Streptozotocin-Induced Diabetic Pregnant Rats Exhibit Signs and Symptoms Mimicking Preeclampsia

Go Ishihara, Yuji Hiramatsu, Hisashi Masuyama, and Takafumi Kudo

The number of patients with hypertension, obesity, diabetes, and hyperlipidemia is increasing. This tendency is observed in pregnant women, in whom many obstetrical and perinatal complications occur. The prevention of these abnormalities is important in reducing perinatal mortality and the risk of coronary disease. We established a pregnant rat model with diabetes and signs and symptoms mimicking preeclampsia. On day 6 of pregnancy, streptozotocin (STZ) or citrate buffer was injected into the tail vein. After STZ administration, plasma glucose was increased within 48 hours and sustained at a high level until day 20 of pregnancy, and plasma insulin was decreased. Fetuses from STZ-treated mothers were growth-restricted, and plasma glucose was 6-fold higher in fetuses of STZ-treated versus control rats. The systolic blood pressure, urinary protein, and hematocrit were increased significantly in STZ-treated rats. Total cholesterol and triglycerides were also elevated in STZ-treated rats, but plasma leptin levels were decreased. The STZ-induced diabetic pregnant rat model exhibited preeclampsia, hemoconcentration, hyperlipidemia, hypoleptinemia, and intrauterine growth restriction. This model closely mimics the features of human pregnancy complicated by diabetes and is useful for the basic study of the pathophysiology of pregnancy with diabetes.

THE NUMBER OF PATIENTS with hypertension, obesity, diabetes, and hyperlipidemia is increasing rapidly in many countries, including Japan. This tendency is also found in pregnant women, and if they are obese and/or diabetic, they may experience many obstetrical and perinatal complications such as preeclampsia, fetal distress, macrosomnia, and an increased rate of cesarean section.[1]

In the nonpregnant patient, the characteristic findings of hyperinsulinemia, impaired glucose tolerance, hyperlipidemia, and hypertension are known as syndrome X.[2] The combination of these abnormalities can place patients at high risk for coronary disease, even if each abnormality is clinically mild. When obesity coexists with syndrome X, this condition has been reported as visceral fat accumulation syndrome,[3] the deadly quartet,[4] or insulin resistance syndrome.[5] It is thought that these conditions are closely interrelated and in the same disease spectrum.

Pregnancy is a physiological state associated with significant changes in appetite, blood pressure, lipid metabolism, and functions regulated in part by hormones. Pregnant women with obesity and/or glucose intolerance tend to develop preeclampsia,[6,7] and impaired glucose tolerance is often found in pregnant women with preeclampsia.[8] The perinatal mortality rate is increased 20-fold for preeclamptic diabetic women compared with those who are normotensive.[9] Hyperlipidemia, a well-established risk factor for coronary heart disease, is common in the second half of pregnancy.[10]

The purpose of this study was twofold. The first was to establish an animal model of pregnancy complicated by diabetes and preeclampsia since there are no reports of a model of this type, and to investigate the pathophysiology of diabetic pregnancy. We used streptozotocin (STZ), which is useful for inducing insulin-dependent diabetes and investigating various complications in nonpregnant laboratory rodents.[11,12] The second purpose was to investigate the metabolism and the effects on the fetus in this diabetic model and to clarify the relationships among diabetes mellitus, hypertension, obesity, and hyperlipidemia in pregnancy.

MATERIALS AND METHODS

Female Wistar rats weighing 200 to 230 g and their fetuses were used in this study. They were kept in a temperature- and light-controlled room with free access to food and water. For breeding, female rats were housed with mature male rats. The vaginal smear was checked the next morning, and the day that a sperm-positive result was obtained was designated day 0 of pregnancy. The pregnant rats were transferred to individual cages during the period of pregnancy.

On day 6 of pregnancy, STZ (Sigma Chemical, St Louis, MO) 30 mg/kg body weight in 0.1 mol/L citrate buffer, pH 4.5, was injected into the tail vein (STZ-treated). Control rats were injected with citrate buffer on the same day. Maternal blood glucose was determined in blood sampled from the tail vein. Diabetes was confirmed 48 hours after STZ administration, and maternal blood glucose was also determined on days 6, 8, 13, and 20 of pregnancy. The blood glucose level was measured by the glucose oxidase method using a Medisafe automated analyzer (Termo, Tokyo, Japan).

The systolic blood pressure of rats in both groups was measured on days 6, 13, and 19 of pregnancy by the tail-cuff method using a Softron BP98A tail-cuff hemodynamometer (Softron, Tokyo, Japan) after the behavior and heart rate of the rat was stabilized. Blood pressure is reported as the mean of at least 3 measurements performed during the same session, which had to vary by less than 5%. Most of the blood pressure values were within the required range while the rats were stable. The rats were transferred to metabolic cages in the evening on day 19 of pregnancy, and 12-hour urine was collected. The urinary protein level was measured by the pyrogallol red method.[13]

On day 20 of pregnancy, the rats were anesthetized with ether and the maternal blood was collected by heart puncture, after which a cesarean section was performed. The fetal weight and placental weight were measured, and the maternal pancreas was removed for immunohistochemical study. The paraffin-embedded pancreas tissue was stained using guinea pig anti-insulin (L1859), rabbit anti-porcine glucagon (L1813), and a Dako System 40 PAP Kit (Dako, Carpinteria, CA). The blood was centrifuged at 3,000 rpm for 10 minutes, and the plasma was stored at −40°C until analysis for insulin, cholesterol, triglyceride, and

From the Department of Obstetrics and Gynecology, Okayama University Medical School, Okayama, Japan.

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Address reprint requests to Yuji Hiramatsu, MD, Department of Obstetrics and Gynecology, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700-8558, Japan.

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leptin. Fetal blood was collected from the jugular vein and the blood glucose level was determined immediately. Plasma insulin and leptin were determined by a rat insulin and leptin enzyme immunoassay system (Amersham, Tokyo, Japan). The intraassay coefficient of variation was 3% and 4%, respectively. Plasma total cholesterol and triglycerides were determined by the Cholesterol-E kit and Triglyceride-E kit (Wako Pure Chemical Industries, Osaka, Japan). The intraassay coefficient of variation for cholesterol and triglyceride was less than 4%.

In this study, we also injected STZ (30 mg/kg body weight) into the nonpregnant rats and measured the systolic blood pressure on days 0, 7, and 13 after STZ administration, the urinary protein level on day 13, and the plasma lipid levels on day 14. The methods used were the same as for pregnant rats.

Statistical Analysis

The results are reported as the mean ± SD. Statistical analysis was performed with the unpaired Student's *t* test for comparison of 2 groups or a multiple-comparison test (Scheffe's test) for comparison of more than 2 groups. Analyses were performed with the software package StatView version 5.0 (Abacus Concepts, Berkeley, CA) on a Macintosh personal computer (Apple Japan, Tokyo, Japan). Statistical significance was set at a P level less than .05.

RESULTS

The changes in plasma glucose for the control and STZ-treated rats are shown in Fig 1. Blood glucose was rapidly elevated after administration of STZ and showed a significantly high level after 7 days in STZ-treated rats (control *v* STZ, 76.6 ± 13.4 *v* 325.2 ± 76.4 mg/dL, $P < .001$), and these differences persisted till term. In nonpregnant rats, blood glucose increased similarly after administration of STZ. The maternal plasma insulin level on day 20 of pregnancy was significantly lower in STZ-treated rats versus control rats (control *v* STZ, 5.25 ± 2.32 *v* 2.95 ± 2.14 ng/mL, $P < .01$; Fig 2). Immunohistochemical staining of STZ-treated rat pancreas also showed a marked decrease in β cells, but no changes were observed in α cells (data not shown).

There were no differences in maternal body weight (control *v* STZ, 215 ± 23 *v* 213 ± 18 g) and litter size (control *v* STZ, 12.6 ± 1.5 *v* 11.8 ± 1.0) between groups. Fetuses from STZ-treated mothers were growth-retarded and had a significantly reduced body weight compared with control fetuses. However, the placental weight was significantly higher in STZ-treated rats compared with control rats. Fetal blood glucose was 6-fold higher in STZ-treated rats versus control rats (Table 1).

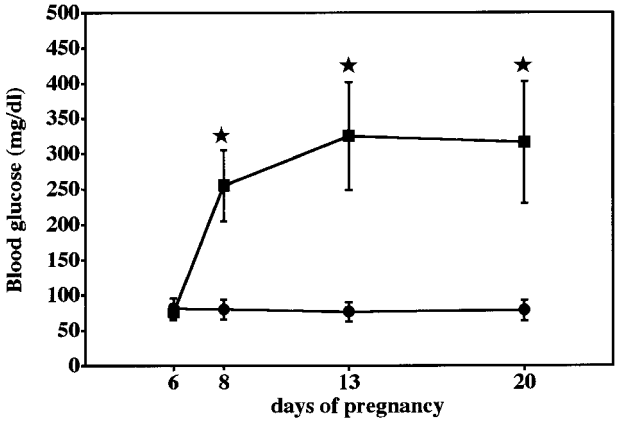

Fig 1. Changes in blood glucose in control (●; n = 15) and STZ-treated (■; n = 13) rats during pregnancy. Citrate buffer or STZ (30 mg/kg body weight) was injected on day 6 of pregnancy. Results are the mean ± SD. ★$P < .001$ *v* control.

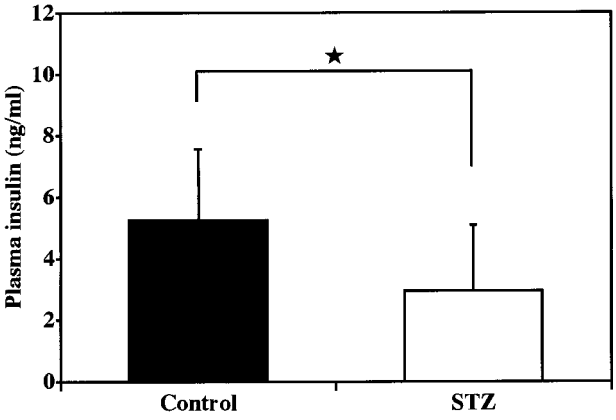

Fig 2. Plasma insulin in control (n = 17) and STZ-treated (n = 14) rats on day 20 of pregnancy. Results are the mean ± SD. ★$P < .01$ *v* control.

After administration of STZ, systolic blood pressure was markedly elevated and the difference persisted till term in STZ-treated rats, but there was no remarkable change in control rats during the experiment (Fig. 3). Systolic blood pressure in nonpregnant STZ-treated rats also showed no change (day 0, 102 ± 7 mm Hg; day 7, 101 ± 10 mm Hg; day 13, 97 ± 6 mm Hg; n = 5). The maternal urinary volume on day 20 of pregnancy was increased in STZ-treated rats (control *v* STZ, 11.0 ± 4.8 *v* 22.8 ± 8.3 mL/12 h, $P < .001$), and urinary protein levels were also higher in STZ-treated rats versus control rats (control *v* STZ, 13.8 ± 11.7 *v* 78.7 ± 69.9 mg/dL, $P < .05$) and nonpregnant STZ-treated rats (15.4 ± 11.2 mg/dL; Fig 4).

Complete blood cell counts are shown in Table 2. No significant differences were observed among the 3 groups for any parameter except the hematocrit, which was significantly higher in STZ-treated rats compared with control and nonpregnant STZ-treated rats ($P < .05$).

Plasma lipid values are listed in Table 3. Triglyceride levels were higher in STZ-treated rats versus control and nonpregnant STZ-treated rats ($P < .001$ and $P < .05$, respectively). Total cholesterol was higher in STZ-treated rats than in control rats ($P < .05$). Plasma leptin in normal pregnancy increased 2.4-fold from a mean of 3.39 ± 0.81 ng/mL before pregnancy to 8.00 ± 3.77 ng/mL on day 20 of pregnancy ($P < .05$; Fig 5), but plasma leptin in STZ-treated rats decreased to 1.75 ± 1.41 ng/mL on day 20 of pregnancy.

DISCUSSION

We have confirmed intrauterine growth restriction of fetuses in our STZ-treated rat model, which exhibited blood glucose of about 320 mg/dL. This model showed the characteristic signs and symptoms resembling preeclampsia, ie, hypertension, proteinuria, hemoconcentration, and hyperlipidemia, and these findings were specific in STZ-treated pregnant rats. However,

Table 1. Maternal, Fetal, and Placental Weight and Fetal Blood Glucose in Both Groups

Parameter	Control		STZ		P
	Mean ± SD	No.	Mean ± SD	No.	
Maternal weight (g)	215 ± 23	14	213 ± 18	15	NS
Fetal weight (g)	3.52 ± 0.25	151	3.27 ± 0.4	161	<.001
Placental weight (g)	0.54 ± 0.07	151	0.61 ± 0.07	161	<.001
Fetal blood glucose (mg/dL)	34.8 ± 7.8	55	213.2 ± 102.3	57	<.001

Abbreviation: NS, not significant.

placental weight was increased, in contrast to the findings usually observed in preeclampsia.

The altered maternal-fetal metabolic fuel relations resulting from diabetes in pregnancy modulate fetal growth. The increases in fetal glucose and insulin availability with maternal diabetes are strongly associated with the development of fetal macrosomnia,[14] but severe diabetes mellitus or diabetes of long duration restrict fetal growth.[15] Rat fetal body weight correlates positively with maternal plasma glucose in diabetic rats with a glucose level less than 220 mg/dL, but it correlates negatively in rats with a level above 220 mg/dL.[16]

The increase in glucose availability to the fetus resulting from severe maternal diabetes downregulates glucose transporter-1, thereby limiting glucose availability to cells, and thus contributes to fetal growth restriction.[17] In most fetal tissue, glucose transporters control glucose entry into cells. Glucose transporter-1 appears to be the predominant isoform in most fetal and neonatal rat tissues, including brain and muscle,[18] and insulin is an important modulator of glucose transport in both the fetus and the adult animal. Changes in the availability of other fuels such as ketones and fatty acids[19] or growth factors such as insulin-like growth factor-I may contribute to fetal growth restriction in diabetic rats.

The incidence of hypertension is known to be increased in diabetic pregnant women.[6] The classic studies by White[20] in a series of 439 diabetic pregnancies showed that insulin-dependent diabetic women are at higher risk to develop hypertension during pregnancy than nondiabetic women. The overall increase in hypertensive complications in pregnant women with diabetes was 24%. Preeclampsia was diagnosed in 13% of the diabetic women, as compared with 10% of the general pregnant population.[21] Hypertension and/or albuminuria was associated with 71% of stillbirths and 52% of neonatal deaths in diabetic pregnancies.[21] Pedersen's study[7] also suggested that insulin-dependent diabetic patients who develop pregnancy-induced hypertension have a significantly higher perinatal morbidity and mortality rate. In our study, signs and symptoms mimicking preeclampsia were observed in STZ-treated rats. These findings suggest that it is very important to control maternal diabetes carefully.

Although the precise mechanisms of preeclampsia are still obscure and require elucidation, it is well established that in preeclampsia there is a relative increase in vasoconstricting, platelet-aggregating prostaglandins such as thromboxane A_2 and prostaglandin F_2, and similar alterations are observed in diabetes.[22] Vascular prostacyclin synthesis is decreased in both patients and experimental animals with diabetes.[23] We believe that these factors contribute to hypertension in STZ-treated rats. Proteinuria, a sign of preeclampsia, is also found in STZ-treated rats. Hypertension accelerates the development of proteinuria and glomerulosclerosis in several types of renal disease. The proteinuria in diabetic nephropathy may arise primarily from a disturbance of the charge barrier of the glomerular basement membrane, and destruction of the size barrier is believed to contribute to the development of proteinuria.[24] It has also been reported that diabetic Wistar rats exhibited a thicker glomerular basement membrane than control rats.[25]

Pregnancy is a hypermetabolic state associated with a physiological increase in maternal body fat and weight. Increased serum triglyceride and total cholesterol levels were observed during pregnancy[26] and in nonpregnant STZ-induced

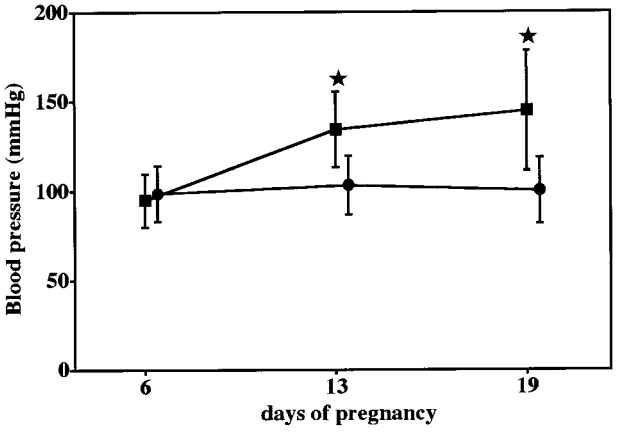

Fig 3. Changes in systolic blood pressure in control (●; n = 17) and STZ-treated (■; n = 14) rats during pregnancy. Citrate buffer or STZ (30 mg/kg body weight) was injected on day 6 of pregnancy. Results are the mean ± SD. ★*P* < .001 *v* control.

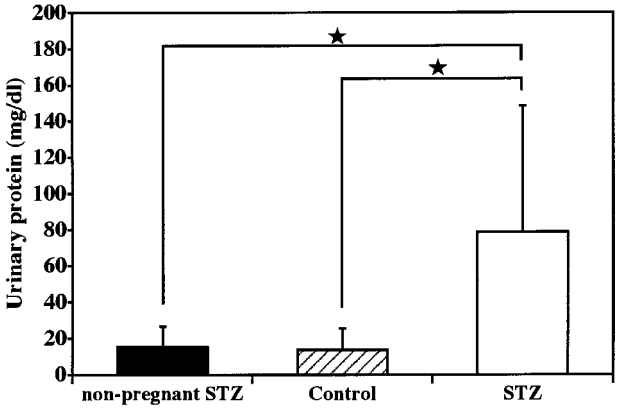

Fig 4. Urinary protein in nonpregnant STZ-treated (n = 4), control (n = 11), and STZ-treated (n = 14) rats on day 19-20 of pregnancy. Results are the mean ± SD. ★*P* < .05.

Table 2. Blood Parameters in the Three Groups

Parameter	Nonpregnant STZ (n = 7)	Control (n = 12)	STZ (n = 13)
White blood cell count (per μL)	4,943 ± 2,020	4,742 ± 1,639	5,438 ± 1,909
Red blood cell count ($\times 10^4$/μL)	568 ± 44	549 ± 34	548 ± 50
Hemoglobin (g/dL)	11.9 ± 0.8	11.5 ± 0.6	11.9 ± 0.8
Hematocrit (%)	34.4 ± 1.2	34.1 ± 1.2	37.5 ± 2.6*
Platelet ($\times 10^4$/μL)	119 ± 17	114 ± 14	101 ± 16

NOTE. Results are the mean ± SD.

*$P < .05$ *v* nonpregnant STZ-treated and control.

diabetic rats.[27] Our study obtained the same findings for diabetic pregnant rats. The hyperphagia of diabetic rats induces hypertrophy of the intestinal mucosa, and cholesterol synthesis is strikingly increased in the gut.[28] Cholesterol absorption is increased due to the enhanced activity of acylcoenzyme A: cholesterol acyltransferase, the enzyme that catalyzes cholesterol esterification.[29] In addition, all of these metabolic changes observed in diabetes and physiological changes in pregnancy may be present also in lipid metabolism in diabetic pregnant rats.

Finally, we investigated the plasma leptin level, which is closely related to obesity. Leptin, a hormone secreted by adipocytes, has a central role in the regulation of appetite and energy and energy expenditure.[30] It has also been implicated in the regulation of reproductive function, promoting maturation of the reproductive system and signaling the presence of adequate maternal fat stores to initiate reproduction.

Serum leptin on day 20 of pregnancy was significantly higher in control rats versus nonpregnant and STZ-treated rats. Rat serum leptin levels were modulated during normal pregnancy, with a 1.8-fold increase at 20 day of pregnancy followed by a decrease just prior to parturition.[31] These changes paralleled the increase in body weight and leptin receptor mRNA in the uterus.[31] In human pregnancy, serum leptin at 36 weeks of pregnancy is significantly higher than the postpartum level and correlates with the weight, body mass index, fat mass, and serum insulin level.[32] Others have also reported that plasma leptin is correlated with plasma insulin in humans and animals.[33,34]

The close association between hyperinsulinemia and hyperleptinemia suggests that ob gene expression may be mediated by insulin.[35] Given the relationship between circulating insulin and leptin concentrations, insulin is considered a candidate hormonal regulator of leptin synthesis and/or secretion. It was recently reported that glucose uptake and metabolism are involved in the regulation of leptin expression and secretion in

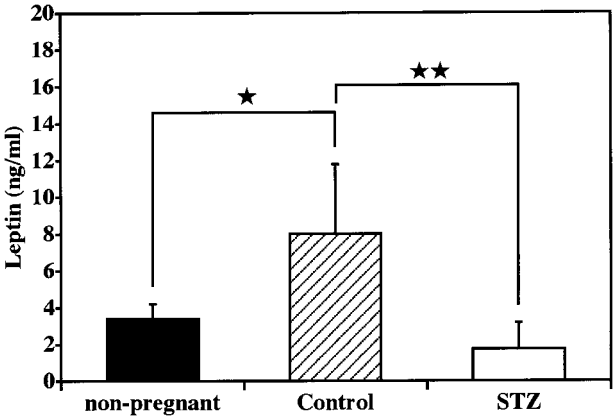

Fig 5. Plasma leptin in nonpregnant (n = 6), control (n = 14), and STZ-treated (n = 14) rats on day 20 of pregnancy. Results are the mean ± SD. ★$P < .05$, ★★$P < .001$.

cultured rat adipocytes.[36] Several studies have found that ob gene expression is increased after administration of insulin or glucose[37] and is decreased in insulin-dependent diabetic animals.[38] The findings of these studies closely agree with our own. In addition, other factors such as progesterone, estrogen, and human placental lactogen, which increase during pregnancy, may affect the expression of the ob gene during pregnancy.

In our study, the circulating plasma leptin concentration was markedly decreased in STZ-treated rats. It has been reported that a decrease in plasma leptin after 2 weeks of diabetes was related to reduced adiposity and inversely correlated with the plasma glucose concentration.[39] These findings suggest that the short-term effect of STZ-induced diabetes to decrease plasma leptin is mainly the result of insulin deficiency and a decrease in glucose uptake and metabolism, whereas in diabetes of longer duration, both reduced adiposity and insulin deficiency contribute to hypoleptinemia.

STZ-induced diabetes is associated with insulin resistance[40] in addition to insulin deficiency, and our model has many features found in human diabetic disease. The STZ-induced diabetic pregnant rat model outlined in this study exhibited signs and symptoms mimicking preeclampsia such as hypertension, proteinuria, hemoconcentration, hyperlipidemia, and intrauterine growth restriction. The mechanism for the control of fetal growth in patients with diabetes is difficult to determine because it depends on the glucose level and the diabetes duration. However, our pregnant animal model closely mimics the features of human pregnancy complicated by diabetes and is useful for the basic study of pregnancy with diabetes.

Table 3. Triglyceride and Total Cholesterol in the Three Groups

	Nonpregnant STZ		Control		STZ	
	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD	No.
Triglyceride (mg/dL)	533.4 ± 140.9	7	380.4 ± 125.8	15	804.5 ± 188.8*†	12
Total cholesterol (mg/dL)	94.2 ± 44.6	7	67.1 ± 21.7	15	107.1 ± 25.5‡	14

*$P < .05$ *v* nonpregnant STZ-treated.

†$P < .001$ *v* control.

‡$P < .05$ *v* control.

REFERENCES

1. Cunningham FG, MacDonald PC, Gant NF, et al: Diabetes, in Cunningham FG, MacDonald PC, Gant NF, et al (eds): Williams Obstetrics (ed 20). Norwalk, CT, Appleton & Lange, 1997, pp 1203-1222

2. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

3. Fujioka S, Matsuzawa Y, Tokunaga K, et al: Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 36:54-59, 1987

4. Kaplan NM: The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Arch Intern Med 149:1514-1520, 1989

5. DeFronzo RA, Ferrannini E: Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

6. Cousins L: Pregnancy complications among diabetic women: Review 1965-1985. Obstet Gynecol Surv 42:140-149, 1987

7. Pedersen J, Molsted-Pedersen L: Prognosis of the outcome of pregnancies in diabetics. A new classification. Acta Endocrinol (Copenh) 50:70-78, 1965

8. Mancuso S, Caruso S, Ferrazzani S, et al: Insulin secretion and resistance in hypertensive pregnant women, in Nakabayashi M, Araki T (eds): Recent Advances in the Pathophysiology of Pregnancy. Tokyo, Japan, Simul International, 1997, pp 141-149

9. Garner P: Type I diabetes mellitus and pregnancy. Lancet 346:157-161, 1995

10. van Stiphout WAHJ, Hofman A, de Bruijn AM: Serum lipids in young women before, during and after pregnancy. Am J Epidemiol 126:922-928, 1987

11. Cortes P, Dumler F, Goldman J, et al: Relationship between renal function and metabolic alterations in early streptozotocin-induced diabetes in rats. Diabetes 36:80-87, 1987

12. Ebara T, Hirano T, Mamo JCL, et al: Hyperlipidemia in streptozotocin-diabetic hamsters as a model for human insulin-deficient diabetes: Comparison to streptozotocin diabetic rats. Metabolism 43:299-305, 1994

13. Fujita Y, Mori I, Kitano S: Color reaction between pyrogallol red–molybdenum (VI) complex and protein. Bunseki Kagaku 32:E379-E386, 1983

14. Pedersen J: The Pregnant Diabetic and Her Newborn (ed 2). Baltimore, MD, Williams & Wilkins, 1977

15. Pedersen JF, Molsted-Pedersen L: Early growth retardation in diabetic pregnancy. BMJ 1:18-19, 1979

16. Kobayashi A, Ueda Y, Morikawa H, et al: Effects of maternal hyperglycemia on fetal growing mechanism. Acta Obstet Gynaecol Jpn 43:289-296, 1991

17. Atkins V, Flozak AS, Ogata ES, et al: The effects of severe maternal diabetes on glucose transport in the fetal rat. Endocrinology 135:409-415, 1994

18. Werner H, Adamo M, Lowe WL, et al: Developmental regulation of rat brain/Hep G2 glucose transporter gene expression. Mol Endocrinol 3:273-279, 1989

19. Freinkel N, Ogata E, Metzger BE: The offspring of the mother with diabetes, in Rifkin H, Porte D (eds): Diabetes Mellitus: Theory and Practice (ed 4). New York, NY, Elsevier, 1990, pp 651-659

20. White P: Pregnancy complicating diabetes. Am J Med 7:609-616, 1949

21. Gabbe SG, Mestman H, Freeman RG, et al: Management and outcome of pregnancy in diabetes mellitus, classes B to R. Am J Obstet Gynecol 129:723-732, 1977

22. Tomasi V, Strano A, Orlandi M, et al: Are the vascular complications of diabetes mellitus preceded by an altered thromboxane/prostacyclin plasmatic ratio? Med Hypotheses 19:229-241, 1986

23. Dadak C, Kefalides A, Sinzinger H, et al: Reduced umbilical artery prostacyclin formation in complicated pregnancies. Am J Obstet Gynecol 144:792-795, 1982

24. Deckert T, Feldt-Rasmussen B, Djurup R, et al: Glomerular size and charge selectivity in insulin-dependent diabetes mellitus. Kidney Int 33:100-106, 1988

25. Cooper ME, Allen TJ, O'Brien RC, et al: Effects of genetic hypertension on diabetic nephropathy in the rat—Functional and structural characteristics. J Hypertens 6:1009-1016, 1988

26. Herrera E, Lasunción MA, Gomez-Coronado D, et al: Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglyceride in pregnancy. Am J Obstet Gynecol 158:1575-1583, 1988

27. Dall'agrio E, Chang F, Chang H, et al: Effect of exercise and diet on triglyceride metabolism in rats with moderate insulin deficiency. Diabetes 32:46-50, 1983

28. Feingold KR, Wiley MH, MacRae G, et al: The effect of diabetes mellitus on sterol synthesis in the intact rat. Diabetes 31:388-395, 1982

29. Jiao S, Matsuzawa Y, Matsubara K, et al: Increased activity of intestinal acyl CoA: cholesterol acyltransferase in rats with streptozocin-induced diabetes and restoration by insulin supplementation. Diabetes 37:342-346, 1988

30. Spiegelman BM, Flier JS: Adipogenesis and obesity: Rounding out the big picture. Cell 87:377-389, 1996

31. Chien EK, Hara M, Rouard M, et al: Increase in serum leptin and uterine leptin receptor messenger RNA levels during pregnancy in rats. Biochem Biophys Res Commun 237:476-480, 1997

32. Butte NF, Hopkinson M, Nicolson MA: Leptin in human reproduction: Serum leptin levels in pregnant and lactating women. J Clin Endocrinol Metab 82:585-589, 1997

33. Havel PJ: Leptin production and action: Relevance to energy balance in humans. Am J Clin Nutr 67:355-356, 1998

34. Ahrén B, Månsson S, Gingerich RL, et al: Regulation of plasma leptin in mice: Influence of age, high-fat diet, and fasting. Am J Physiol 273:R113-R120, 1997

35. McGarry JD: Does leptin lighten the problem of obesity? Curr Biol 5:1342-1345, 1995

36. Mueller WM, Gregoire FM, Stanhope KL, et al: Evidence that glucose metabolism regulates leptin secretion from cultured rat adipocytes. Endocrinology 139:551-558, 1998

37. Mizuno TM, Bergen H, Funabashi T, et al: Obese gene expression: Reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. Proc Natl Acad Sci USA 93:3434-3438, 1996

38. Becker DJ, Ongemba LN, Brichard V, et al: Diet- and diabetes-induced changes of ob gene expression in rat adipose tissue. FEBS Lett 371:324-328, 1995

39. Havel PJ, Uru-Hare JY, Liu T, et al: Marked and rapid decrease of circulating leptin in streptozotocin diabetic rats: Reversal by insulin. Am J Physiol 274:R1482-R1491, 1998

40. Nishimura H, Kuzuya H, Okamoto M, et al: Postreceptor defect in insulin action in streptozotocin-induced diabetic rats. Am J Physiol 256:E624-E630, 1989