Oral Contraceptives, Angiotensin-Dependent Renal Vasoconstriction, and Risk of Diabetic Nephropathy

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OBJECTIVE — Diabetes, the leading cause of end-stage renal disease in the U.S., is believed to involve activation of the renin angiotensin system (RAS) as a risk factor for nephropathy. RAS activation occurs in healthy women using oral contraceptives (OCs), but the effects of OC use on the diabetic kidney are unclear.

RESEARCH DESIGN AND METHODS — Renal plasma flow (RPF) response to captopril, as an index of RAS activity, was investigated in 92 women (41 nondiabetic OC nonusers, 10 nondiabetic OC users, 29 diabetic OC nonusers, and 12 diabetic OC users). Based on the hemodynamic findings, we examined the impact of OC use on the development of nephropathy as a post hoc analysis in an inception cohort of 114 female patients with newly diagnosed type 1 diabetes followed for a median of 20.7 years (range 1–24).

RESULTS — Nondiabetic OC nonusers showed minimal RPF vasodilator response to captopril (9 ± 10 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}, $P = 0.6$). In comparison, nondiabetic OC users showed a significant increase (69 ± 35 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}, $P = 0.02$) ($P = 0.04$ vs. nondiabetic OC nonusers). Diabetic OC nonusers demonstrated the anticipated vasodilator response (58 ± 12 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}, $P < 0.0001$). Diabetic OC users showed the largest responses (84 ± 12 ml $\cdot$ min^{-1} $\cdot$ 1.73 m^{-2}, $P = 0.002$) ($P = 0.04$ vs. diabetic OC nonusers). Plasma renin activity did not vary with OC use ($P = 0.3$). The RPF responses to captopril and angiotensin receptor blocker were highly correlated ($r = 0.72$, $P < 0.001$), suggesting clear involvement of the RAS. In the observational study, 18% (6/33 [95% CI 4.3–32.1]) of OC users developed macroalbuminuria compared with 2% (2/81 [0–5.9]) of OC nonusers ($P = 0.003$, univariate analysis). After adjustment for known risk factors with a Cox regression model, OC use remained a predictor for the development of macroalbuminuria (relative risk 8.90 [95%CI 1.79–44.36], $P = 0.008$).

CONCLUSIONS — The strong association of OC use with angiotensin-dependent control of the renal circulation and the development of macroalbuminuria suggest that OC use may be a risk factor for diabetic nephropathy. Large prospective studies are required to further investigate this relationship.

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Diabetes is the leading cause of end-stage renal disease in the U.S. Moreover, the number of patients diagnosed each year with end-stage renal disease secondary to diabetes is expected to grow from 41,000 in the year 2000 to 300,000 in the year 2030 (1). Activation of the renin angiotensin system (RAS) is associated with progression of both diabetic and nondiabetic kidney disease (2). Numerous studies have demonstrated that RAS blockade lowers the rate of urinary albumin excretion (UAE) and retards decline of renal function in diabetes (3–7). This protective effect probably reflects both the hemodynamic and nonhemodynamic effects of ACE inhibitors and angiotensin II (Ang II) receptor blockers.

Female sex is a protective factor in the development of renal disease (8), but the mechanism remains elusive. Estrogen plays a role in the regulation of and response to some components of the RAS (9). Ingestion of the oral contraceptive (OC) results in elevated circulating RAS components (10–12). This laboratory reported reduced renal blood flow and a blunted renal hemodynamic response to Ang II in healthy women using high–estrogen dose OCs (10). Ingestion of low–estrogen dose OCs also results in RAS activation with respect to the kidney (11,12).

The risks of stroke and myocardial infarction are quoted as being 5–17 times higher in diabetic OC users compared to nondiabetic OC users, (13,14) though a recent meta-analysis examining the risk of stroke in low-estrogen OCs users has cast doubt on this previously widely accepted association (15). Epidemiological and physiological data on OC use and the kidney are limited and conflicting (10–12,16–18).

The strong association between the RAS and diabetic nephropathy raised the question of whether OC use in the setting of diabetes results in increased angiotensin-dependent control of the renal circulation. Our hypothesis was therefore twofold. In our first study, we hypothesized that OC use in diabetic subjects would result in enhanced angiotensin-dependent control of the renal circulation. We examined renal hemodynamic function, at baseline and in response to the ACE inhibitor captopril, in 92 women stratified according to diabetic and OC user status.

The results of our physiology study prompted us to examine the effects of OC use on the diabetic kidney at the population level. Thus, we prospectively examined an inception cohort of patients newly diagnosed with type 1 diabetes (19) to determine if OC use was associated with the development of nephropathy.

RESEARCH DESIGN AND METHODS

Physiology study
Subjects. A total of 41 female nondiabetic OC nonusers, 10 nondiabetic OC users, 29 diabetic (17 type 1) OC nonusers, and 12 female diabetic (11 type 1) OC users were enrolled in the study. Subjects completed an initial medical history, physical examination, electrocardiogram, and laboratory screening. No subject was taking medication other than OCs, insulin, oral hypoglycemic agents, ACE inhibitors, or angiotensin receptor blockers (ARBs). All subjects gave written informed consent. The study protocol was approved by the Brigham and Women's Hospital Institutional Review Board.
Protocol. Subjects were instructed to consume >200 mmol sodium/day for 4 days before the study. Sodium, creatinine, and protein excretion was measured from a 24-h urine collection; no data were excluded because of dietary noncompliance. ACE inhibitors and ARBs were discontinued 2 weeks before the study—a time period we have found to be sufficient before studying angiotensin-dependent control of the renal circulation (20).

Fasting plasma glucose concentrations were measured at the start of the study. Type 1 diabetic subjects received intravenous insulin at $0.015 \text{ units} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, titrated to maintain blood glucose between 80 and 150 mg/dl. Oral hypogly-

cemic agents were withheld that morning in type 2 diabetic subjects, and individuals who required insulin received half their usual morning dose of intermediate-acting insulin. Subjects were studied in the supine position after an 8-h fast. At 8:00 A.M., an intravenous catheter was placed in each arm (for infusion and blood sampling). Blood pressure (BP) was recorded every 15 min by an automatic recording device (Dinamap; Critikon, Tampa, FL). A loading dose of 8 mg/kg of para-aminohippurate (PAH) and 50 mg/kg of inulin, followed by constant infusions of PAH at 12 mg/min and inulin at 30 mg/min for 90 min to establish baseline renal hemodynamic measurements, followed by 25 mg captopril taken orally, was administered. PAH clearance, inulin clearance, and plasma renin activity were measured at baseline and up to 240 min after captopril ingestion. A subset of subjects returned on a separate day for a repeat study, where they received 150 mg irbesartan or 16 mg candesartan instead of captopril.
Analytical methods. Renal clearance studies were assessed with PAH (Clinalfa, Läufelfingen, Switzerland) and inulin (Inutest Polyfructosan, Fresenius Pharma, Linz, Austria) as previously described (21). Serum PAH and inulin were measured by an autoanalyzer. Plasma renin activity was assayed by radioimmunoassay (21). Urinary albumin concentration was measured by immunonephelometry (Behring, Somerville, NJ).
Statistical analysis. The primary analysis tested associations between diabetes, OC use, and renal hemodynamics. Study subject baseline and response to captopril measures were compared using nonparametric methods. The χ^2 test was used to compare frequencies. Friedman's test was used to check for an interaction between diabetes status and OC use. Statistical analyses were performed using Stata (version 8.0; Stata, College Station, TX) with two-tailed significance levels of 0.05.

Population study
Subjects. All newly diagnosed female type 1 diabetic patients, consecutively admitted to the Steno Diabetes Center between 1 September 1979 and 31 August 1984, were included. All patients fulfilled the criteria of newly diagnosed type 1 diabetes: ketonuria, fasting blood glucose >14 mmol/l, underweight/normal body weight, and considered insulin depen-

dent by experienced diabetologists. The hospital ethics committee approved the experimental design. All patients gave written informed consent.
Procedures, measurements, and outcome. Patients attended the outpatient clinic of the Steno Diabetes Center every 3–4 months as part of routine follow-up. Patients were treated according to set principles and guidelines (22). Baseline values were defined as the first assessment 6 months after onset of type 1 diabetes, i.e., after initial glycemic stabilization. Each patient had a 24-h UAE rate determined by enzyme immunoassay (23) measured at least yearly. Micro- and macroalbuminuria were defined as a UAE between 30 and 300 mg/24 h and >300 mg/24 h for at least 1 year in at least two of three consecutive samples (6). BP was measured in the sitting position after resting ~10 min with a standard mercury sphygmomanometer at least annually. Patients were considered smokers if they smoked one or more cigarettes a day. OC use history was collected by questionnaire in 2003. Retinopathy was assessed through dilated pupils and graded as absent, simplex, or proliferative (22).
Statistical analysis. Baseline data are expressed as means and SDs or medians (interquartile ranges). The Wilcoxon rank-sum test was used to compare groups at baseline. The χ^2 test was used to compare frequencies. A Cox proportional hazards regression model evaluated the relative contributions of covariates to the risk of developing macroalbuminuria, correcting for different lengths of follow-up. A stepwise backwards selection model included the following variables: age at onset, smoking status, BP, log UAE, HbA$_{1c}$ (A1C), serum creatinine, serum cholesterol, fasting plasma C-peptide, and OC use. OC use was included as a time-dependent covariate; patients were considered OC users once OC use started. Statistical analyses were performed using SPSS 12.0 (SPSS, Chicago, IL) with two-tailed significance levels of 0.05.

RESULTS

Physiology study
Baseline characteristics. The baseline characteristics of the study groups were similar with a few notable exceptions (Table 1). Among diabetic participants, OC users were younger ($P = 0.002$) than OC nonusers. Diabetic OC users and non-

Table 1—*Baseline characteristics of physiology study subjects*

	Nondiabetic		Diabetic	
	OC nonusers	OC users	OC nonusers	OC users
n	41	10	29	12
Age (years)	39 ± 3	27 ± 2*	38 ± 3	24 ± 2*†
BMI (kg/m²)	26 ± 1.0	29 ± 2.4	29 ± 1.8	26 ± 1.7
Mean arterial pressure (mmHg)‡	84 ± 2	87 ± 2	90 ± 3	83 ± 2
Fasting glucose (mg/dl)	80 ± 2	79 ± 3	144 ± 11†§	150 ± 21†§
A1C (%)	NA	NA	7.7 ± 0.3	7.5 ± 0.3
Known duration of diabetes (years)	NA	NA	14.9 ± 2.8	9.5 ± 1.3
Smoker (%)	0/36 (0)	1/10 (10)	4/25 (14)†	3/12 (25)†
OC estrogen content (μg/tablet)	NA	30.5 ± 2.1	NA	31.0 ± 1.9
OC progesterone content (mg/tablet)	NA	0.36 ± 0.12	NA	0.34 ± 0.11
Plasma renin activity (ng Ang I · ml⁻¹ · h⁻¹)	0.33 ± 0.06	0.52 ± 0.14	0.56 ± 0.15	0.53 ± 0.14
Urine Na (mmol/24 h)	261 ± 18	272 ± 25	217 ± 19	270 ± 28
Urine protein (mg/24 h)	6 ± 1	5 ± 1	75 ± 41†§	94 ± 44†§
Microalbuminuria (%)	0/41 (0)	0/10 (0)	7/21 (33)	6/9 (67)
Glomerular filtration rate (ml · min⁻¹ · 1.73 m⁻²)‡	119 ± 5	131 ± 9	114 ± 6	129 ± 4
RPF (ml · min⁻¹ · 1.73 m⁻²)‡	560 ± 14	623 ± 30	569 ± 25	585 ± 17
Filtration fraction	0.21 ± 0.01	0.19 ± 0.01	0.22 ± 0.02	0.22 ± 0.01

Data are means ± SE unless otherwise indicated. *$P < 0.05$ vs. diabetic OC nonusers. †$P < 0.05$ vs. nondiabetic OC nonusers. ‡Median of readings at $t = -10$, -5, and 0 min. §$P < 0.05$ vs. nondiabetic OC users. NA, not applicable.

users were more likely to smoke ($P = 0.002$ and $P = 0.06$ vs. nondiabetic OC nonusers); however, there were no differences in percentage of smokers in the diabetic and nondiabetic OC user groups ($P = 0.4$).

Renal hemodynamic responses to captopril. Nondiabetic OC nonusers showed the anticipated minimal renal plasma flow (RPF) response to captopril ($9 ± 10$ ml · min⁻¹ · 1.73 m⁻², $P = 0.6$), reflecting RAS suppression by the high salt intake (Fig. 1, Table 2). In comparison, nondiabetic OC users showed a significant increase in the RPF vasodilator response to captopril ($69 ± 35$ ml · min⁻¹ · 1.73 m⁻², $P = 0.02$ vs. baseline, $P = 0.04$ vs. nondiabetic OC nonusers). Diabetes also induced the anticipated enhancement of the renal vasodilator response to captopril in OC nonusers ($58 ± 12$ ml · min⁻¹ · 1.73 m⁻², $P < 0.0001$). When the stimulus of diabetes and OC use was combined, the largest RPF responses were found ($84 ± 12$ ml · min⁻¹ · 1.73 m⁻², $P = 0.002$). The RPF

responses in the diabetic OC users were significantly larger than those observed in the diabetic OC nonusers ($P = 0.04$).

The enhanced renovascular response to captopril in the diabetic OC users compared with diabetic OC nonusers was not reflected in the plasma renin activity, which was not different between the groups ($P = 0.7$), thus indicating only a difference in renal RAS activation at the level of the kidney. Glomerular filtration rate was similarly maintained ($P = 0.4$) in both diabetic OC users ($P = 0.9$ vs. baseline) and diabetic OC nonusers ($P = 0.5$ vs. baseline) in response to ACE inhibition. Filtration fraction decreased in both diabetic OC users ($P = 0.008$ vs. baseline) and nonusers ($P = 0.02$ compared to baseline) in response to captopril, but there was no difference between the groups ($P = 0.1$).

We performed five groups of secondary analyses. First, we compared RPF response to ACE inhibition with RPF response to ARB in a subset of subjects ($n = 25$) and found that $R^2 = 0.52$ ($P < 0.001$), indicating that the mechanism for the enhanced renal hemodynamic response to ACE inhibition was caused predominantly by reduced Ang II formation. Second, in a subgroup analysis of type 1 diabetic subjects (11 OC users, 17 OC nonusers), OC users had a larger change in RPF (83 ml · min⁻¹ · 1.73 m⁻² [95% CI 54–111]) in response to captopril compared with OC nonusers (6 ml · min⁻¹ · 1.73 m⁻² [23–101], $P = 0.097$). Third, using linear regression to adjust for baseline RPF, fasting glucose, and age, we determined that the RPF response to captopril was 44 ml · min⁻¹ · 1.73 m⁻² (95% CI 4–85) greater in diabetic OC users than in diabetic OC nonusers. Fourth, analysis by two-way fixed-effects
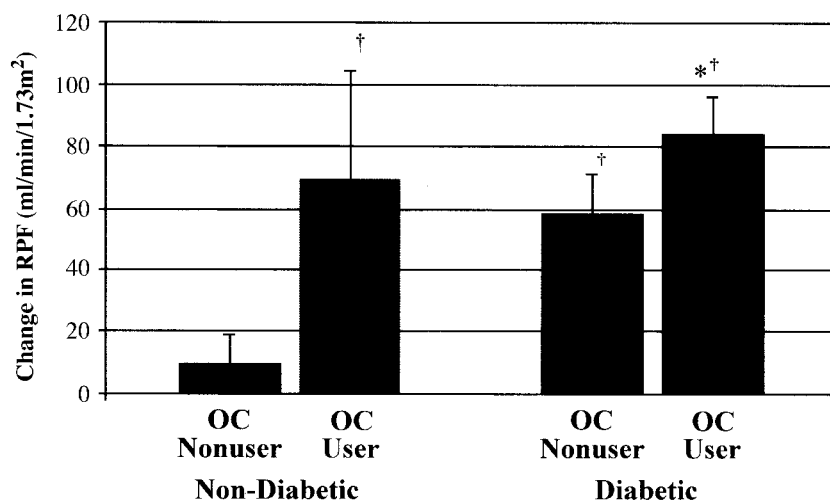

Figure 1—*Physiology study: RPF response to captopril (mean ± SE). *P < 0.05 compared with diabetic OC nonuser; †P < 0.05 compared with nondiabetic OC nonuser.*

Table 2—*Response to captopril*

	Nondiabetic		Diabetic	
	OC nonusers	OC users	OC nonusers	OC users
Mean arterial pressure (mmHg)*	79 ± 2†	82 ± 3	84 ± 3†	75 ± 2†
ΔMean arterial pressure (mmHg)	−5.2 ± 1.7	−3.5 ± 1.9	−6.4 ± 1.2	−7.8 ± 2.0
Plasma renin activity (ng Ang I · ml^{-1} · h^{-1})‡	0.9 ± 0.35†	2.1 ± 1.04†	1.5 ± 0.42†	1.2 ± 0.38†
ΔPlasma renin activity (ng Ang I · ml^{-1} · h^{-1})	0.7 ± 0.30	1.7 ± 0.95	0.9 ± 0.41	0.7 ± 0.28
Glomerular filtration rate (ml · min^{-1} · 1.73m^{-2})*	113 ± 5	137 ± 5	117 ± 6	129 ± 4
ΔGlomerular filtration rate (ml · min^{-1} · 1.73 m^{-2})	−6.1 ± 5.0	1.0 ± 7.2	2.8 ± 3.7	−0.6 ± 1.9
RPF (ml · min^{-1} · 1.73 m^{-2})‡	568 ± 17	692 ± 50†§	627 ± 32†	669 ± 25†‖
ΔRPF (ml · min^{-1} · 1.73 m^{-2})	9 ± 10	69 ± 35§	58 ± 12‖	84 ± 12#**
Filtration fraction	0.19 ± 0.01	0.16 ± 0.01	0.21 ± 0.01†	0.19 ± 0.01†
ΔFiltration fraction	−0.02 ± 0.01	−0.03 ± 0.02	−0.02 ± 0.01	−0.03 ± 0.01

Data are means ± SE unless otherwise indicated. *Mean of two readings at nadir of response to captopril. †$P < 0.05$ vs. baseline value. ‡Mean of two readings at peak response to captopril. §$P < 0.05$ vs. nondiabetic OC nonuser. ‖$P < 0.005$ vs. nondiabetic OC nonuser. #$P < 0.05$ vs. diabetic OC nonuser. **$P < 0.0005$ vs. nondiabetic OC nonuser.

ANOVA suggests that both the diabetes and OC use main effects are significant ($P = 0.006$ for diabetes, $P = 0.01$ for OC use). Finally, we examined the relationship between diabetes and OC use and found no evidence of multiplicative effects ($P = 0.3$).

Population study
Baseline characteristics. The inception cohort comprised 117 patients. Two patients had severe psychiatric disease and one patient had macroalbuminuria due to glomerulonephritis, and these three patients were excluded at baseline. We followed 33 OC users and 81 OC nonusers for a median (interquartile range) of 21.4 (20.1–22.8) and 20.2 (18.5–22.1) years, respectively (total 2,235 patient-years of follow-up). Mean age at start of OC use was 21.5 years (95% CI 15.5–36.2) and mean duration of use was 8.4 years (5.7–11.0).

The baseline characteristics of the two groups are outlined in Table 3. The groups were similar at onset in terms of UAE rate, BP, A1C, and renal function. OC users were younger at the onset of diabetes ($P < 0.001$) and therefore not as tall ($P = 0.06$). Total cholesterol was greater in OC nonusers ($P = 0.002$).

A total of 18% (6/33, 95% CI 4.3–32.1%) of the OC user group progressed to macroalbuminuria compared with 2% (0–5.9%) of the OC nonuser group ($P = 0.003$; Fig. 2). Of the OC user group, 30% (13.8–46.9%) developed microalbuminuria compared with 17% (8.9–25.7%) in the OC nonuser group ($P = 0.1$). Half of

the microalbuminuric patients in each group received RAS blockade.

A Cox proportional hazard model, adjusting for age at onset and BP, revealed OC use to be a predictor of macroalbuminuria development (relative risk 8.90 [95% CI 1.79–44.36], $P = 0.008$). Weight, A1C, log UAE, smoking status, serum cholesterol, and serum creatinine were not significant predictors of the development of macroalbuminuria.

CONCLUSIONS — Our physiology study examined the baseline components of the RAS and the renal hemodynamic response to ACE inhibition and ARB in nondiabetic and diabetic OC users and nonusers. OC use in the setting of diabetes produced an additional effect on angiotensin-dependent control of the renal circulation, noted by intrarenal vasodilator responsiveness to acute ACE inhibition. Both nondiabetic and diabetic OC users have an increased RPF response to captopril compared with their OC nonuser counterparts. Concordance with the response to ARBs confirms that the response to captopril reflects a more activated RAS (24,25). The prospective observational study examined the relationship between OC use by women with type 1 diabetes and the development of macroalbuminuria. After controlling for other known risk factors, OC use remained a significant predictor of development of macroalbuminuria. All patients developing macroalbuminuria fulfilled

Table 3—*Baseline characteristics of inception cohort*

	OC users	OC nonusers	P
n	33	81	—
Age at onset of diabetes (years)	18 ± 8	33 ± 16	<0.001
Height (cm)	160 ± 15	166 ± 8	0.06
Systolic BP (mmHg)	118 ± 14	123 ± 18	NS
Diastolic BP (mmHg)	75 ± 11	78 ± 10	NS
ACE inhibitor or ARB use (%)*	15	15	NS
Antihypertensive use (%)*	21	20	NS
Smoker (%)*	45	52	NS
UAE rate (mg/24 h)	7 (5–12)	9 (5–13)	NS
A1C (%)	9.8 ± 2.3	9.9 ± 2.1	NS
Serum cholesterol (mmol/l)	4.9 ± 1.0	5.8 ± 1.4	0.002
Serum creatinine (μmol/l)	69 ± 12	74 ± 16	NS
Glomerular filtration rate (ml/min)†	87 ± 22	87 ± 22	NS
Fasting plasma C-peptide (nmol/l)	0.19 ± 0.09	0.19 ± 0.08	NS
Follow-up time (years)*	21.4 (20.1–22.8)	20.2 (18.5–22.1)	0.01

Data are means ± SD or median (interquartile range). *Use of antihypertensive treatment or smoking during the follow-up period. †Estimated glomerular filtration rate by the Cockcroft & Gault formula.
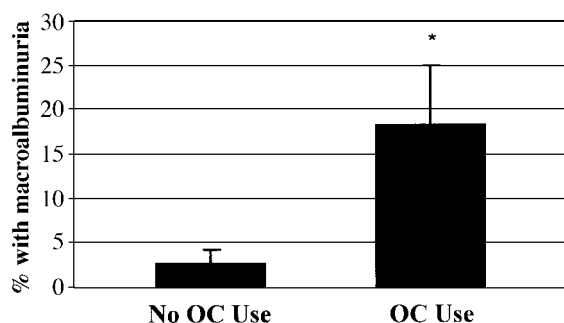
Figure 2—*Inception cohort study: OC use and development of macroalbuminuria (univariate analysis) (mean ± SE). *P < 0.005 compared with no OC use.*

the criteria for diabetic nephropathy: albuminuria (>300 mg/24 h), presence of diabetic retinopathy, and absence of evidence of other kidney disease (26). Macroalbuminuria is a more robust end point than microalbuminuria, reflecting more severe glomerulopathy (27). Finally, identical fractions of microalbuminuric patients received RAS blockade in both groups.

Many factors link RAS activation and diabetic nephropathy. Diabetes itself is associated with RAS activation (2,4,6). Acute hyperglycemia results in an activated RAS in normal and diabetic subjects (28), and poor glycemic control is associated with progression of chronic renal disease in diabetic patients (29–31). However, these factors do not explain the difference in RPF or macroalbuminuria observed between the groups, because diabetic OC users and nonusers in each study had similar fasting plasma glucose and A1C levels. Nephron loss ultimately results in increased Ang II production (32); additionally, proteinuria contributes to kidney damage (33). Thus, if renal disease were already present, the result would be an enhancing effect on the RPF response to ACE inhibition. However, all groups had similar baseline renal hemodynamics, and the two diabetic groups had similar UAE.

ACE inhibition results in elevated bradykinin levels, because ACE is also a kinase II (34). However, bradykinin does not play a significant role in the renal response to ACE inhibition (34). It has been shown that the RPF response to ACE inhibition is highly correlated with that to angiotensin receptor blockade in both healthy (25) and diabetic (24) subjects. Although indirect, this approach to testing the hypothesis that the RPF response to ACE inhibition truly represents activation of the intrarenal RAS is one of the few methods available in humans. The re-

markable concordance in the renal hemodynamic response to ACE inhibition and ARB in our physiology study, coupled with the fact that all groups had similar changes in BP and plasma renin activity in response to captopril, makes it exceedingly likely that the increase in RPF response reflects reversal of an increase in Ang II–mediated renal vascular tone.

The first-generation OC has been associated with biopsy-proven renal damage in the absence of primary renal disease (35), and healthy women ingesting the high-estrogen OC demonstrated decreased renal blood flow and a blunted response to Ang II (10). In contrast, an increased renal hemodynamic response to Ang II in healthy OC users compared with nonusers was described more recently. This discrepancy has been attributed to differences in Ang II preparation and the estrogen dose used in each study. Compared with nonusers, Kang et al. (11) reported elevations in circulating levels of all RAS components as well as BP, renovascular resistance, and filtration fraction, which were partially corrected by losartan. In a study of women with low-estrogen OC–associated hypertension, OC use, independent of BP, was associated with an increased 24-h UAE (18).

Few epidemiological studies have examined OC use and diabetic renal sequelae. In a retrospective case-control study, type 1 diabetic low-dose OC users and nonusers were found to show no difference in mean UAE during an 11.8-year period (16). In contrast, Monster et al. (17) reported an odds ratio of 1.90 for having microalbuminuria in low-dose OC users compared with OC nonusers after adjusting for multiple cardiovascular disease risk factors, including diabetes. This large cross-sectional study showed a trend toward an increased risk of microalbuminuria according to OC estrogen content.

Men are more likely to develop diabetic kidney disease than women (19); as such, our study was biased against detecting a difference in rates of renal disease between OC users and nonusers, because women are relatively protected. Other than an increased risk of albuminuria reported by Monster et al. (17) and Ribstein et al. (18), there is currently no evidence suggesting that OC use predisposes women to renal disease, and it may be that a simultaneous "second hit" to the kidney is required. OC use may therefore have implications for nondiabetic women with increased susceptibility to renal damage, because OC use in nondiabetic women increased angiotensin-dependent control of the renal circulation to levels observed in diabetic OC nonusers.

In practice, diabetic women with albuminuria may be more likely to be prescribed OCs compared with their counterparts with no renal disease to prevent an unplanned pregnancy. Indeed, 18/81 nonusers became pregnant, compared with 17/33 OC users (P = 0.002). However, pregnancy is not a risk factor for the development of diabetic nephropathy (36).

Overwhelming evidence indicates that RAS blockade prevents or delays diabetic nephropathy (2–7), thus raising the question whether activation of the RAS because of OC use may contribute to the initiation or progression of kidney injury in patients with a predisposition to renal disease. There are currently no contraceptive methods that are contraindicated in diabetic women; the same guidelines that apply to healthy women are used for diabetic women (37). In fact, contraception is particularly advocated in this population given the serious complications associated with an unexpected pregnancy.

This study has limitations. It has been reported that circulating RAS components peak during the luteal phase of the menstrual cycle, when plasma estrogen levels are highest (38). While we did not record the menstrual phase, the phase of the menstrual cycle does not affect the hemodynamic responses to RAS components at the tissue level (39). We included both type 1 and 2 diabetic subjects in our physiology study because an activated RAS is thought to be detrimental to kidney function in both populations (2–7). Indeed, OC use has been associated with an increased risk of microalbuminuria in

women with type 2 diabetes (17). OC users were younger than their nonuser counterparts in both studies, reflecting the pattern of OC use in the general population (40). However, even after adjusting for age in the physiology study, there were still clear differences in RPF response to ACE inhibition between the groups. Smoking is hypothesized to lead to β_1 receptor–mediated renin and Ang II production (41), ultimately resulting in renal damage. However, diabetic OC users and nonusers were equally likely to be smokers in the physiology study. The period from onset of diabetes until development of diabetic nephropathy is significantly longer for patients with early onset compared with patients with later onset (42), making our estimate of the risk for development of diabetic kidney disease conservative. Furthermore, the Cox proportional hazards model did not identify A1C as a risk factor for development of diabetic nephropathy in the present female subset ($n = 114$) of the whole inception cohort ($n = 277$) (19). This could be a chance finding, but is more likely because of the lack of power, since A1C was a significant predictor of development of albuminuria in the total cohort (19). The fact that OC use is associated with an increase in BP (11) was accounted for in the Cox proportional hazards model; OC use remained a predictor of albuminuria. Although the numbers in our population study are small, we used baseline predictors to estimate the risk of albuminuria. The use of baseline values (in contrast to follow-up values) in observational studies can result in systematic and substantial underestimation of the strength of the real association between the baseline values and disease—so-called regression dilution bias (43). Our results may therefore be regarded as conservative.

In our study, diabetic OC users had an enhanced level of angiotensin-dependent control of the renal circulation and an adjusted rate of macroalbuminuria development almost nine times that of nonusers. This may have widespread public health implications, because OCs are the preferred method of contraception in at least 20% of diabetic women (40).

Although our sample size was limited, and the effects of duration of OC use and whether the effects of the OC on the kidney persist after discontinuation of the medication are unclear, the biological effects seen in our studies were large. In each case, the study was a product of a specific predefined hypothesis, but clearly large-scale prospective studies will need to be performed before recommendations regarding clinical practice can be made. Meanwhile, caution and surveillance should be applied to OC use in the context of diabetes.

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