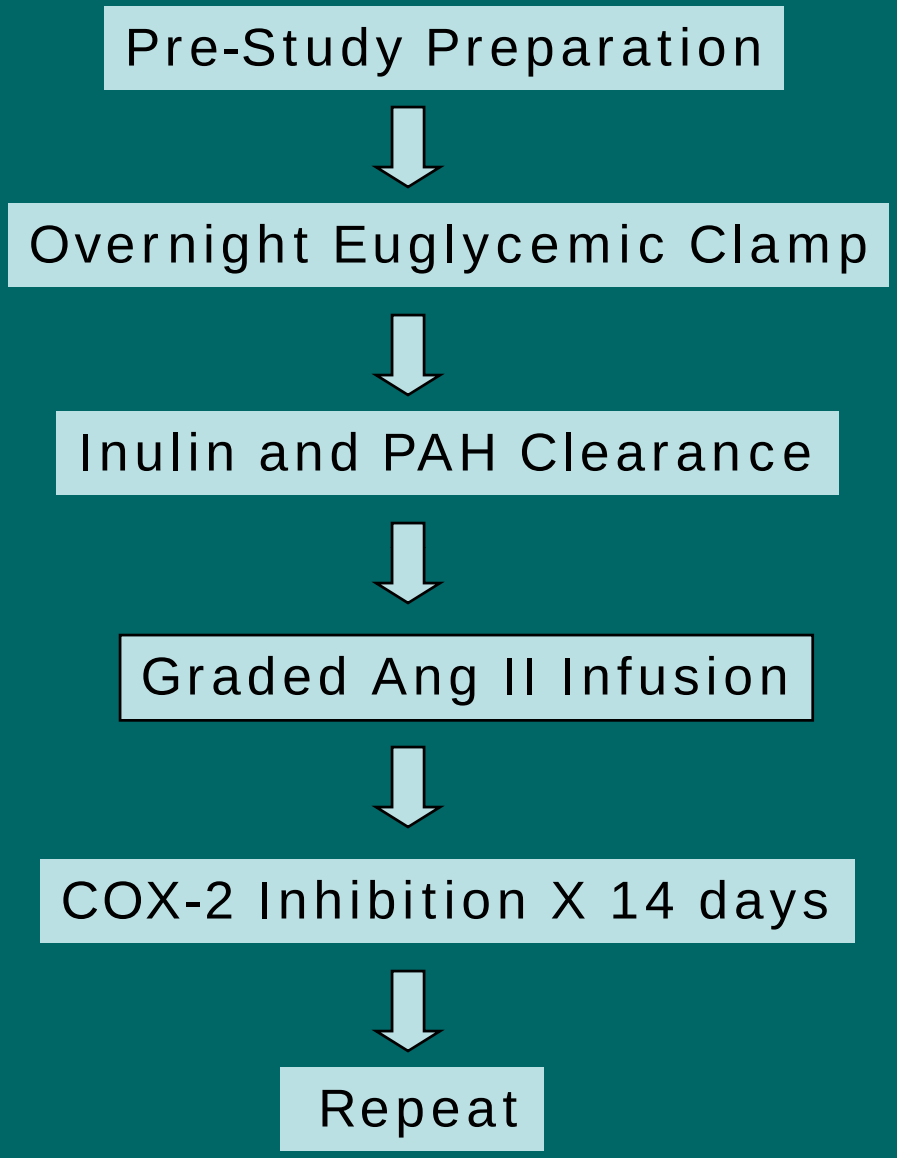


Major findings

- RAS blockade decreased GFR and FF in women compared to men, suggestive of increased efferent vasodilatation

Background

- COX-2 pathway has been implicated in diabetes-mediated renal hemodynamic abnormalities
- COX-2 derived vasodilatory prostaglandins play a more prominent role in female animals
- Are there sex-differences in intrarenal RAS-COX-2 interactions in diabetes?


Pre-Study Preparation
Overnight Euglycemic Clamp
Inulin and PAH Clearance
Graded Ang II Infusion
COX-2 Inhibition X 14 days
Repeat

Impact of Sex on the Responses to COX-2 Inhibition

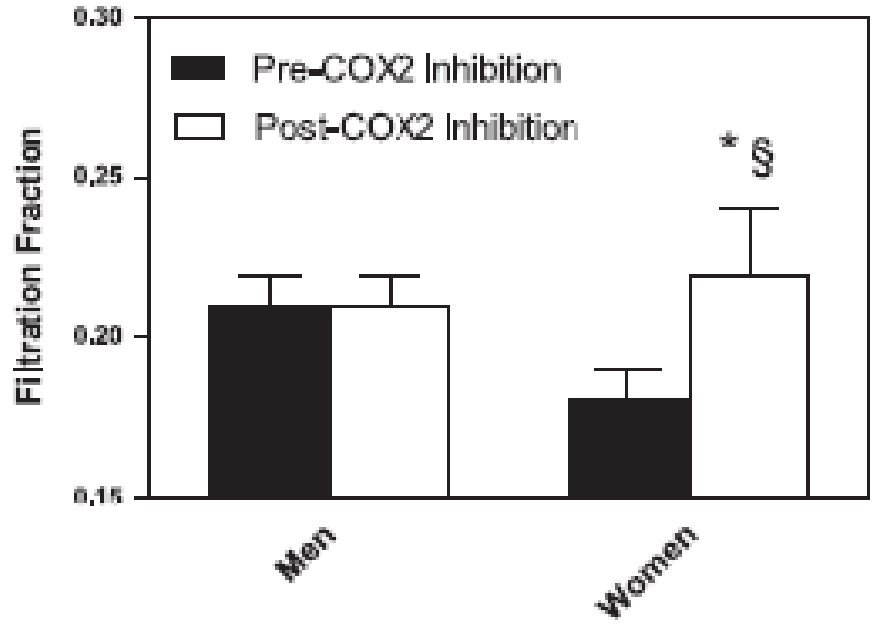


Fig. 1. Effect of cyclooxygenase 2 (COX2) inhibition on filtration fraction (FF) in men and women with type 1 diabetes mellitus (DM) (means ± SE). *$P \leq 0.05$ vs. baseline. §$P \leq 0.05$ vs. response in men.

Cherney D et al: Am J Physiol F1336-F1341, 2008

Impact of Sex on the RAS-COX-2 Interactions

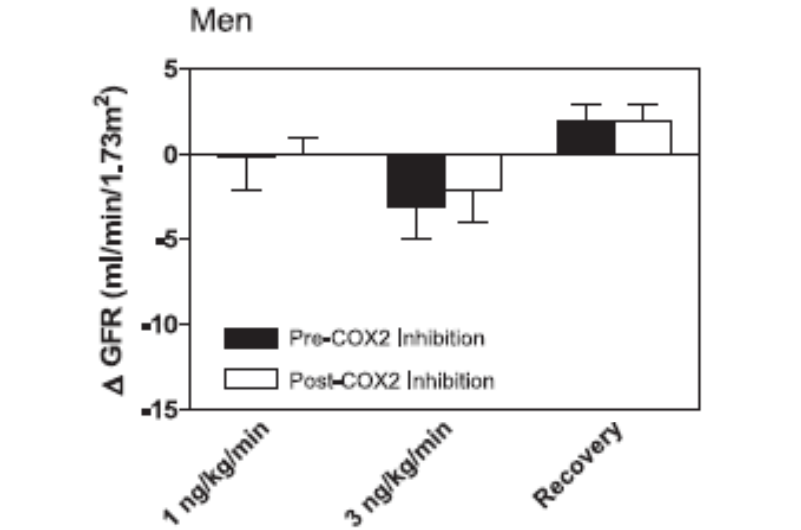


Fig. 2. Change in glomerular filtration rate (GFR; ml·min^{-1}·1.73 m^{-2}) in response to ANG II in men with type 1 DM (means ± SE).

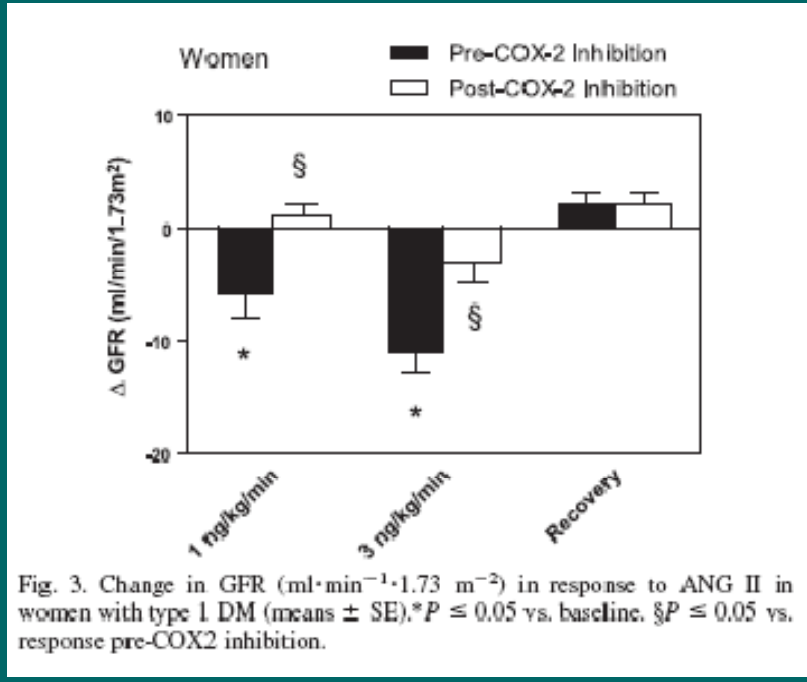


Fig. 3. Change in GFR (ml·min^{-1}·1.73 m^{-2}) in response to ANG II in women with type 1 DM (means ± SE).*$P \leq 0.05$ vs. baseline. §$P \leq 0.05$ vs. response pre-COX2 inhibition.

Cherney D et al: Am J Physiol F1336-F1341, 2008

Major Findings

- COX-2 inhibition resulted in:
  - A significant increase in daytime systolic blood pressure in women as measured by ABPM
  - A significant increase in FF and decline in RBF in women
  - Abolition of Ang II-mediated decline in GFR in women, suggesting removal of post-glomerular vasodilators, resulting in a similar pattern to men

Conclusions

- Findings are suggestive of a COX-2-mediated vasodilatory effect in women, operative at the systemic and the post-glomerular level
- The Ang II response is further indication of loss of post-glomerular vasodilators, resulting in renal vasoconstriction
- These findings confirm that women exhibit increased activity of COX-2 dependent factors in the renal and systemic vasculature compared to men, resulting in modulation of the impact of Ang II.

Background

- Previous studies have shown that estrogen supplementation results in improvement in renal injury in laboratory animals
- What is the evidence for this phenomenon in women ingesting exogenous estrogen?

17β-Estradiol supplementation reduces tubulointerstitial fibrosis by increasing MMP activity in the diabetic kidney

Richard W. Mankhey,[1] Corinne C. Wells,[1] Faizah Bhatti,[1] and Christine Maric[1,2]

Kidney International, Vol. 66 (2004), pp. 2148–2154

Estradiol reverses renal injury in Alb/TGF-β1 transgenic mice

JOEL BLUSH, JUN LEI, WENJUN JU, SHARON SILBIGER, JAMES PULLMAN, and JOEL NEUGARTEN

Divisio *Cardiovascular, Pulmonary and Renal Pathology* *Medicine, Bronx, New Y*

Differential Effects of Continuous and Intermittent 17β-Estradiol Replacement and Tamoxifen Therapy on the Prevention of Glomerulosclerosis

Modulation of the Mesangial Cell Phenotype in Vivo

Oral Contraceptive Use and Hormone Replacement Therapy Are Associated With Microalbuminuria

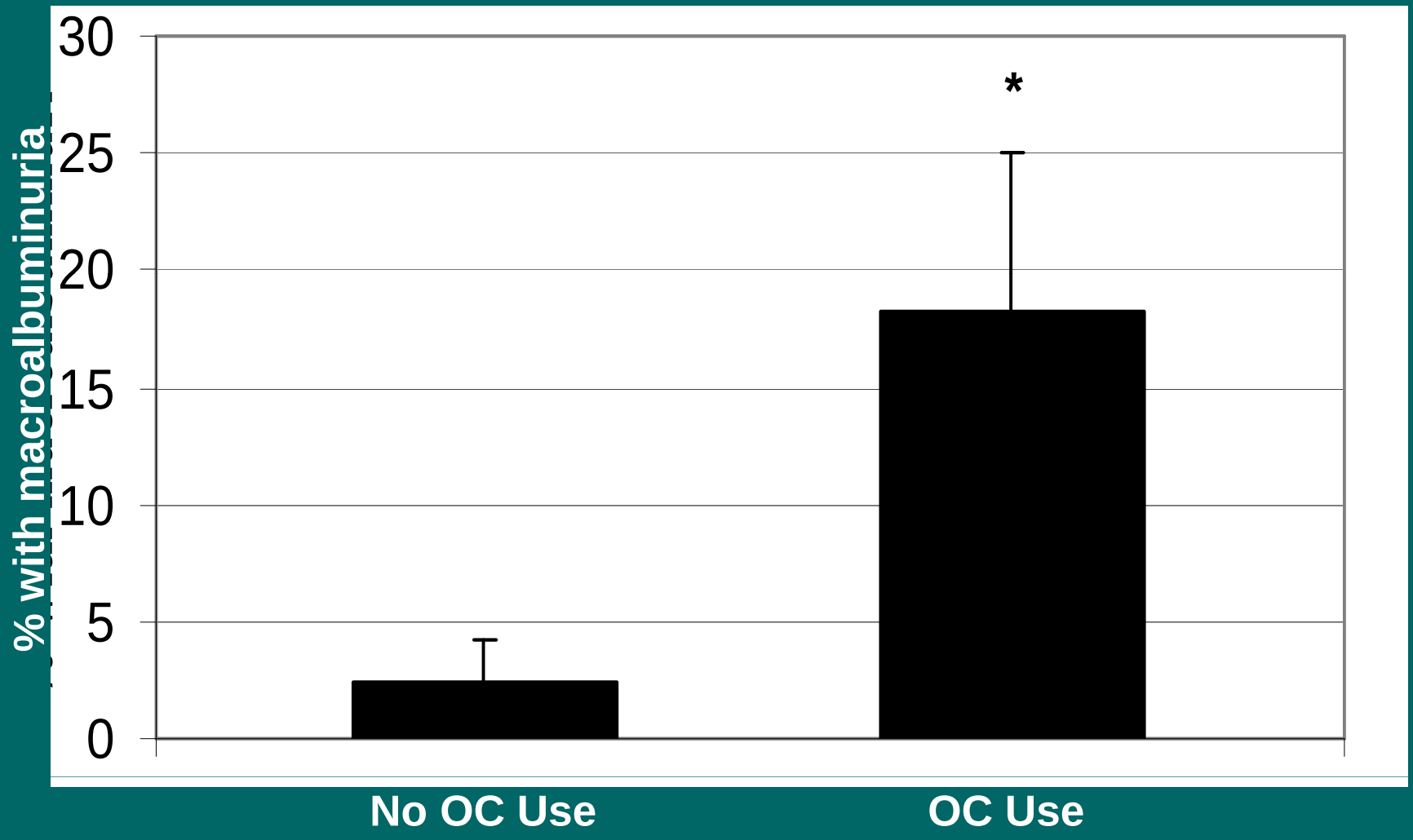
Taco B. M. Monster, MPharmSc; Wilbert M. T. Janssen, MD, PhD; Paul E. de Jong, MD, PhD;
Lolkje T. W. de Jong–van den Berg, MPharmSc, PhD;
for the Prevention of Renal and Vascular End Stage Disease Study Group

Table 3. Crude and Adjusted Odds Ratios (ORs) for Microalbuminuria*

	OR Crude	OR Adjusted†	OR Adjusted‡
Premenopausal, oral contraceptive use vs nonuse	1.60 (1.08-2.38)	1.87 (1.23-2.84)	1.90 (1.23-2.93)
Postmenopausal, hormone replacement therapy use vs nonuse	1.26 (0.73-2.17)	1.83 (1.03-3.25)	2.05 (1.12-3.77)

*Data are given as OR (95% confidence interval).
†Adjusted for age.
‡Adjusted for age, hypertension, diabetes, obesity, hyperlipidemia, and smoking.

OC Use and Diabetic Nephropathy



*p<0.005 compared to OC Nonuser.

Ahmed S et al: Diabetes Care, 2005

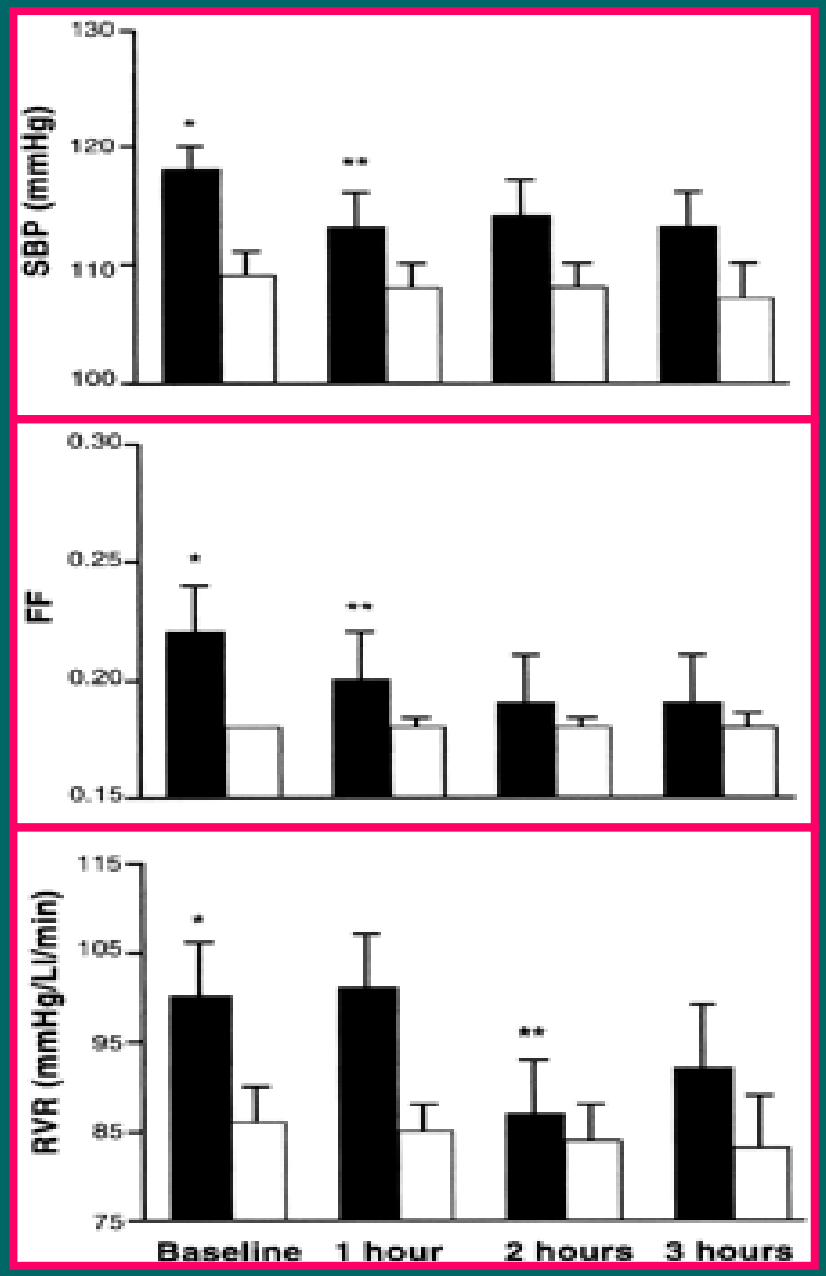
RAS Components (baseline)

PARAMETER	OC NON-USERS	OC-USERS
Angiotensinogen	1231±123	5098±156*
Plasma Renin Concentration	12±2	14±2
Angiotensin II	9±1	26±2*
Plasma Renin Activity	0.57±0.07	3.29±0.02*

Table 1. *Baseline characteristics*

Parameter	OC Users	OC Nonusers	*P*
Age, yr	24 ± 1	25 ± 1	NS
BMI, kg/m^2	23 ± 1	23 ± 1	NS
SBP, mmHg	118 ± 2	109 ± 2	< 0.002
MAP, mmHg	85 ± 2	82 ± 2	NS
$U_{Na}V$, mmol/day	187 ± 18	130 ± 7	< 0.01
Corrected $U_{Na}V$, mmol/kg	3 ± 0.25	2 ± 0.01	< 0.01
$U_{urea}V$, mmol/day	245 ± 24	288 ± 24	NS
Protein intake, g·kg^{-1}·day^{-1}	1 ± 0.06	1 ± 0.06	NS
Hct	0.352 ± 0.005	0.376 ± 0.007	< 0.01

Kang et al: Am J Physiol 280:R807-813, 2001



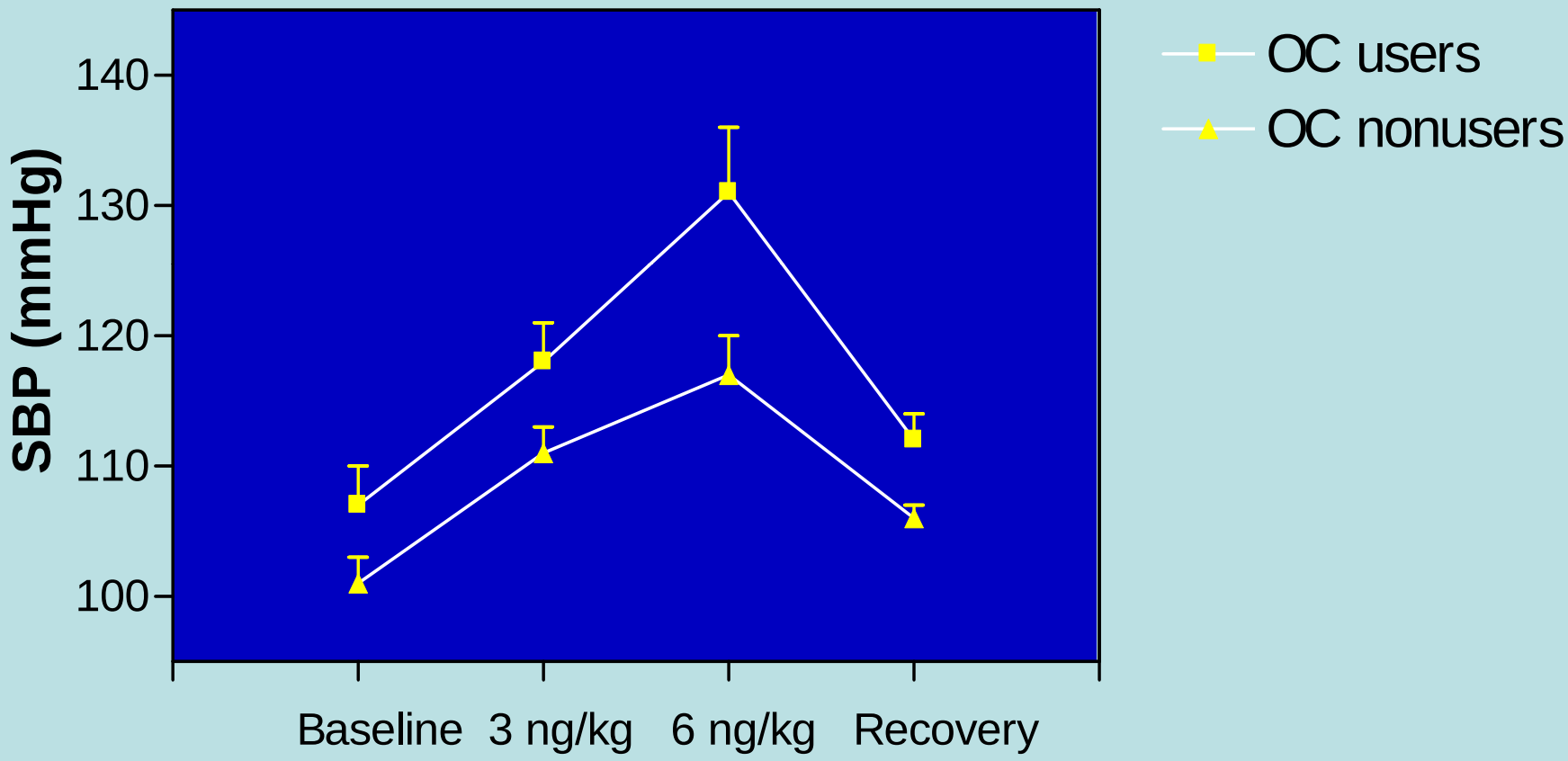
Demonstrates:

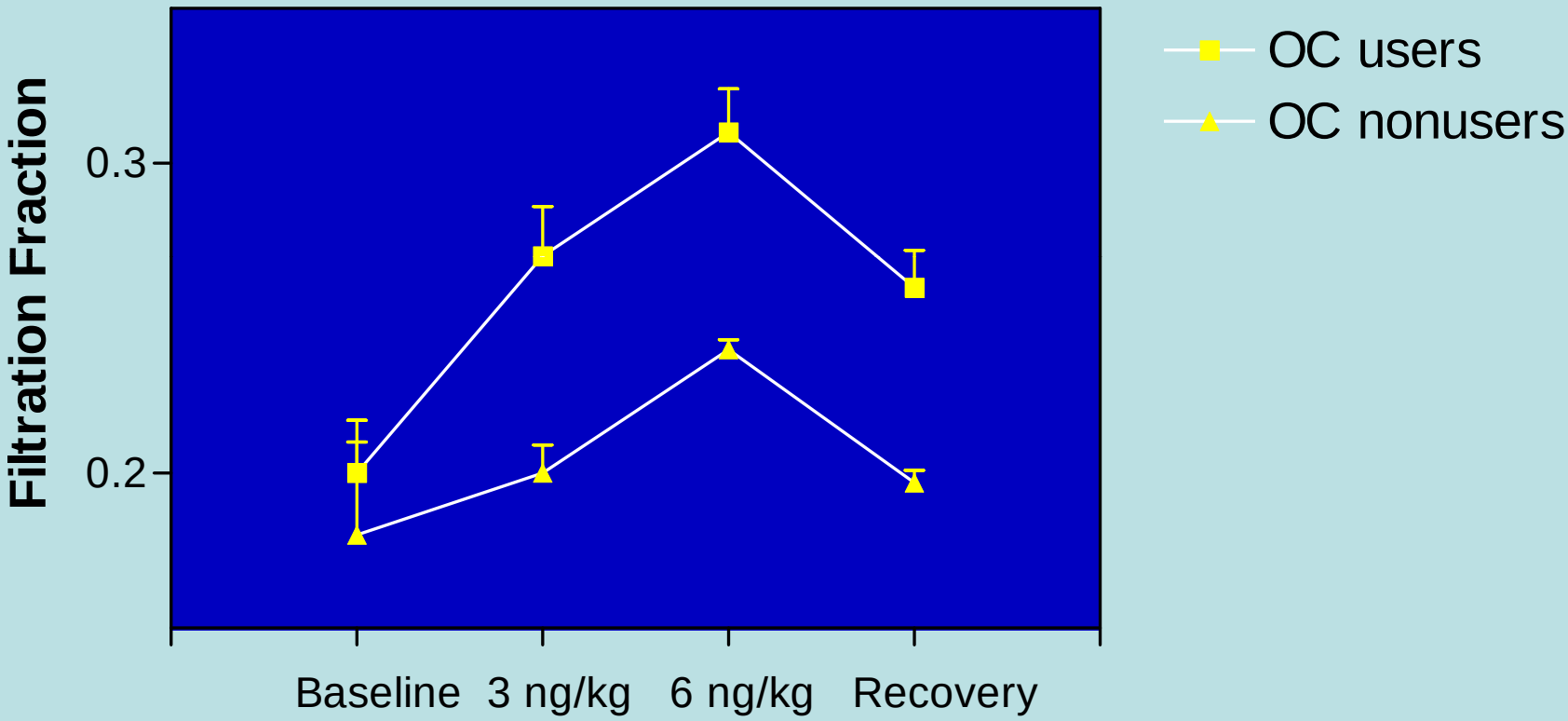
1) Significant baseline renal and peripheral hemodynamic differences exist between OC users vs non-users

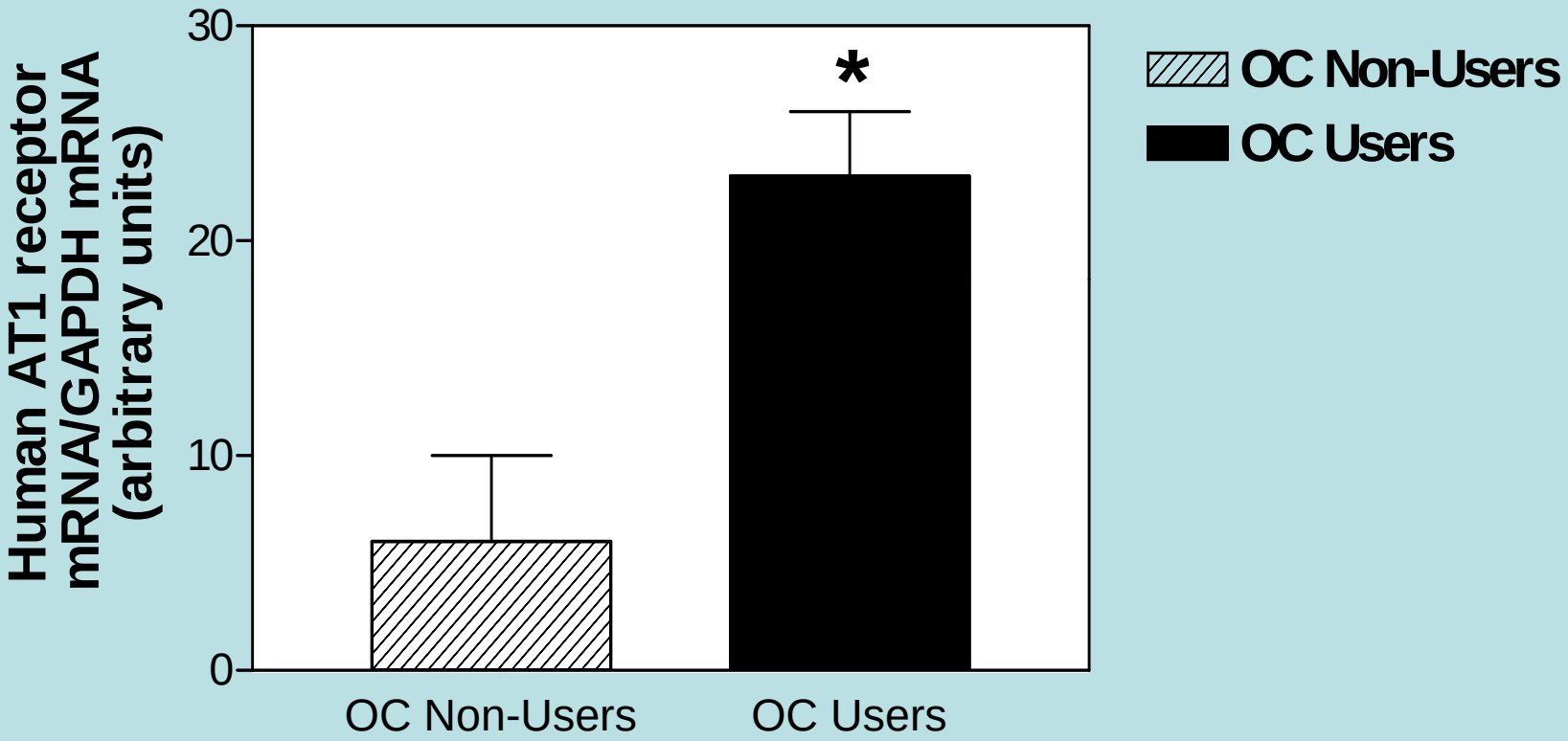
2) The response to RAS blockade is augmented in OC users

3) RAS is activated in OC users

Kang et al: Am J Physiol 280: R807-R813, 2001


SBP (mmHg)
140
130
120
110
100
Baseline
3 ng/kg
6 ng/kg
Recovery
OC users
OC nonusers


Filtration Fraction
0.3
0.2
Baseline
3 ng/kg
6 ng/kg
Recovery
OC users
OC nonusers



Ahmed et al: J Am Soc Nephrol. 15:780-786, 2004

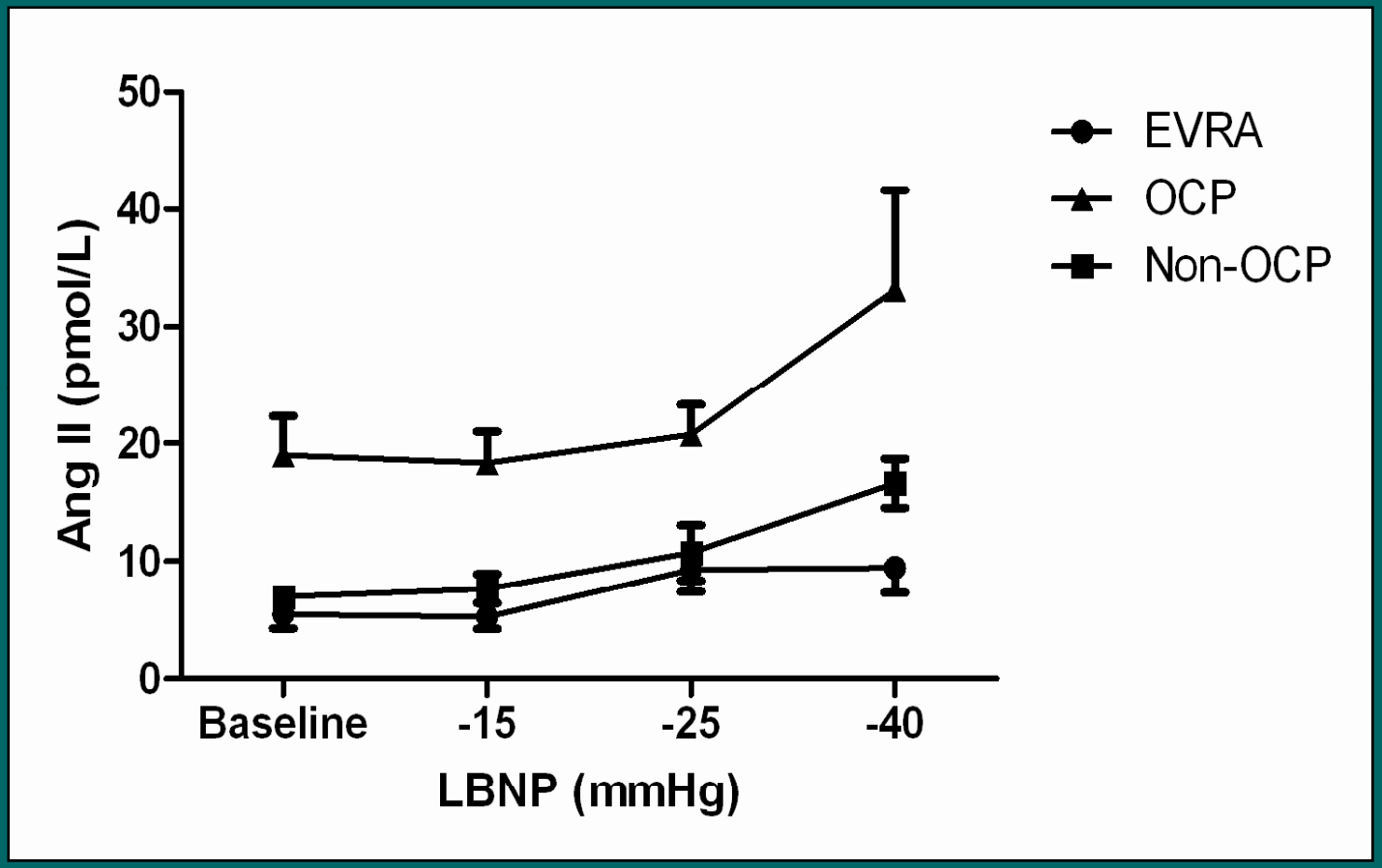
Conclusions

- OC usage results in ⇧ circulating RAS components and significant renal and peripheral hemodynamic changes indicative of RAS activation.
- OC users exhibited an augmented renal and peripheral hemodynamic response to Ang II
- Ang II Type 1 receptor expression was increased in OC users
- OC use results in RAS activation

Why?

- Endogenous vs exogenous
- Type of estrogen
  - Ethinyl estradiol vs 17ß estradiol
- Method of administration
  - Oral → first pass effect?
- Dose?

	EVRA	OCP	Non-OCP
Angiotensinogen (ng/mL)	2305±186	6268±491	1547±68
Renin (ng/L)	5.2±10.9	14.0±2.5	17.1±3.6
Angiotensin II (pmol/L)	6.0±1.3	19.0±3.3	7.0±0.8
Aldosterone (pmol/L)	238±48	287±51	140±19


50
40
30
20
10
0
Ang II (pmol/L)
Baseline
-15
-25
-40
LBNP (mmHg)
EVRA
OCP
Non-OCP

Summary and Conclusions

- Sex differences in RAS function exist
- The impact of endogenous estrogen on RAS function is bi-directional, resulting in blunting
- Sex differences exist in RAS function and RAS-COX-2 interactions in diabetes
- Exogenous estrogen (OC usage) results in RAS activation probably due to the first pass effect.
- OC-mediated RAS activation may be of clinical significance in women with diabetes, renal, cardiac, or vascular disease

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