

Maternal Obesity Is Associated with Dysregulation of Metabolic, Vascular, and Inflammatory Pathways

JANE E. RAMSAY, WILLIAM R. FERRELL, LYNNE CRAWFORD, A. MICHAEL WALLACE, IAN A. GREER, AND NAVEED SATTAR

University Departments of Obstetrics and Gynaecology (J.E.R., I.A.G.), Medicine (W.R.F.), and Pathological Biochemistry (L.C., N.S.), Glasgow Royal Infirmary, Scotland, G31 2ER, United Kingdom

Obesity is increasing in prevalence worldwide and in all age groups. In nonpregnant individuals, obesity is associated with dyslipidemia; hyperinsulinemia; vascular dysfunction; and, more recently, low-grade chronic inflammation. However, whether such effects are sustained during pregnancy has been sparsely investigated but is important to establish, given the association of maternal obesity with numerous adverse metabolic and vascular consequences.

We consecutively recruited 47 healthy women in the third trimester of pregnancy and divided the participants into 2 groups, lean [$n = 24$; median body mass index (BMI), 22.1 kg/m²] and obese ($n = 23$; median BMI, 31.0 kg/m²) around the median first trimester BMI. The age, parity, and smoking history were comparable in both groups. A detailed panel of metabolic and inflammatory parameters was measured and an *in vivo* assessment of endothelial-dependent and -independent microvascular function made using laser doppler imaging. Although low-density lipoprotein cholesterol and glycosylated hemoglobin were similar, fasting triglyceride concentrations were higher [2.70 (interquartile range, 2.3–3.21) vs. 2.20 (IQ range, 2.0–2.6) mmol/liter, $P = 0.02$] and high-density lipoprotein concentrations were lower [1.55 (IQ range, 1.1–1.7) vs. 1.72 (IQ range, 1.4–2.0) mmol/liter, $P = 0.02$] in the obese group. Leptin [55.6 (range, 45–64.4) ng/ml vs. 23.8 (range, 13.2–35.2) ng/ml, $P < 0.0001$]

and fasting insulin [14.5 (range, 11.4–27.3) vs. 6.5 (range, 4.6–9.7) mU/liter, $P < 0.0001$] levels were more than double. Similarly, levels of inflammatory parameters, IL-6 [3.15 (range, 2.4–3.5) vs. 2.1 (range, 1.73–2.85) pg/ml, $P = 0.003$], and sensitive C-reactive protein [4.45 (range, 2.9–6.6) vs. 2.25 (range, 0.92–3.65) mg/ml, $P = 0.0015$] were also substantially elevated. Both endothelial-dependent and -independent vasodilatory responses were significantly reduced in the obese group ($P = 0.0003$ and $P = 0.02$, respectively, ANOVA) and systolic blood pressure was higher ($P = 0.01$). Metabolic factors, C-reactive protein ($r = 0.289$, $P = 0.049$), and insulin ($r = 0.339$, $P = 0.02$) were related inversely to endothelial-dependent function.

These comprehensive data demonstrate that, as in nonpregnant obese individuals, obesity in pregnancy is associated not only with marked hyperinsulinemia (without necessarily glucose dysregulation) and dyslipidemia but also impaired endothelial function, higher blood pressure, and inflammatory up-regulation. Such a spectrum of risk factors may contribute to maternal complications in obese women and, as a result, influence fetal programming of adult vascular disease. Clearly, these data provide further rationale to examine the potential benefits of preconceptual weight loss and antenatal exercise. (*J Clin Endocrinol Metab* 87: 4231–4237, 2002)

OBESITY IS A HEALTH problem of epidemic proportions in Western society, with 10% of children and 20% of adults in the United Kingdom now classified as clinically obese [body mass index (BMI) > 30] (1, 2). In the United States, these figures are even more alarming (3). Worldwide, it is estimated that more than 300 million people are clinically obese. It is now well established that obesity and body fat distribution strongly correlate with deranged metabolic function and are associated with an elevated incidence of cardiovascular disease (4–6).

Obesity in pregnancy has implications for morbidity and mortality in both mother and baby. The risk of maternal hypertensive complications, such as pregnancy-induced hypertension or preeclampsia, are significantly greater if the mother is overweight, as assessed by BMI in early pregnancy, or centrally obese, as assessed by waist circumference (7, 8). Maternal obesity also increases the risk of metabolic complications, such as gestational diabetes. Furthermore, some data suggest that

adiposity in the mother may critically influence the programming of metabolic pathways of her fetus and its risk for diabetes and cardiovascular disease in later life (9).

Although there is abundant evidence concerning the effects of obesity on metabolic pathways and vascular function in the nonpregnant individual, such information is currently sparse with respect to pregnancy. Therefore, the aim of this study was to examine classical (lipids, blood pressure) and novel (inflammation, insulin resistance) cardiovascular risk factors in lean and obese women in the third trimester of pregnancy. To relate these risk factors to blood vessel function, we took advantage of recent developments in non-invasive microvascular assessment to examine endothelial function, using a novel technique ideally suited to the study of pregnant women (10). Our hypothesis was that all of the pathological associations of obesity in nonpregnant subjects would be sustained in pregnant women in the third trimester and, as such, may underlie the elevated risk for pregnancy complications in obese women.

Subjects and Methods

Forty-seven consecutive subjects, in the third trimester of pregnancy, were recruited from antenatal clinics. These women were divided into

Abbreviations: ACh, Acetylcholine; BMI, body mass index; CRP, C-reactive protein; CV, coefficient(s) of variation; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDI, laser doppler imaging; LDL-C, low-density lipoprotein cholesterol; NO, nitric oxide; SNP, sodium nitroprusside; T, T-statistic.

two groups, lean and obese, around the median early pregnancy (10–12 weeks gestation) BMI of 27.7 kg/m². This median value is similar to criteria used in previous studies (11). Our power calculations suggested that 20 women in each group would provide 90% power to detect differences in endothelial function. Women participating in the study were healthy and normotensive, with no significant past medical history, such as peripheral vascular abnormalities, dermatological diseases, or systemic disease processes such as diabetes mellitus, and no relevant complications of pregnancy. In addition, no participant had ongoing infection or a recent history of infection or injury. The study was performed according to the Declaration of Helsinki, and approval was granted by the institutional ethics committee. All women gave written informed consent.

Clinical and laboratory measurements

Women attended, for participation in the study, after an overnight fast (>10 h) and underwent testing between 0900 and 1100 h. Blood pressure was recorded using a standard sphygmomanometer and appropriately sized cuff (Table 1). Fasting blood was withdrawn for lipid profile [via modification of the standard Lipid Research Clinics Protocol; the intraassay and interassay coefficients of variation (CV) for all lipid measures were less than 3%]. Other measures included glycosylated hemoglobin (HbA1c) (HPLC, HA8121 analyzer; Menarini Diagnostics, Berkshire, UK), insulin (a competitive RIA, Coat-A-Count I; DPC, Los Angeles, CA), IL-6 (Quantikine High Sensitivity Human IL-6 Immunoassay; R&D Systems Inc., Oxon, UK), and C-reactive protein (CRP) (double-antibody sandwich ELISA with rabbit antihuman CRP and peroxidase-conjugated rabbit antihuman CRP: DK-2600; DAKO Corp. A/S, Glostrup, Denmark). For the CRP assay, standard curves were linear up to 5 mg/liter and logarithmic thereafter. The interassay and intraassay CV were less than 10%, across the range of measured results. Plasma leptin was measured by an in-house RIA validated thoroughly against the commercially available Linco Research, Inc. assay (12). The intra- and interassay CV were less than 7% and less than 10%, respectively, over the sample concentration range. The detection limit of the assay was 0.5 ng/ml.

Perfusion measurements

Perfusion measurements were also performed after an overnight fast, therefore ensuring no caffeine-containing drinks had been consumed before testing. Also, no over-the-counter medications were taken by any of the participants for at least 48 h before testing. Before testing, a 10-min period of acclimatization was enforced, in a temperature-controlled room. The women lay in a semirecumbent position, with the flexor aspect of the forearm exposed on an armrest. Noninvasive measurement of skin perfusion was performed by means of a laser doppler imaging (LDI) unit (Moor Instruments LTD, Axminster, UK) equipped with a red laser (wavelength, 633 nm; power, 1mW; beam diameter, 1 mm) as previously described (10). The laser is scanned over the area to be examined, and backscattered light is collected by photodetectors and converted into a signal proportional to perfusion, in arbitrary perfusion (flux) units. Twenty repetitive scans with an incremental iontophoretic current protocol were taken (described below). An assessment of the

overall response was defined as the area under the perfusion time curve. As previously described (10), correction for individual variation in skin resistance was performed.

Iontophoresis

This technique is based on the principle that a charged molecule migrates across the skin under the influence of an applied electrical field. (current × time = charge, in coulombs). Iontophoresis of acetylcholine (ACH) examines endothelial function, because binding to muscarinic receptors, with subsequent generation of nitric oxide (NO), requires intact endothelial cells and is therefore said to be endothelium-dependent. Vasodilatation is ultimately mediated by action of NO on vascular smooth muscle (via the cyclic GMP pathway); and so, iontophoresis of sodium nitroprusside (SNP), an NO donor, is used as a so-called endothelium-independent control. Drug delivery was achieved using a battery-powered constant current iontophoresis controller (Moor Instruments LTD MIC-1e). The chambers used for iontophoresis (Moor Instruments LTD ION 6) were constructed of Perspex (internal diameter, 22 mm; area 3.8 cm²) with an internal platinum wire electrode. Two chambers were attached to the skin of the volar aspect of the forearm, avoiding hair, broken skin, and superficial veins. The protocol involved incremental current delivery, with a baseline scan (four scans at 5 μA, four at 10 μA, four at 15 μA, and two at 20 μA), giving a total charge of 8 millicoulombs, followed by five so-called recovery scans. Two and a half milliliters of 1% ACH (Sigma, Poole, UK) was introduced into the anodal chamber while 2.5 ml of 1% SNP (Sigma) was placed in the cathodal chamber. The vehicle for these drugs was 0.5% NaCl in deionized water. Responses were also observed with the vehicle alone as a control experiment. Based on the raw perfusion-time integrals, the mean (± SD) between-day CV for the ACH response, measured in four subjects on 2 separate days, was 6.4 ± 3.3%; whereas the within-day, between-site CV, measured in both forearms on the same morning in four subjects, was 8.9 ± 5.3%.

Statistical analyses

Dose response curves were expressed as mean ± SEM and were compared using two-way ANOVA. Metabolic parameters were compared using the Mann-Whitney *U* test. Univariate analysis relationships were expressed as Pearson's correlation coefficients after log transformation of skewed variables. Linear regression was employed to examine independent associates of endothelial and metabolic measures.

Results

Demographic characteristics of each subject group are given in Table 1. The groups were not significantly different, with regard to age, smoking history, parity, and weeks of gestation. The lean group consisted of 24 women, with a median BMI of 22.15 kg/m² (interquartile range, 20–24); and the obese group consisted of 23 women, with a median BMI of 31 kg/m² (interquartile range, 29–34). Although within normal ranges, diastolic blood pressure recorded in the first trimester and systolic

TABLE 1. Demographic characteristics of study subjects

	Lean n = 24	Obese n = 23	P
1st Trim BMI (kg/m ²)	22.1 (20–24)	31 (29.1–34)	<0.0001
Age (yr)	27 (21.5–32)	30 (25–34)	0.24
Smokers	4	7	0.26 ^a
Parity (primigravidae)	17	15	0.68 ^a
Gestation (wk)	36 (35–37)	35 (34–38)	0.79
1st Trim systolic (mm Hg)	117 (110–125.7)	119 (110–130)	0.5
1st Trim diastolic (mm Hg)	65 (60–72.75)	75 (64–78)	0.035
3rd Trim systolic (mm Hg)	115 (110–120)	130 (120–130)	0.01
3rd Trim diastolic (mm Hg)	70 (62–80)	80 (70–80)	0.2
Gestation of delivery (wk)	40 (39–40)	39 (38–40)	0.44
Birth centile	40 (10–60)	60 (40–90)	0.03
Vaginal delivery	91%	68%	0.07 ^a

All values are medians (interquartile ranges). Statistical analysis performed using Mann-Whitney *U* test. Trim, Trimester.

^a Chi-square test.

blood pressure recorded in the third trimester were significantly higher in obese, compared with lean, subjects. Three women in the lean group subsequently gave birth to babies with birthweights below the 5th centile, and 1 woman from each group went on to develop mild preeclampsia at term. There were no significant differences between gestation of delivery or mode of delivery between the groups, although the birth centile of offspring was significantly greater from the obese women. All babies were liveborn (Table 1).

Plasma analyses

Results of plasma lipid, HbA1c, IL-6, CRP, leptin, and insulin concentrations are displayed in Table 2. Although total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations were not significantly different, fasting plasma triglyceride and very-low-density lipoprotein cholesterol concentrations were significantly higher and high-density lipoprotein cholesterol (HDL-C) concentrations lower in obese women. Insulin and leptin concentrations were more than 2-fold higher in obese women, as compared with lean controls, although the percentage of HbA1c demonstrated no significant difference between the two groups. IL-6 and CRP concentrations were also both significantly higher in the obese group (all $P < 0.05$). These differences persisted after exclusion of the three women with babies weighing less than the 5th centile and the two women who developed preeclampsia. Given that the women were recruited consecutively and they covered a continuous range of BMI, we were able to examine cross-sectional relationships between BMI and other parameters. Simple linear regression analysis of the entire cohort revealed significant correlation of BMI with log triglyceride ($r = 0.326$, $P = 0.025$), log insulin ($r = 0.684$, $P < 0.005$), log leptin ($r = 0.729$, $P < 0.005$), log IL-6 ($r = 0.523$, $P < 0.005$), and log CRP ($r = 0.476$, $P < 0.005$).

Endothelial-dependent vasodilatation

Dose-dependent perfusion response to ACH was significantly greater in the lean, as compared with obese, women (Fig. 1, $P = 0.0003$). Corrected area under the perfusion-time curve for ACH response was also calculated for each individual woman and was used in simple linear regression to consider the relationship of endothelial function to metabolic

parameters. Log CRP and log fasting insulin concentrations inversely correlated with microvascular endothelial function (ACH response) ($r = -0.289$, $P = 0.049$; $r = -0.339$, $P = 0.02$). In addition, CRP correlated strongly with fasting insulin ($r = 0.473$, $P = 0.001$).

Endothelial-independent vasodilatation

There was a small, but significant, difference in dose-dependent perfusion responses to SNP between lean and obese groups (Fig. 2, $P = 0.02$).

Predictors of CRP and fasting insulin

Given the recent evidence for the association of CRP with obesity and its independent prediction of diabetes in the nonpregnant individual (13, 14), we were interested in the correlates of CRP and insulin in pregnancy. Potential correlates examined were based on biological plausibility and previous literature, as above, and included BMI, leptin, insulin, and IL-6. Log leptin was the strongest linear correlate to both CRP ($r = 0.532$, $P < 0.001$, Fig. 3) and fasting insulin ($r = 0.738$, $P < 0.001$, Fig. 4), but other parameters were also correlated (data not shown). We therefore examined the independent predictors of CRP and insulin using step-wise regression analysis. For CRP, leptin [T-statistic (T) = 3.06, $P = 0.004$] and IL-6 (T = 2.16, $P = 0.036$) were retained in the final model and together explained 32% of its variability. For insulin, the independent predictors were again leptin (T = 3.62, $P = 0.001$) and BMI (T = 2.21, $P = 0.032$); and together, they accounted for 57.1% of its variability.

Discussion

We demonstrate, for the first time, that microvascular endothelial function, assessed by a novel noninvasive technique, is impaired in obese pregnant women, as compared with lean counterparts. These obese women also show a perturbed metabolic state, with dyslipidemia, as characterized by higher triglyceride and lower HDL-C concentration, hyperinsulinemia, elevated leptin concentrations, and a low-grade inflammatory response. Interestingly, just as in the prediabetic state, this perturbation occurred in advance of any notable glucose dysregulation, because HbA1c concen-

TABLE 2 Lipid, other metabolic, and inflammatory parameters in lean and obese groups

	Lean n = 24	Obese n = 23	P
Lipids			
Cholesterol (mmol/liter)	6.35 (5.87–6.95)	6.25 (5.6–7)	0.43
LDL-C (mmol/liter)	4.05 (3.75–4.79)	3.9 (3.1–4.7)	0.32
Triglyceride (mmol/liter)	2.17 (1.9–2.6)	2.7 (2.3–3.2)	0.02
VLDL-C (mmol/liter)	0.52 (0.31–0.64)	0.75 (0.6–1)	0.008
HDL-C (mmol/liter)	1.77 (1.46–1.99)	1.55 (1.15–1.7)	0.02
Metabolic			
Insulin (mU/liter)	6.15 (4.47–9.5)	14.2 (11.3–27)	<0.0001
HbA1c (%)	4.4 (4.2–4.7)	4.5 (4.3–4.7)	0.89
Leptin (ng/ml)	23.4 (12.4–30.9)	56.8 (46.2–65.2)	<0.0001
Inflammatory			
CRP (mg/ml)	2.13 (0.89–3.29)	4.45 (3.09–6.78)	0.0002
IL-6 (pg/ml)	2.1 (1.7–2.8)	3.15 (2.36–3.59)	0.0016

All values are medians (interquartile ranges). Statistical analysis performed using Mann-Whitney *U* test. VLDL-C, Very-low-density lipoprotein cholesterol.

FIG. 1. Endothelial-dependent vasodilatation. Dose-dependent perfusion response to ACH in pregnant, lean (n = 24) vs. obese women (n = 23). Data are mean ± SE (SEM). P = 0.0003, ANOVA. PU, Perfusion units; mCOUL, millicoulombs.

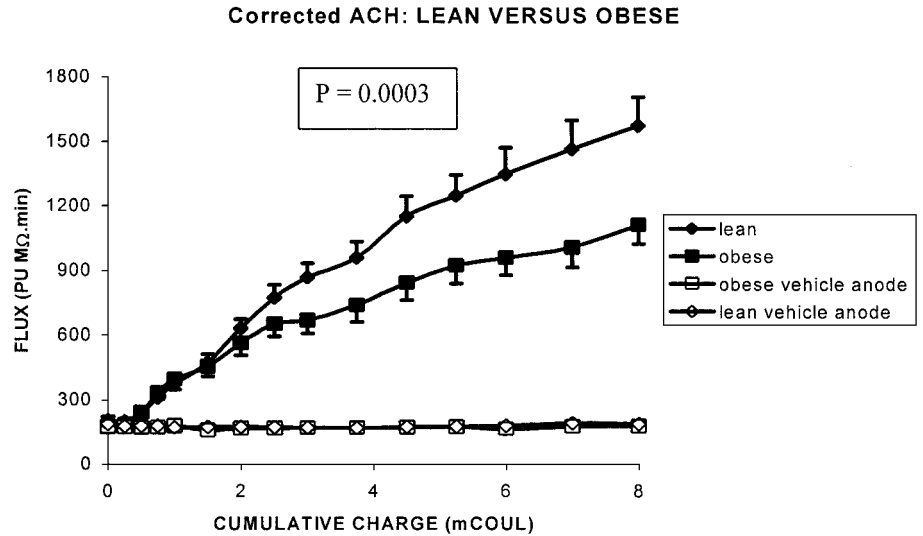


FIG. 2. Endothelial-independent vasodilatation. Dose-dependent perfusion response to SNP in pregnant, lean (n = 24) vs. obese women (n = 23). Data are mean ± SE (SEM). P = 0.02, ANOVA.

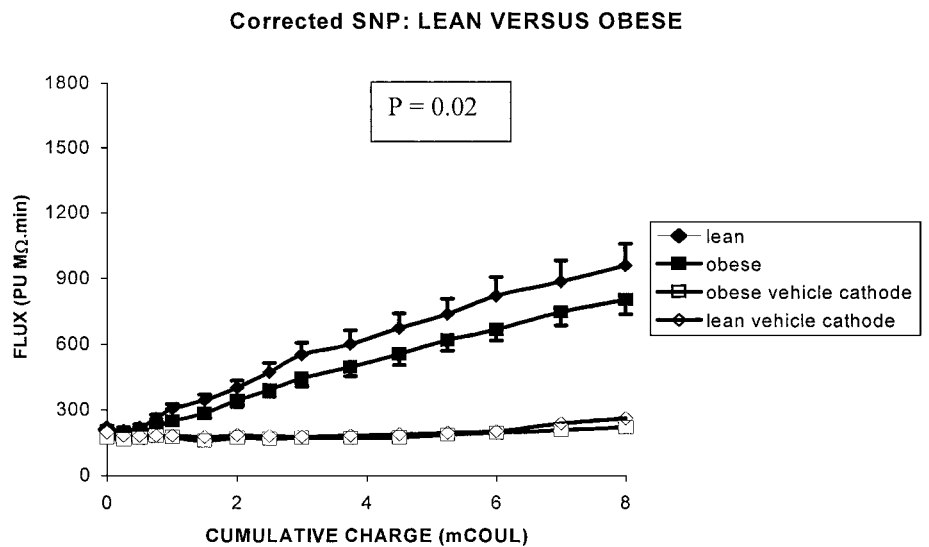
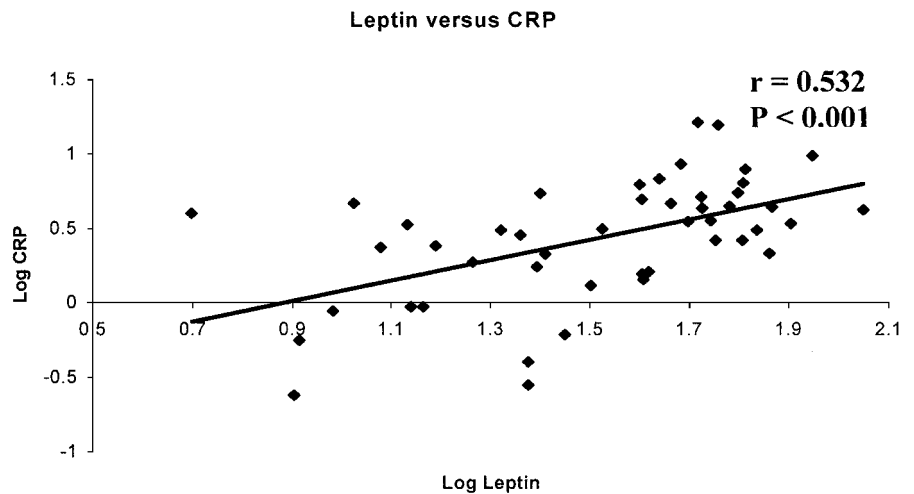


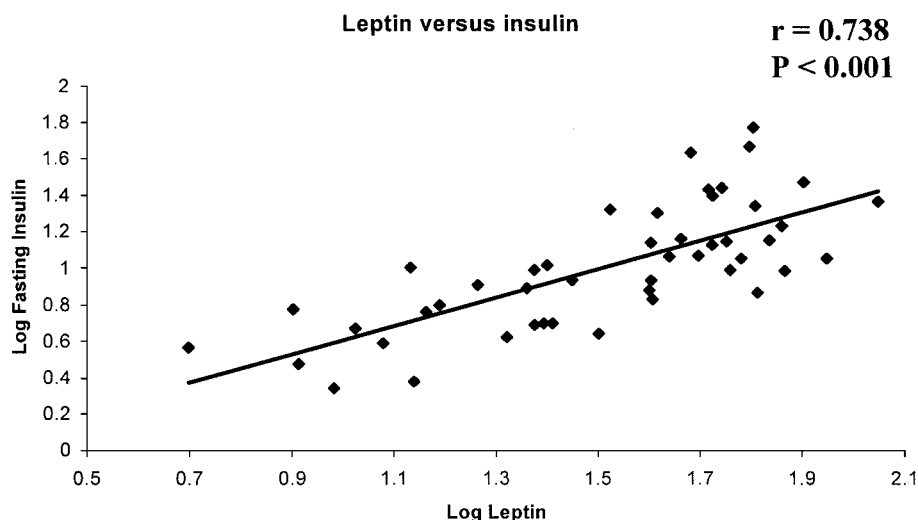
FIG. 3. Positive correlation between log¹⁰ leptin and log¹⁰ CRP concentrations (n = 47, r = 0.532, P < 0.001).



trations were near identical. Thus, just as in the nonpregnant state, obesity in pregnancy results in a plethora of metabolic and vascular abnormalities that could collectively exacerbate

the risk of maternal complications. These observations also indicate that the marked pregnancy-associated hormonal increments, such as estrogen, progesterone, and human pla-

FIG. 4. Positive correlation between \log_{10} leptin and \log_{10} fasting insulin concentrations ($n = 47$, $r = 0.738$, $P < 0.001$).



central lactogen, do not exert an effect to override the metabolic and inflammatory associations of obesity.

Microvascular function was investigated using a well-tolerated *in vivo* method of assessment, ideal for the pregnant patient. LDI, in combination with iontophoresis, examines the cutaneous microcirculation, a robust surrogate marker of vascular function in other vascular beds. Reduced responsiveness to iontophoretic administration of ACH has been observed in diabetes (15–17) and hypercholesterolemia (18); and, in both these conditions, there is a parallel reduction of the ACH response in the forearm circulation (a predominantly skeletal muscle vascular bed), assessed by venous occlusion plethysmography (19, 20). Furthermore, assessment of endothelial vasomotion in peripheral arteries has been shown to correlate with and relate directly to coronary dysfunction (21).

We noted not only a reduced response to ACH (endothelial dependent) but also reduced responses to administration of SNP (endothelial independent). Therefore, impaired perfusion responses in obese subjects may not necessarily represent solely endothelial dysfunction but vascular dysfunction further downstream at the level of the vascular smooth muscle. However, the smaller magnitude of the SNP response, as compared with the ACH response, may suggest endothelial dysfunction to be relatively more important. Other groups using LDI technology to investigate microvascular function in diabetic subjects and healthy relatives of Type II diabetics, a condition strongly linked with obesity, also demonstrated impaired endothelial-independent vascular responses (17, 22).

These observations may have particular significance, with regard to maternal vascular complications. Maternal obesity is established as a significant risk factor in the development of hypertensive complications of pregnancy, particularly preeclampsia (7, 23). Deranged endothelial function is proposed as the pathophysiological mechanism of this condition, and several *ex vivo* techniques have supplied evidence of altered vascular reactivity in preeclampsia (24–26). Thus, the metabolic, inflammatory, and functional abnormalities that we have described, for the first time, in obese pregnancy, may predispose to vascular compromise and may contribute to the mechanism by which maternal adiposity is associated with an elevated risk of preeclampsia.

This report, to the best of our knowledge, is also the first to comprehensively demonstrate that obesity in pregnancy is associated with hypertriglyceridemia and low high-density lipoprotein. This dyslipidemic pattern is consistent with the metabolic syndrome (27, 28). Interestingly, although LDL-C was not raised, smaller, dense, atherogenic low-density lipoprotein species are likely to be elevated, because circulating triglyceride-rich particles drive production of smaller particles from larger more buoyant species (29). Evidence from the cardiovascular arena links such a pattern, directly and indirectly, to endothelial dysfunction and greater oxidative stress (30). Indeed, we and others have shown the same pattern in preeclampsia (31–33). Clearly, prospective studies are required to examine whether the magnitude of the lipid dysregulation in the third trimester, associated with maternal obesity, is more or less exaggerated than would be seen in the nonpregnant state.

Our novel findings of elevated concentrations of IL-6 and CRP in obese pregnant women concur with recent literature implicating adiposity as a key factor in low-grade chronic inflammation (34–36). We found that IL-6 was 50% higher, and CRP was almost double, in the obese pregnant women, relative to the lean group. High CRP correlates with endothelial dysfunction (37, 38) and impaired insulin sensitivity (39, 40) in nonpregnant populations and predicts risk for type 2 diabetes in women (14). In line with these observations, we noted that CRP correlated negatively with ACH-mediated endothelial vasodilatation ($P = 0.049$) and positively with fasting insulin ($P = 0.001$). Thus, obesity-driven inflammation in pregnancy could be implicated in pathogenesis of preeclampsia and gestational diabetes. Indeed, some suggest that preeclampsia is a disease of inflammation (41–43), with endothelial cells in maternal systemic vessels becoming activated and damaged by circulating factors.

As expected, concentrations of leptin, an adipocyte-derived molecule (12, 44), were more than 2-fold higher in the obese women. Leptin is also produced by the placenta (45), so it is not entirely clear, from our data, to what extent the high leptin reflects adipose tissue-derived or placental leptin. However, because leptin independently correlated with both CRP and fasting insulin levels in our cohort, we suggest a dominant role for adiposity. In line with this, we have sug-

gested previously that elevated leptin concentrations in pregnancy could be linked to changes in maternal fat mobilization (46). These combined observations further emphasize the importance of adipose tissue as an active metabolic organ, even in pregnancy (36, 47).

Finally, could the above metabolic and vascular disruption have adverse consequences for fetal programming? First, altered maternal vascular function and dyslipidemia may dysregulate blood and nutrient flow to the developing fetus. Second, a higher inflammatory burden may be damaging, as a recent animal study demonstrated that offspring of rats injected with IL-6, throughout pregnancy, had greater body fat and, in male offspring, reduced insulin sensitivity (48). A proinflammatory phenotype is also linked to miscarriage (49). Finally, data exists linking maternal obesity to cardiovascular and metabolic disease in her offspring. A population-based study from Finland (9) demonstrated a positive relationship, in short mothers, between maternal BMI on admission to the labor ward and future death rate from coronary heart disease in male offspring. Higher adult rates of type 2 diabetes have also been reported in offspring of mothers who were above average weight in pregnancy in a different population (50). Clearly, the dysregulation in several risk factor pathways described herein may be relevant to this process; and importantly, such effects need not include glucose dysregulation. However, this suggestion remains speculative, and more data linking maternal obesity with the health of offspring are needed.

We recognize that our study has limitations, in that it is cross-sectional in nature; and therefore, further prospective data are now required. However, strengths include a comprehensive assessment of a panel of classical and novel risk factors and robust methodology.

In conclusion, these comprehensive data demonstrate, for the first time, that, as in nonpregnant obese individuals, obesity in pregnancy is associated not only with marked hyperinsulinemia (in advance of glucose dysregulation) and deranged lipids but also impaired endothelial function and inflammatory up-regulation. Such perturbation may contribute to the risk for maternal complications in obese women and, as a result, may also be relevant to fetal programming of adult vascular disease. Clearly, these data provide further rationale to examine the potential benefits of preconceptional weight loss and antenatal exercise.

Acknowledgments

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Address all correspondence and requests for reprints to: Dr. Jane Ramsay, University Department of Obstetrics and Gynaecology, 3rd Floor, Queen Elizabeth Building, Glasgow Royal Infirmary, 10 Alexandra Parade, Glasgow, G31 2ER, United Kingdom. E-mail: jer6q@clinmed.gla.ac.uk.

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