

# Inflammation and Glucose Intolerance

## A prospective study of gestational diabetes mellitus

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**OBJECTIVE** — Increased leukocyte count is a marker of inflammation that has been associated with the development of type 2 diabetes in prospective studies. Although gestational diabetes mellitus (GDM) and type 2 diabetes share certain pathophysiological mechanisms, few studies have examined inflammation and risk of GDM.

**RESEARCH DESIGN AND METHODS** — We prospectively examined routine leukocyte counts collected at the first prenatal visit in a cohort of 2,753 nulliparous euglycemic women, 98 (3.6%) of whom were later diagnosed with GDM. Subjects were divided into quartiles of leukocyte count, and the results of third-trimester glucose screening tests and the incidence of GDM among these quartiles were compared. Logistic regression was used to calculate univariate and multivariable-adjusted relative risks (RRs) of GDM according to leukocyte quartiles.

**RESULTS** — Leukocyte counts were increased among women who subsequently developed GDM compared with those who remained free of GDM ( $10.5 \pm 2.2$  vs.  $9.2 \pm 2.2 \times 10^3$  cells/ml;  $P < 0.01$ ). There was a linear increase in postloading mean glucose levels ( $P$  for trend  $<0.01$ ), the area under the glucose tolerance test curves ( $P$  for trend  $<0.01$ ), and the incidence of GDM (quartile 1, 1.1; quartile 2, 2.5; quartile 3, 4.2; and quartile 4, 6.4%;  $P$  for trend  $<0.01$ ) with increasing leukocyte quartiles. In the multivariable-adjusted analysis, the linear trend in the RR of GDM with increasing leukocyte quartiles remained statistically significant (quartile 1, reference; quartile 2, RR 2.3 [95% CI 0.9–5.7]; quartile 3, 3.3 [1.4–7.8]; quartile 4, 4.9 [2.1–11.2];  $P$  for trend  $<0.01$ ).

**CONCLUSIONS** — Increased leukocyte count early in pregnancy is independently and linearly associated with the results of GDM screening tests and the risk of GDM. Although overlap in the leukocyte count distributions precludes it from being a clinically useful biomarker, these data suggest that inflammation is associated with the development of GDM and may be another pathophysiological link between GDM and future type 2 diabetes.

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**G**estational diabetes mellitus (GDM) complicates roughly 4% of pregnancies (1,2). After delivery, women with GDM are at substantially increased risk of developing type 2 diabetes, and they are more likely to display features of the insulin resistance syndrome that are linked to cardiovascular disease, such as hypertension, dyslipidemia, microalbuminuria, and endothelial dysfunction

(3–6). Women with GDM, therefore, represent a high-risk group for cardiovascular disease that can be identified years before the development of adverse events. With the incidence of type 2 diabetes increasing at epidemic proportions (7), further understanding of the mechanisms of GDM that might also contribute to the progression to type 2 diabetes, and more distantly to future cardiovascular disease, is needed so that additional pathophysiological pathways may be targeted for primary prevention.

Insulin resistance and obesity are important pathophysiological contributors to both GDM and type 2 diabetes (8–10). Recent evidence suggests that inflammation is a central feature of the insulin resistance syndrome that is also linked to obesity. For example, physiological studies (11–13) using intravenous glucose tolerance tests (GTTs) or euglycemic-hyperinsulinemic clamps found a direct association between the degree of insulin resistance and levels of inflammatory markers. Inflammatory cytokines such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6, which are secreted by adipose tissue and are constitutively elevated in the serum of obese subjects (14,15), have been shown to induce insulin resistance (16–18). Women with polycystic ovary syndrome, which is associated with insulin resistance, obesity, and increased risk of GDM and type 2 diabetes, also demonstrate increased levels of inflammatory markers (19,20). Conversely, amelioration of insulin resistance through weight loss or administration of insulin sensitizing drugs, such as troglitazone, led to decreased levels of C-reactive protein (CRP) and TNF- $\alpha$  (21–24).

The results of large prospective studies suggest that inflammation may be an underlying pathophysiological mechanism of several of the adverse clinical outcomes associated with obesity and insulin resistance. For example, increased levels of inflammatory markers, such as leukocyte count and CRP, are associated with the future development of type 2 diabetes, cardiovascular disease, and death (11–13,25–29). Although insulin resistance

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**Abbreviations:** CRP, C-reactive protein; GDM, gestational diabetes mellitus; GLT, glucose-loading test; GTT, glucose tolerance test; IL, interleukin; MGH, Massachusetts General Hospital; MOMS, Massachusetts General Hospital Obstetric Maternal Study; TNF, tumor necrosis factor.

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

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and obesity predispose to type 2 diabetes and GDM, few studies have examined the association between inflammation and GDM. Recently, TNF- $\alpha$  was shown (30) to be the primary mediator of the progressive increase in insulin resistance that characterizes normal pregnancy, but there were no differences in early pregnancy TNF- $\alpha$  levels among women who later developed GDM and women with normal glucose tolerance. In a small prospective, nested case-control study (31), increased first trimester CRP levels were associated with subsequent GDM, but the effect was attenuated when the data were adjusted for differences in BMI. Whether inflammation is independently associated with the development of GDM therefore remains unclear. We conducted a prospective cohort study to test the following hypotheses: 1) increased inflammation during early pregnancy, marked by increased leukocyte count, is associated with the development of GDM; 2) the association between inflammation and GDM is independent of obesity; and 3) inflammation and GDM exhibit a dose-response relationship such that increased degrees of inflammation are linearly correlated with increased risk of GDM and the degree of hyperglycemia, ascertained from the results of third trimester GDM screening tests.

## RESEARCH DESIGN AND METHODS

The Massachusetts General Hospital Obstetric Maternal Study (MOMS) is a prospective pregnancy cohort study of all women who receive prenatal care and deliver through the Massachusetts General Hospital (MGH) obstetrics network. The study was designed to prospectively examine early pregnancy risk factors for subsequent adverse outcomes such as GDM. The MGH obstetrics service provides primary prenatal care for an ethnically and socioeconomically diverse population at the main campus in Boston and at its affiliated neighborhood health centers in Chelsea, East Boston, Revere, and Waltham, Massachusetts. Over 97% of women who receive prenatal care through the network deliver at the main MGH campus. All centers use the identical electronic medical record to prospectively document the details of pregnancy, including demographic data, height, weight, blood pressure measurements, routine laboratory and diabetes screening test results,

urinalyses, and maternal and fetal outcomes. All data are entered into the electronic medical record by the subjects' obstetrical health providers at the point of care, ensuring that the data collection is unbiased by knowledge of pregnancy outcome. The content of the electronic medical record is downloaded directly to the MOMS database on a quarterly schedule, and the investigators validate all pregnancy outcomes using research criteria. No additional data were retrospectively abstracted from the hospital paper record for this study. The MGH human research committee approved the study.

Complete blood counts are measured in all women early in pregnancy as part of routine prenatal care at MGH. The primary exposure for this study was the baseline leukocyte count measured at women's first routinely scheduled prenatal visit (typically at 8–12 weeks of gestation). Between October 1998 and January 2002, 3,043 nulliparous women who ultimately delivered a live pregnancy had a baseline leukocyte count measured early in pregnancy. In 180 of these women, the leukocyte measurements were performed before their first routine prenatal visit at an unscheduled walk-in or emergency visit. These women were excluded to prevent confounding by indication that might be introduced by including subjects whose blood counts were ordered for nonroutine reasons that might be independently associated with increased leukocyte count, e.g., for the evaluation of infection or bleeding. Over 96% of women underwent third trimester screening for GDM using 50-g, 1-h nonfasting glucose-loading tests (GLTs) at ~28 weeks of gestation. Women whose 1-h post-GLT plasma glucose level was >7.8 mmol/l underwent a diagnostic 100-g, 3-h GTT 1–2 weeks following the GLT. GDM was diagnosed when two or more of the four hourly postchallenge glucose levels exceeded the following thresholds: fasting >5.3, 1-h >10.0, 2-h >8.6, and 3-h >7.8 mmol/l. Women who did not undergo third-trimester GDM screening ( $n = 110$ ) because they either declined testing, had preexisting diabetes, or were diagnosed with GDM on the basis of clinical signs and symptoms before third-trimester screening were excluded from the study. The final study population consisted of 2,753 women, of whom 98 (3.6%) subsequently developed GDM. Leukocyte counts were measured using a

standard Bayer Advia 120 Hematology System (Bayer Group, Leverkusen, Germany) with inter- and intra-assay coefficients of variation <3%. Glucose was assayed using standard glucose oxidase techniques with inter- and intra-assay coefficients of variation <3%.

The primary analyses focused on whether increased baseline leukocyte count (primary exposure) was associated with the development of GDM (primary outcome), and whether the association was characterized by a dose-response relationship such that graded increases in levels of the exposure were associated with graded increases in hyperglycemia and risk of GDM. To determine whether concurrent infection might account for differences in leukocyte counts, we compared the prevalence of urinary tract or other clinically evident infections at the time of the leukocyte sampling in all 98 women who subsequently developed GDM and in 98 of the 2,655 women who remained free of GDM. The non-GDM group was randomly selected after being matched to the GDM women according to the date of their blood sampling ( $\pm 1$  day) in order to eliminate confounding by different seasonal infection rates. There were no differences in infection rates comparing the GDM and non-GDM groups (8 of 98 vs. 7 of 98;  $P = 1.00$ ), and there was no association between infection and leukocyte counts (Spearman correlation = 0.04;  $P = 0.6$ ), suggesting that confounding by concurrent infection was unlikely.

Comparisons of baseline and delivery characteristics between women who subsequently developed GDM and those who remained free of GDM were performed using two-sample  $t$  tests for continuous variables or Fisher's exact test for categorical variables. A two-sample  $t$  test was used to compare mean baseline leukocyte counts between women who developed GDM and those who did not. To examine a potential dose-response association between baseline leukocyte count and the degree of glucose intolerance, the non-GDM group was further subdivided into a normal GLT group ( $n = 2,299$ ) and an abnormal GLT but normal GTT group ( $n = 356$ ). The linear trend in mean leukocyte counts comparing the normal GLT, abnormal GLT–normal GTT, and the GDM groups was examined. Pairwise linear correlations between leukocyte count and GLT glucose levels and GTT

area under the curve (32) were also examined.

To further examine the association between baseline leukocyte count and risk of developing GDM, subjects were divided into quartiles according to the distribution of leukocyte counts among the non-GDM women as has been done in prior prospective studies of inflammation and risk of type 2 diabetes (12,33). Results of GDM screening tests and the incidence of GDM according to leukocyte quartile were compared. The relative risks (RRs) of developing GDM comparing each of the upper quartiles with the lowest leukocyte count quartile (the reference group) were calculated using logistic regression. To determine whether there was a significant graded increase in risk of GDM with increasing leukocyte quartiles, the *P* value for the linear trend of the RRs was calculated. Multivariable logistic regression was used to calculate the RRs for GDM among the upper leukocyte quartiles adjusted for potential confounding. Potential confounding factors that were examined in the multivariable analysis included those known to be associated with GDM, such as age, race, BMI (weight in kilograms divided by the square of height in meters, multiple gestation, chronic hypertension, and hypertensive disorders of pregnancy (preeclampsia: blood pressure  $\geq 140/90$  mmHg after 20 weeks of gestation with proteinuria, either  $\geq 2+$  by dipstick or  $\geq 300$  mg/24 h in the absence of infection, or gestational hypertension: blood pressure  $\geq 140/90$  mmHg after 20 weeks of gestation without proteinuria) (34), and those associated with the leukocyte measurement itself, such as smoking, blood pressure, and gestational age at the time of blood sampling (35,36). All analyses were performed using Intercooled STATA statistical package (STATA, College Station, TX). Statistical significance was inferred from two-sided *P* values  $< 0.05$ .

**RESULTS**— Baseline and delivery characteristics of women who developed GDM and women who remained free of GDM are presented in Table 1. The mean gestational age at the time of the leukocyte sampling was between 10 and 12 weeks for both groups. Compared with non-GDM women, those who subsequently developed GDM were older, heavier, and had higher baseline blood pressure. Asian women and women with a history of

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

	GDM	Non-GDM	* <i>P</i>
<i>n</i>	98	2,655	
Baseline characteristics at the first prenatal visit			
Gestational age (weeks)	10.8 $\pm$ 2.7	11.7 $\pm$ 4.5	0.05
Age (years)	31.6 $\pm$ 5.8	29.2 $\pm$ 5.9	$< 0.01$
Race (%)			$< 0.01$
Non-Hispanic Caucasian	61	72	
African American	10	5	
Hispanic	10	14	
Asian	19	9	
Systolic blood pressure (mmHg)	117 $\pm$ 11	113 $\pm$ 12	$< 0.01$
Diastolic blood pressure (mmHg)	74 $\pm$ 9	71 $\pm$ 9	$< 0.01$
BMI (kg/m <sup>2</sup> )	28.3 $\pm$ 7.2	24.6 $\pm$ 4.9	$< 0.01$
Smoking (%)			NS
Never	42	49	
Stopped	28	21	
Current	30	30	
Number of fetuses (%)			$< 0.01$
Singleton	92	97	
Multiple	8	3	
Hypertension			
Chronic (%)	15.0	6.4	$< 0.01$
Gestational (%)	9.2	7.0	NS
Preeclampsia (%)	11.2	4.1	$< 0.01$
Delivery characteristics			
Gestational age (weeks)	38.4 $\pm$ 2.5	39.5 $\pm$ 1.8	$< 0.01$
Cesarean section (%)	40	25	$< 0.01$
Birth weight (g)	3,407 $\pm$ 604	3,357 $\pm$ 537	NS

Data are means  $\pm$  SD, unless otherwise indicated. \**P* for overall comparisons.

chronic hypertension were more likely to develop GDM. The risk of preeclampsia among women with GDM was almost threefold higher than that of the non-GDM women. Mean birth weight was not significantly increased among women with GDM, likely because they delivered  $> 1$  week earlier, had a higher prevalence of preexisting chronic hypertension, and a greater incidence of preeclampsia.

Women who developed GDM displayed increased mean leukocyte count at their first prenatal visit compared with non-GDM women ( $10.5 \pm 2.2$  vs.  $9.2 \pm 2.2 \times 10^3$  cells/ml;  $P < 0.01$ ). Within the non-GDM group, women with an abnormal GLT ( $> 7.8$  mmol/l) but normal GTT had significantly increased leukocyte counts compared with women with a normal GLT ( $9.6 \pm 2.2$  vs.  $9.2 \pm 2.1 \times 10^3$  cells/ml;  $P < 0.01$ ). There was a linear trend between increasing mean leukocyte count and worsening degree of glucose intolerance going from normal glucose tolerance (normal GLT) to abnormal GLT

but normal GTT to GDM (*P* for linear trend  $< 0.01$ ). Within the entire study population, leukocyte counts were linearly correlated with GLT glucose levels ( $r = 0.14$ ;  $P < 0.01$ ), and among the 454 women who underwent a GTT, leukocyte counts were linearly correlated with the area under the GTT glucose curve ( $r = 0.14$ ;  $P < 0.01$ ).

To examine the risk of GDM according to baseline leukocyte counts in further detail, subjects were divided into quartiles of leukocyte count based on the distribution among the non-GDM women (quartile 1,  $< 7.9$ ; quartile 2,  $7.9$ – $9.0$ ; quartile 3,  $9.1$ – $10.4$ ; and quartile 4,  $> 10.4 \times 10^3$  cells/ml). Table 2 presents the results of GDM screening tests and the incidence of GDM according to leukocyte quartiles. There was a statistically significant linear trend between increasing leukocyte quartiles and GLT results, GTT area under the curve, and GDM incidence (Table 2). The crude and multivariable-adjusted RRs of GDM expressed accord-

**Table 2—Leukocyte counts, glucose levels, and incidence of GDM according to leukocyte quartile**

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P for linear trend
n	662	715	668	708	
Leukocyte count range (× 10 <sup>3</sup> cells/ml)	<7.9	7.9–9.0	9.1–10.4	>10.4	—
GLT glucose (mmol/l)	6.1 ± 1.4	6.3 ± 1.4	6.4 ± 1.5	6.7 ± 1.5	<0.01
GTT area under the curve (mmol/l * 3 h)	20.7 ± 3.8	21.8 ± 3.6	22.3 ± 4.7	22.7 ± 4.5	<0.01
GDM (%)	1.1	2.5	4.2	6.4	<0.01

Data are means ± SD, unless otherwise indicated.

ing to leukocyte quartile are presented in Fig. 1. In the unadjusted analysis, there was a linear increase in the RR of GDM with increasing leukocyte quartile (quartile 1, reference; quartile 2, RR 2.4 [95% CI 1.0–5.8]; quartile 3, 4.1 [1.8–9.4]; and quartile 4, 6.4 [2.8–14.2]; P for linear trend <0.01). After adjusting for age, race, BMI, smoking, multiple gestation, baseline blood pressure, chronic hypertension, hypertensive disorders of pregnancy, and gestational age at the time of the leukocyte sampling, the linear relationship between increasing leukocyte quartile and increased GDM risk persisted (quartile 1, reference; quartile 2, 2.3 [0.9–5.7]; quartile 3, 3.3 [1.4–7.8]; and quartile 4, 4.9 [2.1–11.2]; P for linear trend <0.01). Other factors independently associated with increased risk of

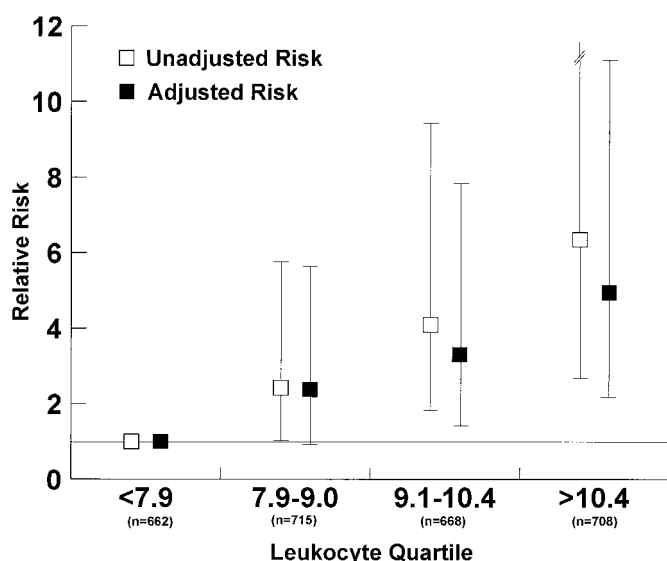
developing GDM in the multivariable model included older maternal age (RR 1.08 per year increase [95% CI 1.04–1.13]; P < 0.01), Asian race (4.9 compared with Caucasians [2.6–9.1]; P < 0.01), African-American race (3.2 compared with Caucasians [1.5–7.0]; P < 0.01), multiple gestation (2.5 compared with singletons [1.1–5.6]; P = 0.03), and increased BMI (1.10 per unit increase [1.06–1.13]; P < 0.01).

**CONCLUSIONS**— In this prospective cohort of nulliparous women, increased early pregnancy leukocyte count was associated with increased risk of developing GDM. This effect was independent of known risk factors for GDM, such as advanced maternal age, non-Caucasian race, obesity, hypertension, and multiple

gestation. There was a linear increase in GDM risk with increasing leukocyte quartiles, a linear correlation between leukocyte counts and results of third-trimester GLTs and GTTs, and a graded increase in mean leukocyte count with increased degrees of glucose intolerance, all of them suggesting a dose-response relationship between inflammation and glucose tolerance during pregnancy. The results of this study, which parallel prior prospective studies outside pregnancy, provide further support to the hypothesis that inflammation contributes to the development of glucose intolerance in pregnancy.

Cytokine-induced insulin resistance appears to be a primary mechanism underlying the association between inflammation and glucose intolerance. IL-6 and TNF-α, which are secreted in response to infection, tissue injury, and perhaps pregnancy (30,37), directly inhibit insulin-stimulated tyrosine phosphorylation at the insulin receptor (16,18). Inflammatory cytokines also stimulate secretion of cortisol and growth hormone, counter-regulatory hormones that contribute to insulin resistance and hyperglycemia (27). In addition, catecholamines stimulate IL-6 release from hepatocytes and adipose tissue (38), and excess basal catecholamine levels characterize obesity and type 2 diabetes (39). Adipose cells are a primary source of basal IL-6 and TNF-α secretion, and increased adiposity leads to increased circulating levels of these cytokines, suggesting that inflammation is one mechanism linking obesity with increased insulin resistance (14,15). Increased leukocyte count is a component of the acute phase response that is stimulated by IL-6 (12), and single nucleotide polymorphisms in the IL-6 gene, which lead to increased IL-6 levels, also lead to increased leukocyte count and decreased insulin sensitivity (40).

Several large, prospective studies examined the risk of developing type 2 diabetes according to baseline leukocyte count. In a prospective study of Pima Indians (12), increased baseline leukocyte count was associated with greater decline in insulin sensitivity and increased incidence of type 2 diabetes during 5 years of follow-up. There was no effect of inflammation on insulin secretion, indicating that inflammation contributes to diabetes via effects at the target tissues of insulin (12). In the Atherosclerosis Risk in Communities study of >12,000 Caucasian and



**Figure 1—Crude and multivariable-adjusted RRs of developing GDM according to quartiles of early pregnancy leukocyte count, with quartile 1 serving as the reference group. White and black boxes indicate the point estimates of the RRs from the unadjusted and multivariable-adjusted analyses, respectively.**

African-American men and women, leukocyte count in the highest versus lowest quartile was independently associated with a 50% increased incidence of type 2 diabetes during the subsequent 7 years of follow-up (41). In an additional prospective study from the National Health and Nutrition Examination Survey Epidemiologic Follow-up Study, increased leukocyte counts were independently associated with a 70% increased risk of type 2 diabetes in women (42). Importantly, in each of these studies, the mean leukocyte counts that were associated with increased risk of type 2 diabetes were within the normal range, as they were in this study. Likewise, recent prospective studies of CRP (29,33) utilized high-resolution assays because small increases in baseline CRP levels, well within the previously reported normal range, were nonetheless associated with significantly increased risk of future type 2 diabetes and cardiovascular disease. The results of the current study add to the growing body of epidemiological evidence that supports subclinical inflammation as a potential mechanism of diabetes.

In a prior prospective, case-control study nested within the same MOMS cohort (31), we identified a significant univariate association between increased CRP and subsequent GDM, but the effect was mitigated when adjusted for BMI. Although these results appear contradictory to the current data, differences in statistical power may account for the apparent discrepancy. In that study, CRP levels tracked closely with BMI ( $r = \sim 0.4$ ) and because case subjects were significantly heavier than control subjects, CRP levels were similarly increased among case subjects, a difference that was partially attenuated when adjusted for BMI. In this larger study, increased power along with less correlation between leukocyte counts and BMI ( $r = 0.16$ ) allowed for identification of independent effects of both leukocyte count and BMI on the risk of GDM. These data are similar to other large prospective studies (12,41) in which the associations between leukocyte count and type 2 diabetes were not obscured by differences in BMI. Indeed, in one study (43), the strongest association between inflammation and diabetes existed in lean subjects, confirming that inflammation contributes to the development of glucose intolerance independent of obesity.

Several features of our results warrant emphasis. First, we excluded women with

“early GDM,” that is, women in whom GDM was diagnosed because of signs and symptoms of diabetes before third trimester screening. While these women are technically labeled as GDM, many may have had diabetes before pregnancy or at the time of the blood sampling. Given the known association between increased leukocyte count and diabetes, excluding these women likely underestimated the true difference in mean leukocyte counts between the GDM and non-GDM groups. In addition, for all of the primary analyses the non-GDM group included 356 women who failed the GLT, some of who also had one abnormal GTT glucose level. This abnormal GLT-normal GTT subgroup had a significantly higher mean baseline leukocyte count than the non-GDM subgroup with normal GLTs. Had we compared women with GDM to only those with completely normal glucose tolerance, as has been done in prior studies (31,44), the difference in mean leukocyte counts would have been magnified further.

Second, while several prospective studies have identified an association between increased levels of inflammatory markers and the development of diabetes, in many of these the primary measure of effect was the relative risk of diabetes comparing members of the uppermost versus the lowest inflammatory marker quantile. In few studies was there evidence of intermediate risk in the intermediate quartiles and thus, a statistically significant linear increase in relative risk with increasing levels of the inflammatory exposure, as was observed in this study. This dose-response relationship between exposure and outcome supports the hypothesis that inflammation contributes mechanistically to the development of glucose intolerance. Additional dose-response observations from this study corroborate the hypothesis. For example, there was significant correlation between the level of the inflammatory exposure and the degree of glucose intolerance as defined by the screening GLT and GTT results: women with normal GLTs displayed the lowest mean leukocyte count at baseline, those with abnormal GLTs but normal GTTs demonstrated intermediate levels and women with GDM had the highest leukocyte counts. Furthermore, there was significant linear correlation between leukocyte counts and both GLT glucose levels and GTT area under

the curve. Although the leukocyte – GLT glucose correlation may appear relatively weak at first inspection ( $r = 0.14$ ), it is more noteworthy when considering that marked variance in the GLT glucose levels, owing in part to the lack of standardization of this initial screening test (i.e., fasting versus nonfasting, time of day, etc.), would tend to obscure the linear correlation we nonetheless observed. We conclude that women who develop GDM display increased inflammation during early pregnancy roughly 20 weeks before the GDM is diagnosed. Furthermore, the degree of inflammation at baseline is linearly associated with the degree of hyperglycemia and the degree of risk for developing GDM.

Third, the magnitudes of the relative risk estimates were substantially greater in this pregnancy study compared with prior studies of type 2 diabetes. For example, in prior studies, the greatest relative risk for type 2 diabetes comparing the upper leukocyte quartile with the reference group was 1.7, whereas in this study, the maximum relative risk from the multivariable analysis was nearly fivefold. The explanation for the markedly increased point estimates observed in this study of GDM remains unclear. It is possible that inflammation is more strongly associated with GDM, perhaps because the physiological increase in insulin resistance associated with normal pregnancy exerts additive effects with subclinical inflammation that together manifest as GDM. Alternatively, one could speculate that different durations between the exposure and outcome may account for the differences. For example, during the long duration between the leukocyte measurement and the development of type 2 diabetes (years) several additional factors beyond inflammation and various levels of inflammation would have the opportunity to influence the risk of diabetes and thus mitigate the risk estimate attributable to the initial leukocyte measurement. Conversely, such misclassification of the exposure would be less likely in this study given the comparatively short duration between the leukocyte measurement and the development of GDM (months).

A valid criticism of this study is that leukocyte count is a relatively nonspecific marker of inflammation that is influenced by several factors, including infection, certain medications, and gestational age (45). We acknowledge that residual con-

founding by these and other unknown factors cannot be definitively excluded. However, imprecision or misclassification in the measurement of the primary exposure would preferentially bias the results toward the null, and thus, identifying statistically significant associations despite this limitation bolsters rather than weakens the results (46). Furthermore, there were no differences in infection rates at the time of the leukocyte sampling comparing the GDM and non-GDM groups, and there was no association between infection and leukocyte counts. Finally, we attempted to minimize confounding by concurrent infection by excluding women whose leukocyte counts were measured before routine screenings. These tests were more likely to have been ordered by practitioners in response to clinical signs and symptoms, such as vaginal bleeding or infection, and were thus prone to confounding by indication. Regarding the timing of blood sampling, leukocyte count did increase with gestational age ( $r = 0.07$ ;  $P < 0.01$ ), and we accounted for this effect in the multivariable model.

A second important limitation is that although mean leukocyte counts were statistically increased in women who later developed GDM, the significant overlap in the leukocyte distributions and the relatively small absolute difference in levels preclude this marker from useful clinical application in individual patients. The purpose of this study, like prior studies of leukocyte counts and risk of type 2 diabetes, however, was not to identify a novel screening or diagnostic test but rather to explore mechanisms of disease. It is noteworthy, however, that a relatively non-specific early pregnancy marker, such as leukocyte count, can be shown to be associated with pregnancy complications that develop several months later. This suggests that the application of novel, highly sensitive genomic or proteomic technologies for early pregnancy diagnostic and prognostic purposes offers promise for future clinical utility.

Inflammation appears to be a component of the pre-diabetes phenotype during pregnancy, just as it is for type 2 diabetes. In the future, perhaps early pregnancy measurement of more precise inflammatory markers (e.g., cytokine profiles) may be clinically useful in predicting which women will develop GDM. Likewise, whether inflammatory markers

may help identify which women with GDM are at greatest risk of recurrent GDM or progression to type 2 diabetes postpartum is worthy of further investigation. Finally, it is important to note that we have demonstrated increased inflammation and its impact on clinical outcomes in a significantly younger population than has been examined in most diabetes and cardiovascular studies. With recent advances in the primary prevention of type 2 diabetes and cardiovascular disease, the results of this study of GDM should have important implications on risk factor modification strategies for a variety of clinicians engaged in women's health, beyond obstetricians and diabetologists.

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