Plasma Endothelin in Normal and Diabetic Pregnancy

KERSTIN WOLFF, MD, PHD
KJELL CARLSTRÖM, PHD
FREJ FYHRQUIST, MD, PHD
ANETTE HEMSÉN, MSC, PHD
NILS-OLOV LUNELL, MD, PHD
HENRY NISELL, MD, PHD

OBJECTIVE — To examine endothelin-1 (ET-1) concentrations longitudinally throughout pregnancy in healthy and insulin-dependent diabetic women and to evaluate the relationship between ET-1 and big ET-1 in normal pregnancy.

RESEARCH DESIGN AND METHODS — Venous blood samples were obtained consecutively in gestational weeks 18, 28, and 38 from 40 healthy women with uneventful pregnancies and 24 pregnant women with IDDM. By radioimmunoassay, plasma ET-1 and big ET-1 were analyzed in the healthy women and plasma ET-1 in the diabetic women.

RESULTS — In the diabetic pregnant women, plasma ET-1 levels were significantly higher than in healthy pregnant women during the entire observation period ($P < 0.001$), but did not change with advancing gestational age. Five of the diabetic, but none of the healthy pregnant women, developed preeclampsia. ET-1 levels did not differ between the diabetic women who developed preeclampsia and those who did not. Plasma ET-1 levels in healthy pregnant women were within the range of those in healthy nonpregnant women and did not change during pregnancy. The big ET-1 levels increased and the ET-1/big ET-1 ratio decreased significantly during the observation period.

CONCLUSIONS — Plasma ET-1 levels do not change with advancing gestational length. During normal pregnancy, the ET-1/big ET-1 ratio decrease, indicating a suppressed converting enzyme activity or altered clearance of ET-1. Pregnant women with IDDM have markedly elevated ET-1 levels. Although diabetic women with and without preeclampsia did not differ with respect to endothelial dysfunction, as reflected by elevated ET-1 concentration, we cannot exclude that altered endothelial function may be of importance for the increased frequency of preeclampsia in pregnant IDDM patients.

Much attention has been paid to the importance of endothelin-1 (ET-1) during pregnancy and delivery. On account of its contractile effect on the myometrium, involvement of ET-1 in the initiation and maintenance of labor has been proposed (1). It has also been suggested that in view of its potent vasoconstrictive properties, ET-1 may be of pathogenetic importance in the development of pregnancy-associated complications with reduced uteroplacental blood flow, such as preeclampsia (2).

ET-1 is produced from a biologically inactive propeptide, big ET-1, by enzymatic cleavage. Inhibitors of this cleavage have been suggested as effective means of preventing production of endothelin in circumstances where it may play a pathogenetic role (3). The ratio between the concentrations of ET-1 and big ET-1 reflects the conversion of big ET-1 to ET-1. This ratio is reduced in nonpregnant patients with diabetes (4) and in normal pregnant women, as compared with healthy nonpregnant women, but increased in preeclamptic women, as compared with healthy pregnant subjects (5).

During normal pregnancy, the cardiovascular system undergoes functional changes characterized by an expanded plasma volume, reduced peripheral resistance, increased cardiac output, and decreased sensitivity of the vascular bed to vasoconstrictors, resulting in a reduction of blood pressure (6). In women who develop preeclampsia, this physiological adaptation fails to occur, resulting in increased vascular tone and an increased sensitivity to vasopressors, with a consequent rise in blood pressure. There is an accumulation of evidence that endothelial cell dysfunction is involved in the pathogenesis of this pregnancy-specific disorder (7,8). In this context, it is of interest that women with microvascular diseases such as diabetes, possibly due to underlying endothelial dysfunction (10), run an increased risk of developing preeclampsia (11). ET-1 is considered a marker of endothelial injury, and the elevated plasma ET-1 concentrations found in preeclamptic women are regarded as the consequence of endothelial damage (12). In fact, endothelial cell injury in diabetes and preeclampsia might have an additive effect and possibly explain the increased frequency of preeclampsia in diabetic pregnancy.

The aim of the present study was twofold. First, to compare the plasma ET-1 concentrations between healthy pregnant women and pregnant women with IDDM at different gestational ages and, second, to evaluate the effect of gestational age on ET-1 and its precursor, big ET-1.

RESEARCH DESIGN AND METHODS

— Initially, 40 healthy pregnant women and 24 pregnant women with IDDM with a mean disease duration of 12 years (range 1–27 years) were consecutively recruited to this longitudinal prospective study. The healthy pregnant women were recruited from the antenatal care unit and the IDDM subjects from the antenatal high-risk unit, covering a catchment area of ~500,000 people. All of the diabetic subjects and 37 of the healthy pregnant women fulfilled the sampling procedure at all three sampling occasions at 18, 28, and 38 weeks of gestation. Three healthy women withdrew from the last sampling at 38 weeks. One of them had a preterm delivery and two had moved to other districts. The plasma concentrations

From the Department of Obstetrics and Gynecology (K.W., K.C., N.-O.L., H.N.), Karolinska Institute, Huddinge University Hospital, Huddinge; the Division of Pharmacology (A.H.), Karolinska Institute, Stockholm, Sweden; and the Unit of Clinical Physiology (F.F.), Minerva Institute for Medical Research, Helsinki, Finland.
Address correspondence and reprint requests to Kerstin Wolff, MD, Department of Obstetrics and Gynecology, Huddinge University Hospital, S-141 86 Huddinge, Sweden.
Received for publication 18 June 1996 and accepted in revised form 6 November 1996.
ET-1, endothelin-1; RIA, radioimmunoassay.

Table 1—*Plasma levels of ET-1 in pregnant women and levels of ET-1, big ET-1, and the ET-1/big-ET-1 ratio in healthy pregnant women at gestational week 18, 28 and 38*

Gestational age (weeks)	ET-1 (pmol/l)		Big ET-1 (pmol/l)	ET-1/big ET-1
	Diabetic	Healthy	Healthy	Healthy
18	2.45 ± 0.17	1.36 ± 0.08	2.22 ± 0.13	0.65 ± 0.05
28	2.45 ± 0.15	1.24 ± 0.06	2.54 ± 0.15	0.52 ± 0.04
38	2.73 ± 0.19	1.28 ± 0.09	2.82 ± 0.21	0.49 ± 0.04‡
Nonpregnant women		1.16 ± 0.06§		

Data are means ± SE. $P < 0.01$ vs. ET-1 concentration in healthy pregnant women during the same gestational week. $P < 0.05$ vs. big ET-1 in the 18th gestational week. ‡$P < 0.05$ vs. ET-1/big ET-1 in the 18th gestational week. §From Fyhrquist et al. (13).

of the substances analyzed in these women did not differ significantly from those in the other normal pregnant women at any sampling time and were therefore included in the study. The remaining healthy pregnant women sustained a normal pregnancy. Among the diabetic patients, seven exhibited vasculopathy (i.e., White's Class D; mild retinopathy, $n = 7$, or hypertension, $n = 1$), whereas none showed nephropathy. In previously normotensive women, preeclampsia was defined as blood pressure ≥140/90 and proteinuria exceeding 300 mg/24 h after 20 weeks of gestation. In one patient with chronic hypertension, superimposed preeclampsia was defined as a blood pressure increase of >30/15 mmHg compared with first-trimester recordings and addition of proteinuria, as above.

Blood samples were obtained from an antecubital vein, collected into heparinized tubes and centrifuged, and the plasma was separated and stored at −20°C until analyzed. The study was approved by the local ethics committee and all women gave their informed consent to participate.

Plasma ET-1 was determined by radioimmunoassay (RIA) after solid-phase extraction on Bondelut C18OH microcolumns, as described by Fyhrquist et al. (13). The immunoreactive material detected in human plasma from nonpregnant and pregnant women by this method was shown by high-power liquid chromatography to coelute with synthetic ET-1, but not with ET-2, ET-3, or big ET-1. The limit of detection was 0.3 fmol/tube, and the within- and between-assay coefficients of variation were 12 and 17%, respectively.

Before assay of big ET-1, the samples were extracted with 2 ml acid ethanol. After centrifugation, the supernatants were decanted and dried in a 54°C water bath under a nitrogen stream. The RIA was performed in a 100 mmol/l phosphate buffer, pH 7.4, containing 50 mmol/l NaCl, 0.1% NaN_3, and 0.1% bovine serum albumin. The samples were incubated for 3 days at 4°C with B6 rabbit antiserum (14) before addition of iodinated big ET-1 (Amersham, Bucks, U.K.), followed by incubation for 1 more day. Bound and free fractions were then separated by a solid-phase second antibody technique (SacCel, IDS, Boldon, U.K.). The cross-reactivity of the B6 antiserum was as follows: big ET-1 (1–38), 100%; big ET-1 (22–38), 35%; ET-1, <0.007%.

Detection limit and between-assay coefficients of variation were 0.4 fmol/tube and 6%.

Statistical analysis

Statistical analyses were performed by analysis of variance with repeated measurements followed by Student's t test for dependent and independent variables corrected for multiple comparisons. Frequencies were compared by Fisher's exact test. P values <0.05 were considered statistically significant. Data are expressed as means ± SE or as medians and ranges, where appropriate.

RESULTS — Among the diabetic women, those with vasculopathy ($n = 7$) did not differ from those without vasculopathy ($n = 17$) with regard to the plasma ET-1 concentration on any sampling occasion (18 weeks: 2.32 ± 0.29 vs. 2.48 ± 0.20; 28 weeks: 2.41 ± 0.21 vs. 2.47 ± 0.19; 38 weeks: 3.06 ± 0.33 vs. 2.61 ± 0.22). These subgroups were therefore pooled in the subsequent analysis.

The mean plasma ET-1 levels in the diabetic pregnant women were significantly higher (approximately twice those in the healthy pregnant women) on all three sampling occasions ($P < 0.001$). The ET-1 concentration in the diabetic group did not change during pregnancy. Five of the diabetic patients developed preeclampsia (21%). As shown in Table 2, the mean ET-1 levels in these five women did not differ significantly from those of the diabetic women who did not develop preeclampsia. Age, parity, and duration of IDDM did not differ significantly between the two subgroups. On the contrary, women with vasculopathy tended to have an increased risk for developing preeclampsia (3/7 vs. 2/17; $P = 0.13$, Fisher's exact test).

Mean concentrations of ET-1, and big ET-1 and the range for plasma ET-1 in healthy nonpregnant women, using the same method and laboratory (13), are given in Table 1. The plasma ET-1 levels in healthy pregnant women were within the range of those in normal nonpregnant women (13) and, as for the IDDM pregnant women, did not change during pregnancy.

Big ET-1 was significantly higher than ET-1 ($P < 0.01$). Contrary to ET-1, the big ET-1 levels tended to increase with increasing length of gestation and were significantly higher in the 38th gestational week than in the 18th week ($P < 0.05$). Consequently the ET-1/big ET-1 ratio decreased from week 18 to week 38 ($P < 0.05$).

CONCLUSIONS — The complication of a diabetic pregnancy by preeclampsia is of concern because of the greatly increased fetal morbidity and mortality seen in this situation. Several reports demonstrate an increased incidence of preeclampsia in diabetic pregnancies (15–17). In our limited material, women with vasculopathy, i.e., retinopathy or hypertension, showed an increased incidence of preeclampsia compared with those without vascular complications. This is in accordance with the results of a meta-analysis demonstrating a doubling of the preeclampsia rate in White Classes D, F, and R compared with White Classes B and C (17). It has been argued that the high incidence of preeclampsia was related to inclusion of Class F diabetic patients in previous studies. Many IDDM patients with nephropathy are hypertensive and show an aggravation of these symptoms during pregnancy. Thus, it is impossible, with certainty, to make the diagnosis of superimposed preeclampsia in Class F diabetic patients. In a prospective study, Garner et al. (18) demonstrated that even when Class F diabetic patients and those with chronic hypertension were excluded, the overall incidence of preeclampsia still was

considerably increased in diabetic subjects as compared with the nondiabetic incidence. Thus, vasculopathy does not seem to be a prerequisite for the development of preeclampsia in women with IDDM.

Endothelial cell dysfunction probably plays an important role in the pathogenesis of preeclampsia (7–9) and is a well-recognized feature of diabetes. Thus, increased plasma ET-1 concentrations have been demonstrated in nonpregnant patients with IDDM (19). Furthermore, Drury et al. (20) have shown that this patient group has an increased vasopressor response to angiotensin II. Altered endothelial function is regarded as an early sign of vascular disease developing even before clinical signs, such as hypertension or vasculopathies, are evident. Accordingly, Stroes et al. (21) demonstrated endothelial dysfunction with impaired endothelium-dependent vasodilatation in hypercholesterolemic patients at risk for arteriosclerosis but without evident arterial disease. Our finding of similar ET concentrations in subjects with and without vasculopathy is in agreement with this concept.

After completion of blood sampling from the IDDM patients, we were intrigued by their relatively high ET-1 concentrations and felt it important to compare them with those of healthy pregnant women. Because at that time we had the possibility of determining big ET-1 concentrations, this assay also was included in the normal pregnancy group. Accordingly, we found that pregnant women with IDDM had approximately twice the ET-1 concentration found in normal pregnancy. None of the normal pregnant women, but five (21%) of the patients in the diabetic group, developed preeclampsia. Because the mean ET-1 levels in these five women did not differ significantly from those in the diabetic women who did not develop preeclampsia, it may be concluded that ET-1 levels in pregnant diabetic women cannot be used as markers for prediction of preeclampsia. This finding also suggests that factors other than endothelial dysfunction, as reflected by elevated ET-1 concentration, are necessary to develop preeclampsia in pregnant patients with IDDM. Increased thromboxane formation reflecting platelet activation has been demonstrated in diabetic pregnancy (22).Whether this augmented platelet activation is triggered by endothelial injury or is due to an inherent platelet dysfunction in preeclampsia remains to be elucidated.

Previous investigations of ET-1 concentrations in plasma during normal pregnancy have yielded contradictory results, some showing increasing levels throughout pregnancy (23,24) and others no change (24,25) or even a decrease (26). These studies, however, were cross-sectional. We have chosen to follow up the same women consecutively on three occasions during their pregnancies to determine whether any longitudinal changes in circulating ET levels occurred and to try to clarify the activity of the endothelin system at different gestational lengths.

The plasma ET-1 levels during normal pregnancy were similar to those in nonpregnant humans using the same assay method and laboratory (13) and did not change during pregnancy, whereas the big ET-1 levels tended to increase with advancing pregnancy. Accordingly, the ratio of plasma ET-1 to plasma big ET-1 decreased throughout pregnancy, implying a reduction of converting enzyme activity. The plasma half-life of big ET-1 in the human circulation is considerably longer than that of ET-1, and measurements of plasma big ET-1 levels might therefore be the best approach for investigating the secretory activity of the ET-1 system (14). Our results thus indicate that the activity of this system increases throughout normal pregnancy. Increased clearance of ET-1 during pregnancy may offer an alternative explanation for the decreased ET-1/big ET-1 ratio. Whether pulmonary or renal clearance, which are of great importance for the ET-1 degradation (28,29), is affected by pregnancy is presently unknown.

In conclusion, the plasma ET-1 levels during pregnancy do not change with gestational length, either in healthy or diabetic women. Pregnant women with IDDM have markedly elevated ET-1 levels, which may be caused by endothelial cell damage. Although the ET-1 concentration showed no relationship to the presence of diabetic vasculopathies or the incidence of preeclampsia, we cannot exclude that endothelial dysfunction, as reflected by increased ET-1 concentration, contributes to the increased frequency of preeclampsia seen in women with diabetes.

Table 2—*Clinical characteristics of the IDDM patients with and without preeclampsia*

	Preeclampsia	No preeclampsia
n	5	19
Age (years)	29 (26–33)	29 (18–37)
Nulliparity	2/5	8/19
Vasculopathy	3	4
Duration of diabetes (years)	17 (6–27)	11 (1–21)
BMI	25.6 ± 1.9	25.9 ± 3.6
ET-1 concentration 18 weeks (pmol/l)	2.49 ± 0.30	2.42 ± 0.19
ET-1 concentration 28 weeks (pmol/l)	2.35 ± 0.28	2.47 ± 0.17
ET-1 concentration 38 weeks (pmol/l)	2.98 ± 0.37	2.68 ± 0.22

Data are *n*, means ± SE, or median (range).

Acknowledgments— This study was supported by grants from the Anders Otto Swärd Foundation for Medical Research, the General Maternity Hospital Memory Foundation, the Swedish Society of Medicine, and the Swedish Medical Research Council (grant 9512).

References

1. Word A, Kamm KE, Casey ML: Contractile effects of prostaglandins, oxytocin and endothelin-1 in human myometrium in vitro: refractoriness of myometrial tissue of pregnant women to prostaglandins E_2 and $F_{2\alpha}$. *J Clin Endocrinol Metab* 75:1027–1032, 1992
2. Wolff K, Nisell H, Modin A, Lundberg JM, Lunell NO, Lindblom B: Contractile effects of endothelin-1 and endothelin 3 on myometrium and small intramyometrial arteries of pregnant women at term. *Gynecol Obstet Invest* 36:166–171, 1993
3. Opgenorth TJ, Wu-Wong JR, Shiosaki K: Endothelin-converting enzymes. *FASEB J* 6:2653–2659, 1992
4. Tsunoda K, Abe K, Sato T, Yokosawa S, Yoshinaga K: Decreased conversion of big endothelin-1 to endothelin-1 in patients with diabetes mellitus. *Clin Exp Pharmacol Physiol* 18:731–732, 1991
5. Sudo N, Kamoi K, Ishibashi M, Yamaji T: Plasma endothelin-1 and big endothelin-1 levels in women with pre-eclampsia. *Acta Endocrinol* 129:114–120, 1993
6. Friedman SA, Taylor R, Roberts J: Pathophysiology of preeclampsia. *Clin Perinatol* 18:661–682, 1991
7. Rodgers GM, Taylor RN, Roberts JM:

Preeclampsia is associated with a serum factor cytotoxic to human endothelial cells. *Am J Obstet Gynecol* 159:908–914, 1988
8. Roberts JM, Taylor RN, Musci TJ, Rodgers G, Hubel C, McLaughlin M: Preeclampsia: an endothelial cell disorder. *Am J Obstet Gynecol* 161:1200–1204, 1989
9. Kamoi K, Sudo N, Ishibashi M, Yamaji T: Plasma endothelin-1 levels in patients with pregnancy-induced hypertension. *N Engl J Med* 323:1486–1487, 1990
10. Durante W, Sen AK, Sunahara FA: Impairment of endothelium dependent relaxation in aortae of spontaneous diabetic rats. *Br J Pharmacol* 94:463–468, 1988
11. Garner PR, D'Alton ME, Dudley DK, Huard P, Hardie M: Preeclampsia in diabetic pregnancies. *Am J Obstet Gynecol* 163:505–508, 1990
12. Zeeman GG, Dekker GA, van Geijn HP, Kraayenbrink AA: Endothelial function in normal and pre-eclamptic pregnancy: a hypothesis. *Eur J Obst Gynecol Reprod Biol* 43:113–122, 1992
13. Fyhrquist F, Saijonmaa O, Metsärinne K, Tikkanen I, Rosenlöf K, Tikkanen T: Raised plasma endothelin-1 concentration following cold pressor test. *Biochem Biophys Res Commun* 169:217–221, 1990
14. Hemsén A, Ahlborg G, Ottosson-Seeberger A, Lundberg JM: Metabolism of big endothelin-1 (1–38) and (22–38) in the human circulation in relation to production of endothelin-1 (1–21). *Regul Pept* 55:287–297, 1995
15. Kyle GC: Diabetes and pregnancy. *Ann Intern Med* 59 (Suppl. 3):1–12, 1963
16. Gabbe S, Mestman J, Freeman R, Goebelsmann UT, Lowensohn RI, Nochimson D, Cetrulo C, Quilligan EJ: Management and outcome of pregnancy in diabetes mellitus. *Am J Obstet Gynecol* 129:723–732, 1977
17. Cousins L: Pregnancy complications among diabetic women: review 1965–1985. *Obstet Gynecol Surv* 42:140–149, 1987
18. Garner PR, D'Alton ME, Dudley DK, Huard P, Hardie M: Preeclampsia in diabetic pregnancies. *Am J Obstet Gynecol* 163:505–508, 1990
19. Takahashi K, Ghatei MA, Lam HC, O'Halloran DJ, Bloom SR: Elevated plasma endothelin in patients with diabetes mellitus. *Diabetologia* 33:306–310, 1990
20. Drury PL, Smith PM, Ferris JB: Increased vasopressor responsiveness to angiotensin II in type 1 (insulin-dependent) diabetic patients without complications. *Diabetologia* 27:174–179 1984
21. Stroes ESG, Koomans HA, Bruin de TWA, Rabelink TJ: Vascular function in the forearm of hypercholesterolemic patients off and on lipid-lowering medication. *Lancet* 346:467–471, 1995
22. Van Assche FA, Spitz B, Hanssens M, Van Geet C, Arnout J, Vermylen J: Increased thromboxane formation in diabetic pregnancy as a possible contributor to preeclampsia. *Am J Obstet Gynecol* 168:84–87, 1993
23. Usuki S, Satoh T, Sawamura T, Suzuki N, Shigemitsu S, Yanagisawa M, Goto K, Onda H, Fujino M, Masaki T: Increased maternal plasma concentration of endothelin-1 during labor pain or on delivery and the existence of a large amount of endothelin in amniotic fluid. *Gynecol Endocrinol* 4:85–97, 1990
24. Clark BA, Halvorson L, Sachs B, Epstein FH: Plasma endothelin levels in preeclampsia: elevation and correlation with uric acid levels and renal impairment. *Am J Obstet Gynecol* 166:962–968, 1992
25. Dekker GA, Kraayenbrink AA, Zeeman GG, van Kamp GJ: Increased plasma levels of the novel vasoconstrictor peptide endothelin in severe pre-eclampsia. *Eur J Obst Gynecol Reprod Biol* 40:215–220, 1991
26. Ihara Y, Sagawa N, Hasegawa M, Okagaki A, Li XM, Inamori K, Itoh H, Mori T, Saito Y, Shirakami G, Nakao K, Imura H: Concentrations of endothelin-1 in maternal and umbilical cord blood at various stages of pregnancy. *J Cardiovasc Pharmacol* 17 (Suppl. 7):S443–S445, 1991
27. Iwata I, Takagi T, Yamaji K, Tanizawa O: Increase in the concentration of immunoreactive endothelin in human pregnancy. *J Endocrinol* 129:301–307, 1991
28. Sirviö ML, Metsärinne K, Saijonmaa O, Fyhrquist F: Tissue distribution and half-life of 125-I-endothelin in the rat: importance of pulmonary clearance. *Biochem Biophys Res Commun* 167:1191–1195, 1990
29. Sirviö ML, Saijonmaa O, Metsärinne K, Fyhrquist F: Degradation of endothelin-1 by rat lung, kidney and liver. *Regul Pept* 55:219–225, 1995

Be Part of the Team Coming Close to the Cure
Join the American Diabetes Association

American Diabetes Association.

1. Name: _____

Address: _____ City _____ State _____ Zip _____

Degrees: _____ / _____ Home Phone _____ Office _____ Fax _____

2. Primary Area of Focus: _____ Clinical Practice _____ Research _____ Education _____ Industry

3. Choose Your Membership Category

Category I: Please note, physicians must join this category

Dues:

- ❑ Domestic Members: $125
- ❑ Overseas Members: $180
- ❑ Domestic in Training Dues: $65
- ❑ Overseas in Training Dues: $95

Journal Selection (choose one):

- ❑ Diabetes **(monthly)**: Basic research on the pathophysiology of diabetes
- ❑ Diabetes Care **(monthly)**: Clinical care and research

Category II:

Dues:

- ❑ Domestic Members: $75
- ❑ Overseas Members: $115
- ❑ Domestic in Training Dues: $37
- ❑ Overseas in Training Dues: $45

Journals (includes journals listed below):

Diabetes Spectrum **(quarterly)**: Education and counseling strategies

Diabetes Forecast **(monthly)**: Lifestyle magazine for patients and their families

**If you received your first professional degree, diploma, or certificate in the last five years, you qualify for 50% savings offered with In-Training Membership. Just fill out information below.*

University or college attended: _____ Degree(s) _____ Date Earned: _____

4. Order Additional Publications at "Members-only" Rates: Domestic/International Rates *(only single subscriptions available for each of the following journals)*

	Dom/Intern'l		Dom/Intern'l		Dom/Intern'l
❑ Diabetes Care (monthly)	$75/$130	❑ Diabetes Reviews (quarterly)	$45/$65	❑ Diabetes Spectrum (quarterly)	$30/45
❑ Diabetes (monthly)	$75/$130	❑ Clinical Diabetes (bi-monthly)	$15/$21	❑ Diabetes Forecast (monthly)	$12/$37

5. Select One FREE Council Membership:

Extra Council Memberships are $25 each.

- ❑ Behavioral Medicine and Psychology **(PP)**
- ❑ Diabetes in Pregnancy **(BB)**
- ❑ Complications **(TT)**
- ❑ Education **(SS)**
- ❑ Exercise **(XX)**
- ❑ Foot Care **(RR)**
- ❑ Molecular, Cellular and Biochemical Aspects of Diabetes **(MM)**
- ❑ Clinical Endocrinology, Health Care Delivery and Public Health **(FF)**
- ❑ Diabetes in Youth **(EE)**
- ❑ Epidemiology and Statistics **(CC)**
- ❑ Immunology, Immunogenetics and Transplantation **(JJ)**
- ❑ Nutritional Sciences and Metabolism **(NN)**

6. Mark Your Primary Work Setting:

- ❑ University/Academic (1)
- ❑ Private/Group Practice (2)
- ❑ Hospital (3)
- ❑ HMO (4)
- ❑ Public Health (5)
- ❑ Government (7)
- ❑ Pharmacy (8)
- ❑ Pharmaceutical/Manufacturing (9)
- ❑ Nursing Home (10)
- ❑ Home Health (11)

7. Circle one Primary (P) and one Secondary (S)Specialty

P	S	AD Administration	P	S	IU Immunology	P	S	PH Public Health
P	S	BC Biochemistry	P	S	ME Metabolism	P	S	PM Pharmacy
P	S	CA Cardiology	P	S	NE Nephrology	P	S	PN Pediatric Endocrinology
P	S	DE Dermatology	P	S	NR Neurology			
P	S	DO Dentistry	P	S	NS Nursing	P	S	PO Podiatry
P	S	ED Education	P	S	NU Nutrition	P	S	PR Pedorthic Management
P	S	EN Adult Endocrinology	P	S	OG Obstetrics/Gyn.			
P	S	EP Epidemiology	P	S	OP Ophthalmology	P	S	PS Psychiatry
P	S	EX Exercise Physiology	P	S	OR Orthopedics	P	S	PT Pathology
P	S	FP Family Practice	P	S	OS Osteopathy	P	S	PX Physical Therapy
P	S	GE Geriatrics	P	S	OT Optometry	P	S	PY Physiology
P	S	GP General Practice	P	S	PA Pharmacology	P	S	SU Surgery
P	S	GT Genetics	P	S	PC Psychology	P	S	SW Social Work
P	S	IM Internal Medicine	P	S	PE Pediatrics	P	S	UR Urology

8. Enclose Payment and Mail Today!

❑ New Membership or ❑ Renewing Membership

Member Dues:

Category I $_____ or **Category II** $_____

Additional Subscriptions: $_____ $_____

Additional Councils _____ x $25 = $_____

Taxes: Canadians must add 7% GST tax $_____

TOTAL ENCLOSED: $_____

Dues amount set aside for publications are:

- Category I (Diabetes or Diabetes Care): $75
- Category II (Diabetes Spectrum): $30 (Diabetes Forecast): $12

Payment Options: Mail payment and application to:

American Diabetes Association.
Professional Section Membership
1660 Duke Street
Alexandria, VA 22314

❑ Check: *payable to the American Diabetes Association*

❑ Charge my ❑ VISA ❑ MasterCard ❑ American Express

Account No.# _____

Expiration Date _____

Date: _____

Signature: _____

JCAD497

For fast service, call or send a fax to our membership department:

Phone: (703) 549-1500
Fax: (703) 549-6995

Allow 4-6 weeks to process your order. Prices are subject to change. Payment must accompany order in US funds drawn on US bank.

Two Reasons to Join ADA Today:

Fax your credit card order to (703) 549-6995

1 Choose Your Membership Category

2 Receive Members-only Discounts (on meetings, subscriptions & more)

Introducing ADA's Membership Categories

Fulfill Your Information Needs-
The American Diabetes Association offers two low-priced Professional Section membership options that let you receive the publications ***you want***—at a price that fits your budget.

Category I-Entitles you to choose between *Diabetes* or *Diabetes Care*, plus the opportunity to subscribe to additional ADA journals at reduced member prices. This category is recommended for physicians

Category II-Entitles you to *Diabetes Spectrum* and *Diabetes Forecast*, plus the opportunity to subscribe to additional ADA journals at reduced member prices.

Plus Enjoy Additional Member Benefits-
Both membership categories offer a wide range of benefits, including discounts on ADA's Scientific Sessions, Postgraduate Course, and other educational programs; the Professional Section *Membership Directory*; eligibility for ADA research grants and awards; one free Professional Section Council membership; local ADA affiliate membership; the *Professional Section News*; and *Clinical Practice Recommendations* and a discount to **BRS Colleague.**

In-training Membership Rates-
You are eligible to become a Member-In-Training if you have received your first professional degree within the last five years. This qualifies you for dues at half-price. Also, you will be eligible to subscribe to additional ADA journals at the same reduced rates as other members.

Check These Exclusive Member Benefits

☑ **Your Choice of Publications**

Diabetes—the world's most-cited journal of diabetes research brings you the latest findings from the world's top scientists.

Diabetes Care—the premier journal of clinical diabetes research and treatment. *Diabetes Care* keeps you current with original research reports, news and commentaries, case studies and reviews.

Diabetes Reviews—the comprehensive review articles in ADA's newest journal are a convenient way for the busy clinician to keep up-to-date on what's truly new in research.

Diabetes Spectrum—translates research into practice for nurses, dietitians, and other health-care professionals involved in patient education and counseling.

Clinical Diabetes—for the primary-care provider as well as other diabetes specialists, this newsletter offers articles and abstracts highlighting recent advances in diabetes treatment.

Diabetes Forecast—ADA's magazine for patients and their families features advice on diet, exercise, and other lifestyle changes, plus the latest developments in new technology and research. It is a valuable tool for patient education.

☑ **Professional Section Report**
This quarterly newsletter highlights Professional Section events and other ADA news.

☑ **FREE Council Membership**
Professional Section Councils give you an opportunity to network with members from different specialties who share your interest in a specific area of diabetes research or care. One free Council membership is included with your membership. Additional Council memberships are available for $25 each.

☑ **Membership Directory Listing**
Your link to a valuable network of more *than twelve thousand* diabetes experts. Locate your colleagues by specialty, location, and Professional Section Council membership.

☑ **Eligibility for ADA Research Grants and Awards**
An exclusive benefit. Only members of the Professional Section are eligible to receive ADA grants that support diabetes research. In addition, annual awards are presented to physicians, diabetes educators, and researchers to honor outstanding performance.

☑ **Discounts on ADA Scientific and Medical Programs**
Save on registration for ADA's Scientific Sessions, Postgraduate Course, and ADA-sponsored symposia. ADA meetings are accredited for CME credits.

☑ **Local Affiliate Membership**
Your Professional Section membership also entitles you to membership in your local ADA affiliate where you can participate in patient and professional education programs, network with other professionals, and actively participate in shaping the future of ADA.

☑ **Free copy of Clinical Practice Recommendations**
This extensive guide details the current ADA standards of clinical care. The Position Statements in *Clinical Practice Recommendations* are convenient and important resources for all health-care professionals who care for people with diabetes.

In hypertension
...even for your complex patients

NEW
ONCE-A-DAY

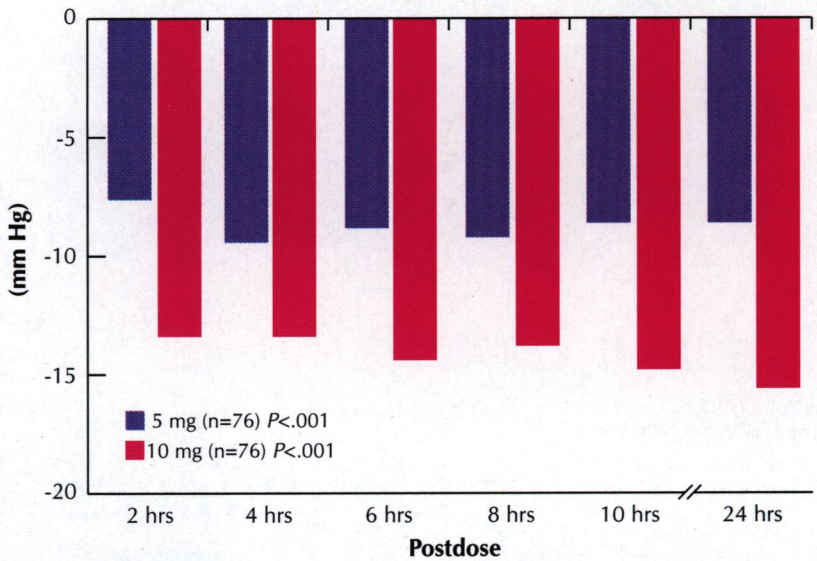
24-Hour Control
With Confidence

New, Once-a-day
DynaCirc CR®
(isradipine) 5 mg and 10 mg GITS controlled release tablets

EFFECTIVE 24-HOUR HYPERTENSION CONTROL

- Convenient, once-daily dosing with DynaCirc CR® (isradipine)
- Constant drug release with the GITS* delivery system
- Sustained blood pressure reduction, consistent over 24 hours[1]

Mean change from baseline in sitting diastolic blood pressure over 24 hours[1]



At 6 weeks in intent-to-treat patients. A similar pattern was observed with sitting systolic blood pressure: reduction at 24 hours was -11.0 mm Hg, 5 mg; -20.0 mm Hg, 10 mg.

* Gastrointestinal Therapeutic System

THE PROVEN SAFETY OF DYNACIRC® (ISRADIPINE)

- Well tolerated
  - In elderly patients[2]
  - In renally impaired and diabetic patients[3-6]
  - In patients with underlying cardiovascular conditions[3,7*]
- No known clinically significant drug interactions
  - With the medications most commonly prescribed for the elderly[8]
  - No alterations of digoxin or warfarin clearance
- Mild and transient side effects
  - The most common are headache (lower than placebo at both 5 and 10 mg), edema (8.9% at 5 mg; 12.7% at 10 mg; 3.6% placebo), and dizziness

* Caution should be exercised when using the drug in congestive heart failure patients, particularly in combination with a beta blocker.

New, Once-a-day

DynaCirc CR®

(isradipine) 5 mg and 10 mg GITS controlled release tablets

In hypertension...

THE CONFIDENCE OF 24-HOUR EFFICACY AND PROVEN SAFETY

NEW
ONCE-A-DAY

- Once-daily dosing
- Effective, consistent 24-hour hypertension control with the GITS delivery system
- Safe to use in the elderly and in patients with concomitant conditions
- No known clinically significant drug interactions
- Side effects are mild and transient, the most frequent being headache, edema, and dizziness
- Priced 10% to 20% lower than either Norvasc® or Procardia XL®*

* Based on AWP comparison, which is a published list price and may not represent actual price paid by pharmacies and consumers. Norvasc® is a registered trademark of Pfizer Labs Division, Pfizer Inc; Procardia XL® is a registered trademark of Pratt Pharmaceuticals, a division of Pfizer Inc.

Please see adjacent brief summary of prescribing information.

New, Once-a-day

DynaCirc CR®
(isradipine) 5 mg and 10 mg GITS controlled release tablets

REFERENCES

1. Chrysant SG, Cohen M. Sustained blood pressure control with controlled-release isradipine (isradipine-CR). *J Clin Pharmacol.* 1995;35:239-243. **2.** Cohen ID, Carr AA, Blecker D, et al. DYNAMICS trial of isradipine in hypertension with and without coexisting diseases. *Clin Geriatr.* 1994;2:36-52. **3.** Marcus AO. Antihypertensive therapy with the calcium channel blocker isradipine: an appropriate choice for the diabetic patient with hypertension. Review. *Curr Ther Res.* 1993;54:763-778. **4.** Berg KJ, Holdaas H, Endresen L, et al. Effects of isradipine on renal function in cyclosporin-treated renal transplanted patients. *Nephrol Dial Transplant.* 1991;6:725-730. **5.** Francischetti EA, da Silva IB, Fagundes VG. Effects of long-term administration of isradipine on renal hemodynamics and sodium metabolism. *J Cardiovasc Pharmacol.* 1992;19(suppl 3):90-92. **6.** Persson B, Andersson OK, Wysocki M, et al. Renal and hemodynamic effects of isradipine in essential hypertension. *Am J Med.* 1989;86(suppl 4A):60-64. **7.** Grossman E, Messerli FH, Oren S, et al. Cardiovascular effects of isradipine in essential hypertension. *Am J Cardiol.* 1991;68:65-70. **8.** Data from *PDDA Top 100 Drug Uses in Patients 65 Years or Older.* March 1995 to March 1996.

SANDOZ
SANDOZ PHARMACEUTICALS CORPORATION
EAST HANOVER, NEW JERSEY 07936

DCR-0996-02 12/96

DynaCirc CR® *(isradipine)* Controlled Release Tablets

BRIEF SUMMARY: Please see package insert for full prescribing information.

Caution: Federal law prohibits dispensing without prescription.

INDICATIONS AND USAGE: Hypertension: DynaCirc CR® (isradipine) is indicated in the management of hypertension. It may be used alone or concurrently with thiazide-type diuretics.

CONTRAINDICATIONS: DynaCirc CR® (isradipine) is contraindicated in individuals who have shown hypersensitivity to any of the ingredients in the formulation.

WARNINGS: None.

PRECAUTIONS: General: Blood Pressure: Because DynaCirc CR® (isradipine) decreases peripheral resistance, like other calcium blockers DynaCirc CR® (isradipine) may occasionally produce symptomatic hypotension. However, symptoms like syncope and severe dizziness have rarely been reported in hypertensive patients administered DynaCirc CR® (isradipine), particularly at the initial recommended doses (*see DOSAGE AND ADMINISTRATION in the full prescribing information*).

Use in Patients with Congestive Heart Failure: Although acute hemodynamic studies in patients with congestive heart failure have shown that immediate-release DynaCirc® (isradipine) reduced afterload without impairing myocardial contractility, it has a negative inotropic effect at high doses *in vitro* and possibly in some patients. Caution should be exercised when using DynaCirc CR® (isradipine) in congestive heart failure patients, particularly in combination with a beta-blocker.

Peripheral Edema: Peripheral edema, when it occurs, is usually mild to moderate in severity. It is a localized phenomenon thought to be associated with vasodilation of arterioles and other small blood vessels, and not due to left ventricular dysfunction or generalized fluid retention. Peripheral edema is dose-related with an incidence ranging from approximately 9% at 5 mg, 13% at 10 mg, 16% at 15 mg, and 36% at the highest dose studied (20 mg once-daily). With patients whose hypertension is complicated by congestive heart failure, care should be taken to differentiate this edema from the effects of decreasing left ventricular function. Although the frequency of edema is correlated with dose, no DynaCirc CR® (isradipine) treated patients discontinued the short-term (6 weeks or less), placebo-controlled hypertension studies as a result of edema. Less than 5% of DynaCirc CR® (isradipine) treated patients in long-term studies discontinued due to edema.

Other: As with any other non-deformable material, caution should be used when administering DynaCirc CR® (isradipine) in patients with pre-existing severe gastrointestinal narrowing (pathologic or iatrogenic). There have been reports of obstructive symptoms in patients with known strictures associated with ingestion of other GITS products.

Information for Patients: DynaCirc CR® (isradipine) Controlled Release Tablets should be swallowed whole. Do not chew, divide or crush tablets. Do not be concerned if you occasionally notice in your stool something resembling a tablet. In DynaCirc CR® (isradipine), the medication is contained within a nonabsorbable shell that has been specially designed to slowly release the drug for your body to absorb. When this process is completed, the empty tablet shell is eliminated in the stool.

Drug Interactions: Nitroglycerin: Immediate-release DynaCirc® (isradipine) has been safely coadministered with nitroglycerin.

Hydrochlorothiazide: A study in normal healthy volunteers has shown that concomitant administration of immediate-release DynaCirc® (isradipine) and hydrochlorothiazide does not result in altered pharmacokinetics of either drug. In a study in hypertensive patients, addition of isradipine to existing hydrochlorothiazide therapy did not result in any unexpected adverse effects, and isradipine had an additional antihypertensive effect.

Propranolol: In a single dose study in normal volunteers using immediate-release DynaCirc® (isradipine), co-administration of propranolol had a small effect on the rate but no effect on the extent of isradipine bioavailability. Significant increases in AUC (27%) and C_{max} (58%) and decreases in t_{max} (23%) of propranolol were noted in this study.

Digoxin: The concomitant administration of immediate-release DynaCirc® (isradipine) and digoxin in a single-dose pharmacokinetic study did not affect renal, non-renal and total body clearance of digoxin.

Fentanyl Anesthesia: Severe hypotension has been reported during fentanyl anesthesia with concomitant use of a beta blocker and a calcium channel blocker. An increased volume of circulating fluids might be required if such an interaction were to occur.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Treatment of male rats for 2 years with 2.5, 12.5, or 62.5 mg/kg/day isradipine admixed with diet (approximately 6, 31, and 156 times the maximum recommended daily dose based on a 50 kg man) resulted in dose dependent increases in the incidence of benign Leydig cell tumors and testicular hyperplasia relative to untreated control animals. These findings, which were replicated in a subsequent experiment, may have been indirectly related to an effect of isradipine on circulating gonadotropin levels in the rats; a comparable endocrine effect was not evident in male patients receiving therapeutic doses of the drug on a chronic basis. Treatment of mice for two years with 2.5, 15, or 80 mg/kg/day isradipine in the diet (approximately 6, 38, and 200 times the maximum recommended dose based on a 50 kg man) showed no evidence of oncogenicity. There was no evidence of mutagenic potential based on the results of a battery of mutagenic tests. No effect on fertility was observed in male and female rats treated with up to 60 mg/kg/day isradipine.

Pregnancy: Pregnancy Category C: Isradipine was administered orally to rats and rabbits during organogenesis. Treatment of pregnant rats with doses of 6, 20, or 60 mg/kg/day produced a significant reduction in maternal weight gain during treatment with the highest dose (150 times the maximum recommended human daily dose) but with no lasting effects on the mother or the offspring. Treatment of pregnant rabbits with doses of 1, 3, or 10 mg/kg/day (2.5, 7.5, and 25 times the maximum recommended human daily dose) produced decrements in maternal body weight gain and increased fetal resorption at the two higher doses. There was no evidence of embryotoxicity at doses tested. In a peri/postnatal administration study in rats, reduced maternal body weight gain during late pregnancy at oral doses of 20 and 60 mg/kg/day isradipine was associated with reduced birth weights and decreased peri and postnatal pup survival.

There are no adequate and well controlled studies in pregnant women. The use of DynaCirc CR® (isradipine) during pregnancy should only be considered if the potential benefit outweighs potential risks.

Nursing Mothers: It is not known whether DynaCirc® (isradipine) is excreted in human milk. Because many drugs are excreted in human milk, and because of the potential for adverse effects of DynaCirc® (isradipine) on nursing infants, a decision should be made as to whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use: Safety and effectiveness have not been established in children.

ADVERSE REACTIONS: In a controlled clinical trial with DynaCirc CR® (isradipine), dose-related edema occurred at an incidence of approximately 9% at 5 mg, 13% at 10 mg, 16% at 15 mg, and 36% at the highest dose studied (20 mg), was mild to moderate in severity, and was not related to age or gender. The incidences of elicited or volunteered adverse reactions (excluding non-drug related) are based on 6-week multicenter, placebo-controlled, double-blind hypertension studies. Less than 1% of DynaCirc CR® (isradipine) or placebo-treated patients discontinued from these studies due to adverse reactions. The following adverse reactions have been reported by 1% or greater for patients receiving DynaCirc CR® (isradipine) at any dose (N=422): edema 15.2%, headache 13.0%, dizziness 4.7%, fatigue 4.3%, abdominal discomfort 2.8%, flushing 1.9%, constipation 1.7%, palpitations 1.2%, nausea 1.2%, and abdominal distention 1.2%. The following adverse experiences were reported in 0.5%-1.0% or less of DynaCirc CR® (isradipine) or immediate-release DynaCirc® (isradipine) treated patients in hypertensive studies, or were noted in postmarketing experience with immediate-release DynaCirc® (isradipine) Capsules. More serious events are shown in italics. The relationship of these adverse experiences to isradipine administration is uncertain. **Skin:** pruritus, *urticaria*. **Musculoskeletal:** backache/pain, joint pain, neck pain/sore/stiff, legs ache/pain, cramps of legs/feet. **Respiratory:** dyspnea, nasal congestion, cough. **Cardiovascular:** epistaxis, tachycardia, chest pain, shortness of breath, hypotension, *syncope, atrial or ventricular fibrillation, myocardial infarction, heart failure*. **Gastrointestinal:** diarrhea, vomiting, appetite increased or decreased. **Urogenital:** pollakiuria, impotence, dysuria, nocturia. **Central Nervous:** drowsiness, insomnia, lethargy, nervousness, libido decrease/frigidity, impotence, depression, *paresthesia* (which includes numbness and tingling), *transient ischemic attack, stroke*. **Autonomic:** dry mouth, hyperhidrosis, visual disturbance. **Miscellaneous:** weight gain, throat discomfort, *drug fever, leukopenia, elevated liver function tests*.

No gastrointestinal bleeding has been reported in clinical trials with DynaCirc CR® (isradipine) Controlled Release Tablets. In a long-term (one-year) DynaCirc CR® (isradipine) open-label, hypertension trial, the adverse events reported were generally the same as those seen in the short-term placebo-controlled studies. About 6% of DynaCirc CR® (isradipine) treated patients discontinued the long-term trial due to adverse reactions.

Store and Dispense: Below 86°F (30°C) in a tight container, protected from moisture and humidity.

Sandoz Pharmaceuticals Corporation, East Hanover, New Jersey 07936
REV: AUGUST 1996

P37022904