First-Trimester C-Reactive Protein and Subsequent Gestational Diabetes

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OBJECTIVE — Systemic inflammation is associated with the development of type 2 diabetes. We tested the hypothesis that increased inflammation, measured early in pregnancy, is associated with the subsequent development of gestational diabetes mellitus (GDM), a precursor of type 2 diabetes.

RESEARCH DESIGN AND METHODS — We conducted a prospective nested case-control study in a pregnancy cohort. First-trimester C-reactive protein (CRP) levels were measured using a high-resolution assay in 43 women who subsequently developed GDM and in a random sample of 94 women who remained euglycemic throughout pregnancy. Median CRP levels were compared using Wilcoxon's rank-sum test. Logistic regression was used to compute unadjusted and multivariable-adjusted odds ratios for developing GDM among CRP tertiles.

RESULTS — First-trimester CRP levels were significantly increased among women who subsequently developed GDM compared with control subjects (3.1 vs. 2.1 mg/l, $P < 0.01$). The risk of developing GDM among women in the highest CRP tertile compared with the lowest tertile was 3.2 (95% CI 1.2–8.8). After adjusting for age, race/ethnicity, smoking, parity, blood pressure, and gestational age at CRP sampling, the risk of developing GDM among women in the highest compared with the lowest tertile was 3.6 (95% CI 1.2–11.4). When BMI was included in the model, however, the association between increased CRP and GDM was attenuated (odds ratio for the highest compared with lowest tertile 1.5 [95% CI 0.4–5.5]).

CONCLUSIONS — In women who develop GDM, there is evidence of increased inflammation during the first trimester. This association is mediated in part by increased BMI. Larger studies are needed to verify these results.

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Gestational diabetes mellitus (GDM), defined as the new onset or new diagnosis of glucose intolerance during pregnancy, complicates ~4% of pregnancies (1). This estimate will likely increase in the future, given the alarming increase in rates of obesity and type 2 diabetes among young women in the U.S. (2). Like type 2 diabetes, GDM results from a combination of increased insulin resistance and impaired pancreatic insulin secretion (3,4), and women with a history of GDM are at significantly increased risk of developing type 2 diabetes in the future (5).

Type 2 diabetes is an important cause of cardiovascular disease, and the latter is a leading cause of death among patients with diabetes (6). Although effective strategies for primary prevention of cardiovascular disease continue to evolve, by the time type 2 diabetes is diagnosed, many patients already manifest established cardiovascular disease (7). Therefore, effective prevention in patients destined to develop diabetes requires earlier intervention against cardiovascular risk factors or primary prevention of diabetes itself. Recent studies involving ACE inhibitors (8), statins (9), and lifestyle modification (10) offer potentially promising means to prevent diabetes in high-risk individuals. As the role of these interventions become more clearly defined, further understanding of early mechanisms of type 2 diabetes and its cardiovascular complications is needed to identify additional targets for primary prevention.

Inflammation, marked by increased serum levels of C-reactive protein (CRP), is an important independent risk factor for cardiovascular disease (11–15). Inflammation is also associated with insulin resistance (16–18), and prospective studies indicate that increased inflammation at baseline is an independent risk factor for the future development of type 2 diabetes (19–23). The molecular basis for the link between inflammation and diabetes likely relates to the actions of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α, which induce insulin resistance and stimulate the acute phase inflammatory response (24–27). Collectively, these data suggest that inflammation is a distinct feature of the atherogenic, prediabetic state. Although GDM represents a unique precursor of type 2 diabetes, whether inflammation is similarly associated with the development of GDM is unknown. Therefore, we tested the hypothesis that increased CRP levels, measured early in pregnancy, are associated with the subsequent development of GDM.

RESEARCH DESIGN AND METHODS

— The Massachusetts General Hospital Obstetric Maternal Study (MOMS) was developed in 1998 to

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Abbreviations: CRP, C-reactive protein; CV, coefficient of variation; GDM, gestational diabetes mellitus; GLT, glucose-loading test; GTT, glucose tolerance test; IL-6, interleukin-6; MOMS, Massachusetts General Hospital Obstetric Maternal Study; OR, odds ratio; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

prospectively study metabolic and hypertensive disorders of pregnancy (28). All women who receive prenatal care at the Massachusetts General Hospital or its affiliated health centers are eligible for inclusion in the MOMS cohort. Potential subjects are offered inclusion at their first prenatal visit, which is typically between 10 and 12 weeks' gestation. Nonfasting serum samples are collected at the conclusion of the first prenatal visit from women who provide written informed consent to participate in the study. The serum samples are frozen at −80°C and aliquots are thawed only for laboratory analysis. Baseline, intrapartum, and postpartum data are collected prospectively from all participants at the point of care by the patients' caregivers. Trained clinical staff enters the data into a computerized electronic record, which serves as the primary medical record used by the Department of Obstetrics and its affiliates. Baseline data collected during the first prenatal visit (when blood is obtained) include age, race, past medical and obstetric history, medications, smoking, height (obtained from patient self-report), weight (directly measured), and blood pressure. Blood pressure, urinalysis and other laboratory test results are recorded at each subsequent prenatal visit. Details of pregnancy complications and outcomes are documented in the electronic record and ascertained after delivery using research-specific criteria. The prospective design of the MOMS cohort, for which all women are recruited long before adverse pregnancy outcomes manifest, ensures that at the time of enrollment no subjects are known to have GDM. The institution's human subjects committee approved the study.

All women in the MOMS cohort without a history of antenatal type 1 or type 2 diabetes underwent routine GDM screening with the 50-g oral glucose-loading test (GLT) between 24 and 28 weeks' gestation. In the nonfasting state, subjects consumed 50 g of oral glucose beverage and glucose levels were assayed in 1-h postloading plasma samples. Women whose 1-h postloading plasma glucose level was >7.8 mmol/l were considered to be at increased risk for GDM and underwent a diagnostic, fasting, 100-g, 3-h oral glucose tolerance test (GTT) within 1–2 weeks following the GLT. Glucose levels were assayed in baseline fasting plasma samples and in three postchallenge plasma samples collected at 1-h intervals. Women were diagnosed with GDM if two or more of the four GTT glucose levels exceeded the American Diabetes Association (ADA) criteria (1): fasting >5.3 mmol/l; 1-h postchallenge >10.0 mmol/l; 2-h postchallenge >8.6 mmol/l; 3-h postchallenge >7.8 mmol/l.

Between September 1998 and July 2001, 2,251 of the 3,320 women enrolled in the MOMS cohort completed their pregnancy. Overall, ~60% of eligible women enrolled during the study period, and participants and nonparticipants, were similar with regard to baseline and delivery characteristics, as well as rates of GDM. Among these 2,251 women, GDM was diagnosed in 69. Using the American College of Obstetrics and Gynecology's criteria to define GDM yielded the identical 69 cases (29). This incidence of 3.1% mirrors rates reported from other cohorts (1). Five women enrolled after 14 weeks' gestation and were excluded. Ten women were diagnosed with GDM early in pregnancy before routine screening because of signs and symptoms of hyperglycemia or because of overt hyperglycemia (>11.1 mmol/l) identified in random, nonfasting blood tests. Although these women have GDM according to strict ADA definition (1), they were excluded from this study to avoid potential confounding by preexisting diabetes that may have been present at the initial prenatal visit when serum was obtained. Given the association of hypertension and preeclampsia with increased CRP levels (28,30), seven women with GDM were excluded because of a history of chronic hypertension or because they later developed preeclampsia or gestational hypertension. Next, individual prenatal flow sheets of each of the remaining 48 potential subjects were manually reviewed. Based on this review, two women were excluded because of a past medical history of chronic inflammatory disease, and an additional two were excluded because of active infection at the first prenatal visit when serum was collected. Finally, one subject had insufficient serum volume for CRP assay, leaving a final sample of 43 cases of GDM for the current study. Baseline and delivery characteristics of these 43 women were similar to the characteristics of women who declined enrollment in MOMS and subsequently developed GDM (data not shown).

There were 2,182 women enrolled in MOMS who completed pregnancy and remained free of GDM. Women whose 1-h post-GLT glucose level was >7.8 mmol/l but whose GTT results did not meet criteria for GDM (0 or 1 abnormal glucose levels) were excluded. Next, the identical algorithm used to select cases was used to narrow the group of control subjects. Women who enrolled after 14 weeks, or delivered before 30 weeks' gestation, those who had a history of chronic hypertension, type 1 or type 2 diabetes, and those who developed hypertensive disorders of pregnancy were excluded, leaving 1,672. From this pool of eligible control subjects, 96 (2 for each case) were randomly selected for the current study using random number–generating software. Upon manual review of each of the potential control subjects' prenatal flow sheets, one woman was excluded because of a history of chronic inflammatory disease and another because of active infection at the time of her first prenatal visit, leaving a final sample of 94 control subjects.

Dedicated study technicians who were blinded to clinical details processed all samples identically. Frozen serum samples were sent on dry ice by overnight courier to Quest Diagnostics (Cambridge, MA). Technicians blinded to GDM status tested batched samples containing a random mix of case and control subjects for CRP using the high-resolution N-latex CRP monoassay. This assay has a sensitivity of 0.2 mg/dl, an intra-assay coefficient of variation (CV) of 4.4%, and an interassay CV of 5.7%. All glucose levels were measured using standard glucose oxidase assays with an intra- and interassay CV of <2%.

Analyses were performed with STATA statistical package (STATA, College Station, TX). Continuous variables were compared using two sample Student's *t* tests or Wilcoxon's rank-sum test, and categorical variables were compared using Fisher's exact test. Due to the rightward skew of the CRP distributions, we compared median CRP levels among case and control subjects using Wilcoxon's rank-sum test. Correlation between CRP and selected covariates was calculated with Spearman coefficients. Study subjects were divided into tertiles based on the distribution of CRP among control subjects. Logistic regression was used to calculate unadjusted and multivariable-adjusted odds ratios (ORs) and 95% CIs for developing GDM with the lowest tertile serving as the reference group. In the

Table 1—*Baseline and delivery characteristics according to pregnancy outcome*

	GDM	Control subjects	*P**
n	43	94	
Baseline characteristics			
Age (years)	32.3 ± 5.6	32.6 ± 5.1	NS
Race ethnicity (%)			<0.01
White non-Hispanic	63	83	
Hispanic	21	3	
African American	7	2	
Asian	5	5	
Other/Unknown	4	6	
Gestational age at 1st prenatal visit (weeks)	10.2 ± 1.9	10.6 ± 1.5	NS
Parity (%)			NS
0	63	67	
1–2	30	30	
>2	7	3	
Smoking (%)			NS
Never	70	72	
Current	14	16	
Before pregnancy	16	12	
BMI (kg/m^2)	29 ± 7	25 ± 4	<0.01
Systolic blood pressure (mmHg)	115 ± 12	115 ± 11	NS
Delivery characteristics			
Gestational age at delivery (weeks)	38.1 ± 2.1	39.7 ± 1.5	<0.01
Cesarean delivery (%)	36	16	<0.01
Birth weight (g)	3520 ± 521	3522 ± 532	NS

Values for continuous measurements are mean ± SD. *NS, not statistically significant.

multivariable models, age, blood pressure, and gestational age at enrollment and BMI were modeled as continuous variables, while parity (0, 1–2, or >2), race/ethnicity (nonwhite/Hispanic or white/non-Hispanic) and smoking (never, remote, or current) were modeled as categorical variables. Two-tailed *P* values <0.05 were considered statistically significant.

RESULTS — Baseline and delivery characteristics of all subjects are presented in Table 1. The average gestational age at the time of the first prenatal visit when serum was sampled for CRP was between 10 and 11 weeks and was no different among case and control subjects. Women who subsequently developed GDM were heavier and more likely to be nonwhite or of Hispanic ethnicity. There was no difference in age, smoking, parity, and blood pressure among case and control subjects. Women with GDM were more likely to deliver by cesarean at younger gestational ages. Despite their significantly younger gestational age at delivery, babies born to mothers with GDM had similar birth weight as babies born to euglycemic mothers. Three women delivered twins, and two of these women developed GDM.

Women who developed GDM displayed significantly increased first-trimester CRP levels compared with control subjects (3.1 vs. 2.1 mg/l; $P < 0.01$). Among all subjects, there was significant correlation between subjects' first-trimester CRP levels and their 1-h post-loading glucose levels ($r = 0.26$; $P < 0.01$). Among women diagnosed with GDM, there was a trend in favor of a correlation between CRP levels and area under the GTT curve ($r = 0.29$; $P = 0.07$). CRP was significantly correlated with BMI ($r = 0.55$; $P < 0.01$) and systolic blood pressure ($r = 0.26$; $P < 0.01$). Of the 43 subjects with GDM, 5 required insulin. The median CRP level among these subjects was 13.0 mg/l.

Subjects were divided into CRP tertiles using cut points defined by the distribution of CRP among control subjects (tertile 1, <1.3; tertile 2, 1.3–4.0; tertile 3, >4.0 mg/l). The unadjusted and multivariable-adjusted risk of developing GDM within the upper CRP tertiles relative to the lowest tertile is displayed in Table 2. In the unadjusted model, subjects in the second (OR 2.2 [95% CI 0.8–6.0]) and third (OR 3.2 [95% CI 1.2–8.8]) tertiles were at increased risk of developing GDM compared with subjects in the lowest tertile (*P* for linear trend <0.01). After adjusting for age, race, ethnicity, smoking, parity, blood pressure, and gestational age at CRP sampling, women in the second (OR 2.0 [95% CI 0.7–5.9]) and third (OR 3.6 [95% CI 1.2–11.4]) tertiles remained at increased risk for GDM compared with the lowest tertile (*P* for linear trend <0.01). When BMI was added to the model, the risk of GDM among the upper CRP tertiles was attenuated. Although the ORs for the upper CRP tertiles in that model suggested increased risk of GDM compared with the reference group, the effect was not statis-

Table 2—*Relative risk of developing GDM according to CRP tertiles with tertile 1 serving as the reference group*

	Tertile 1	Tertile 2	Tertile 3	*P* for linear trend
n	39	52	46	
CRP range (mg/l)	<1.3	1.3–4.0	>4.0	
Unadjusted				
OR	1.0	2.2	3.2	<0.01
95% CI		0.8–6.0	1.2–8.8	
Multivariable adjusted*				
OR	1.0	2.0	3.6	<0.01
95% CI		0.7–5.9	1.2–11.4	
Multivariable and BMI adjusted				
OR	1.0	1.4	1.5	0.13
95% CI		0.4–4.4	0.4–5.5	

*Adjusted for age, race/ethnicity, smoking, parity, blood pressure, and gestational age at CRP sampling.

tically significant (P for linear trend = 0.13). In this multivariable model, only BMI (OR 1.2 per unit increase [95% CI 1.1–1.3]; $P < 0.01$), and nonwhite race/Hispanic ethnicity (OR 3.7; 95% CI 1.1–12.7; $P = 0.03$) were independently associated with GDM.

CONCLUSIONS — In this prospective study we identified an association between first-trimester inflammation, marked by increased CRP levels, and subsequent risk of GDM. This effect was independent of established risk factors for GDM such as age, multiparity, smoking, non–white race/Hispanic ethnicity (31). The increased risk of GDM was attenuated, however, when we adjusted for BMI, which was highly correlated with CRP. These observations highlight an important association between obesity, inflammation, and risk of developing GDM that previously has not been studied in detail.

Increased serum CRP is a sensitive index of systemic inflammation that has emerged as an independent risk factor for cardiovascular disease (11–15). Growing evidence similarly implicates inflammation in the pathogenesis of type 2 diabetes. Inflammation is directly correlated with insulin resistance, as measured by the intravenous GTT (18) or the euglycemic-hyperinsulinemic clamp (32), and with other features of the insulin resistance syndrome, such as obesity, hypertension, and microalbuminuria (17,18, 32–34). Furthermore, women with the polycystic ovary syndrome, a disorder characterized by insulin resistance and increased risk of type 2 diabetes and cardiovascular disease, display significantly increased CRP levels compared with eugonadal women (32). In cross-sectional studies, patients with established (16) or newly diagnosed type 2 diabetes (35) displayed increased levels of inflammatory markers compared with control subjects. In prospective studies, increased white blood cell count, IL-6 and CRP levels were independently associated with increased risk of developing type 2 diabetes (19–23). The results of this study lend further support to the hypothesis that inflammation contributes to the pathogenesis of glucose intolerance.

The association we identified between increasing CRP levels and subsequent GDM was attenuated when BMI was added to the multivariable model. There are several potential interpretations for this observation. First, one could argue that there is no association between CRP and GDM—rather, CRP confounds the true association between obesity and GDM. This is unlikely, however, given the strong independent association between CRP and type 2 diabetes (23) and the similar pathogenesis of GDM and type 2 diabetes. An alternate interpretation is that insufficient power limited our ability to detect an independent association between CRP and GDM that truly exists. As in other studies (23,36,37), CRP was highly correlated with BMI, but in this study, there was a large difference in mean BMI comparing case with control subjects. The net result is that a greater sample of cases would have been required to identify an effect of CRP on GDM independent of BMI, assuming one exists.

A third interpretation is that rather than confounding the association, BMI and CRP might share a causal diabetogenic pathway; therefore, including BMI in the multivariable model would represent an overadjustment that could potentially obscure an important association that truly exists (20,38). Several lines of evidence support this. In epidemiological studies, significant correlation between BMI and CRP has been consistently observed (36,37), including a recent prospective study in which increased CRP was associated with future type 2 diabetes in women (23). In that study, correlation (r) between BMI and CRP was 0.57. From a physiological standpoint, adipose tissue is a major source of basal IL-6 and TNF-α secretion (27). These inflammatory cytokines contribute to increased insulin resistance (24–26) and stimulate hepatic CRP production. Furthermore, TNF-α, and not other maternal or placental hormones such as cortisol or human placental lactogen, was recently shown to be the primary mediator of progressive insulin resistance in pregnancy, both in normal pregnancy and in pregnancies complicated by GDM (39). Therefore, it appears that obesity, inflammation, and diabetes might share a causal pathway. Adjusting for BMI in a model in which the primary exposure of interest is CRP and the primary outcome is biologically linked to insulin resistance may therefore be inappropriate.

This study has several strengths. The prospective design of the MOMS cohort allowed for "real-time" collection of clinical data at the point of care by subjects' caregivers, thereby limiting bias. At the same time, the nested case-control approach allowed CRP levels to be measured in all eligible cases of GDM within the cohort but only in a representative, random subset of control subjects. In addition, the MOMS database was used to exclude women with disorders known to be associated with increased CRP and GDM, such as connective tissue disease, infection, chronic hypertension, and preeclampsia (28). Furthermore, to limit potential confounding by prevalent diabetes, we excluded women in whom GDM was diagnosed before GLT and GTT screening, and thus might have been present when serum was sampled for CRP. Based on the known association between prevalent diabetes and increased inflammation, one would expect women with early GDM to have increased CRP levels. Therefore, by excluding these women we were more likely to underestimate the true difference in CRP levels among cases of GDM, as defined by ADA criteria, and control subjects.

This study has certain limitations. First, we only measured CRP at one juncture in pregnancy and although there is little short-term variation in CRP levels (40), CRP does increase during the course of normal gestation (41). This is, however, one of the first studies to examine the association between CRP and GDM and we adjusted for gestational age of CRP sampling in the analysis. Second, because of the small sample size examined in this nested case-control study, we were unable to identify appropriate cut points to assess the positive and negative predictive values of CRP as a marker for GDM. However, the emphasis of this study was on expanding our understanding of disease mechanisms through the identification of an association not previously reported, rather than evaluating a novel screening or diagnostic method. Larger studies would be needed to examine CRP as a screening method for GDM. Until then, early-pregnancy CRP testing remains a research tool. Third, we were unable to analyze anthropometric measures beyond BMI, such as waist circumference or waist-to-hip ratio. Fourth, the average age of MOMS participants is older than other pregnancy cohorts. Although we did adjust for age, this attribute should be considered when assessing the generalizability of the results. Finally, because collection of clinical data for the MOMS

cohort ends with the postpartum visit 6–8 weeks after delivery, we were unable to determine whether increased first-trimester CRP levels are associated with progression to type 2 diabetes or its cardiovascular complications. Likewise, whether elevated CRP levels persist during the postpartum period after pregnancy complicated by GDM is also unknown and worthy of further investigation.

The incidence of type 2 diabetes is increasing at an alarming rate, particularly among young women (2). GDM is a unique prediabetes state that shares common risk factors with type 2 diabetes (31), and similar alterations in carbohydrate metabolism (4). The results of this study provide further support to the hypothesis that inflammation contributes to the development of glucose intolerance. Furthermore, in this population of young, otherwise healthy women, we detected during early pregnancy increased levels of an important and potentially modifiable risk factor for future cardiovascular disease, months before GDM develops and years before type 2 diabetes or cardiovascular disease develops. While regular postpartum GTT is universally recommended for the early diagnosis of diabetes in women with GDM (1), these results suggest that perhaps postpartum CRP screening might also have a role in the future. The emergence of data that suggest that part of the cardioprotective benefit of statins and renin-angiotensin system blockers is conferred by anti-inflammatory effects would support this approach (42,43). Furthermore, thiazolidinediones and metformin, which increase insulin sensitivity, also reduce CRP levels (44), suggesting an additional potential benefit of these agents in this population. Further studies are required to confirm these results and to define a potential role of CRP screening for diabetes, both during and after pregnancy.

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References

1. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 25 (Suppl. 1):S5–S20, 2002
2. Mokdad AH, Ford ES, Bowman BA, Nelson DE, Engelgau MM, Vinicor F, Marks JS: Diabetes trends in the U.S.: 1990–1998. *Diabetes Care* 23:1278–1283, 2000
3. Buchanan TA, Kjos SL: Gestational diabetes: risk or myth? *J Clin Endocrinol Metab* 84:1854–1857, 1999
4. Catalano PM, Huston L, Amini SB, Kalhan SC: Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *Am J Obstet Gynecol* 180:903–916, 1999
5. Metzger BE, Cho NH, Roston SM, Radvany R: Prepregnancy weight and antepartum insulin secretion predict glucose tolerance five years after gestational diabetes mellitus. *Diabetes Care* 16:1598–1605, 1993
6. Kannel WB, McGee DL: Diabetes and cardiovascular disease: the Framingham study. *JAMA* 241:2035–2038, 1979
7. Muggeo M: Accelerated complications in type 2 diabetes mellitus: the need for greater awareness and earlier detection. *Diabet Med* 15:S60–S62, 1998
8. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G: Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med* 342:145–153, 2000
9. Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, Isles C, Lorimer AR, Macfarlane PW, McKillop JH, Packard CJ, Shepherd J, Gaw A: Pravastatin and the development of diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. *Circulation* 103:357–362, 2001
10. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403, 2002
11. Koenig W, Sund M, Frohlich M, Fischer HG, Lowel H, Doring A, Hutchinson WL, Pepys MB: C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation* 99:237–242, 1999
12. Gussekloo J, Schaap MC, Frolich M, Blauw GJ, Westendorp RG: C-reactive protein is a strong but nonspecific risk factor of fatal stroke in elderly persons. *Arterioscler Thromb Vasc Biol* 20:1047–1051, 2000
13. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH: Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 336:973–979, 1997
14. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH: Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 98:731–733, 1998
15. Rost NS, Wolf PA, Kase CS, Kelly-Hayes M, Silbershatz H, Massaro JM, D'Agostino RB, Franzblau C, Wilson PW: Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke* 32:2575–2579, 2001
16. Pickup JC, Mattock MB, Chusney GD, Burt D: NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* 40:1286–1292, 1997
17. Frohlich M, Imhof A, Berg G, Hutchinson WL, Pepys MB, Boeing H, Muche R, Brenner H, Koenig W: Association between C-reactive protein and features of the metabolic syndrome: a population-based study. *Diabetes Care* 23:1835–1839, 2000
18. Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM: Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation* 102:42–47, 2000
19. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G: Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* 353:1649–1652, 1999
20. Ford ES: Leukocyte count, erythrocyte sedimentation rate, and diabetes incidence in a national sample of us adults. *Am J Epidemiol* 155:57–64, 2002
21. Vozarova B, Weyer C, Lindsay RS, Pratley RE, Bogardus C, Tataranni PA: High white blood cell count is associated with a worsening of insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:455–461, 2002
22. Barzilay JI, Abraham L, Heckbert SR, Cushman M, Kuller LH, Resnick HE, Tracy RP: The relation of markers of inflammation to the development of glucose disorders in the elderly: the Cardiovascular Health Study. *Diabetes* 50:2384–2389, 2001
23. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM: C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 286:327–334,

2001
24. Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE: Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* 9:414–417, 2001
25. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G: Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* 280:E745–E751, 2001
26. Fernandez-Real JM, Vayreda M, Richart C, Gutierrez C, Broch M, Vendrell J, Ricart W: Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women. *J Clin Endocrinol Metab* 86:1154–1159, 2001
27. Mohamed-Ali V, Pinkney JH, Coppack SW: Adipose tissue as an endocrine and paracrine organ. *Int J Obes* 22:1145–1158, 1998
28. Wolf M, Kettyle E, Sandler L, Ecker JL, Roberts J, Thadhani R: Obesity and preeclampsia: the potential role of inflammation. *Obstet Gynecol* 98:757–762, 2001
29. ACOG Practice Bulletin: Clinical management guidelines for obstetrician-gynecologists. Number 30: September 2001 (replaces Technical Bulletin Number 200, December 1994) gestational diabetes. *Obstet Gynecol* 98:525–538, 2001
30. Rohde LE, Hennekens CH, Ridker PM: Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *Am J Cardiol* 84:1018–1022, 1999
31. Solomon CG, Willett WC, Carey VJ, Rich-Edwards J, Hunter DJ, Colditz GA, Stampfer MJ, Speizer FE, Spiegelman D, Manson JE: A prospective study of pregravid determinants of gestational diabetes mellitus. *JAMA* 278:1078–1083, 1997
32. Kelly CC, Lyall H, Petrie JR, Gould GW, Connell JM, Sattar N: Low grade chronic inflammation in women with polycystic ovarian syndrome. *J Clin Endocrinol Metab* 86:2453–2455, 2001
33. Festa A, D'Agostino R, Howard G, Mykkanen L, Tracy RP, Haffner SM: Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: the Insulin Resistance Atherosclerosis Study. *Kidney Int* 58:1703–1710, 2000
34. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW: C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 19:972–978, 1999
35. Temelkova-Kurktschiev T, Henkel E, Koehler C, Karrei K, Hanefeld M: Subclinical inflammation in newly detected type II diabetes and impaired glucose tolerance. *Diabetologia* 45:151, 2002
36. Ford ES: Body mass index, diabetes, and C-reactive protein among U.S. adults. *Diabetes Care* 22:1971–1977, 1999
37. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB: Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 282:2131–2135, 1999
38. Rothman KJ, Greenland S: *Modern Epidemeology*. Boston, MA, Little, Brown, 1998
39. Kirwan JP, Hauguel-De Mouzon S, Lepercq J, Challier JC, Huston-Presley L, Friedman JE, Kalhan SC, Catalano PM: TNF-α is a predictor of insulin resistance in human pregnancy. *Diabetes* 51:2207–2213, 2002
40. Macy EM, Hayes TE, Tracy RP: Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. *Clin Chem* 43:52–58, 1997
41. Romem Y, Artal R: C-reactive protein in pregnancy and in the postpartum period. *Am J Obstet Gynecol* 151:380–383, 1985
42. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, Gotto AM, Jr: Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med* 344:1959–1965, 2001
43. Rahman ST, Lauten WB, Khan QA, Navalkar S, Parthasarathy S, Khan BV: Effects of eprosartan versus hydrochlorothiazide on markers of vascular oxidation and inflammation and blood pressure (renin-angiotensin system antagonists, oxidation, and inflammation). *Am J Cardiol* 89:686–690, 2002
44. Chu NV, Kong AP, Kim DD, Armstrong D, Baxi S, Deutsch R, Caulfield M, Mudaliar SR, Reitz R, Henry RR, Reaven PD: Differential effects of metformin and troglitazone on cardiovascular risk factors in patients with type 2 diabetes. *Diabetes Care* 25:542–549, 2002