In Preeclampsia, Maternal Third Trimester Subcutaneous Adipocyte Lipolysis Is More Resistant to Suppression by Insulin Than in Healthy Pregnancy

Shahzya S. Huda, Rachel Forrest, Nicole Paterson, Fiona Jordan, Naveed Sattar, Dilys J. Freeman

Abstract—Obesity increases preeclampsia risk, and maternal dyslipidemia may result from exaggerated adipocyte lipolysis. We compared adipocyte function in preeclampsia with healthy pregnancy to establish whether there is increased lipolysis. Subcutaneous and visceral adipose tissue biopsies were collected at caesarean section from healthy (n=31) and preeclampsia (n=13) mothers. Lipolysis in response to isoproterenol (200 nmol/L) and insulin (10 nmol/L) was assessed. In healthy pregnancy, subcutaneous adipocytes had higher diameter than visceral adipocytes (P<0.001). Subcutaneous and visceral adipocyte mean diameter in preeclampsia was similar to that in healthy pregnant controls, but cell distribution was shifted toward smaller cell diameter in preeclampsia. Total lipolysis rates under all conditions were lower in healthy visceral than subcutaneous adipocytes but did not differ after normalization for cell diameter. Visceral adipocyte insulin sensitivity was lower than subcutaneous in healthy pregnancy and inversely correlated with plasma triglyceride (r=−0.50; P=0.004). Visceral adipose tissue had lower ADRB3, LPL, and leptin and higher insulin receptor messenger RNA expression than subcutaneous adipose tissue. There was no difference in subcutaneous adipocyte lipolysis rates between preeclampsia and healthy controls, but subcutaneous adipocytes had lower sensitivity to insulin in preeclampsia, independent of cell diameter (P<0.05). In preeclampsia, visceral adipose tissue had higher LPL messenger RNA expression than subcutaneous. In conclusion, in healthy pregnancy, the larger total mass of subcutaneous adipose tissue may release more fatty acids into the circulation than visceral adipose tissue. Reduced insulin suppression of subcutaneous adipocyte lipolysis may increase the burden of plasma fatty acids that the mother has to process in preeclampsia. (Hypertension. 2014;63:1094-1101.) • Online Data Supplement

Key Words: adipocytes • lipolysis • preeclampsia • pregnancy

Maternal metabolism during pregnancy adapts to support fetal growth and development. All women increase maternal fat stores in early pregnancy to meet the fetoplacental and maternal metabolic demands of late gestation and lactation.1,2 Total fat increases to a peak toward the end of the second trimester before diminishing, corresponding to a period of increased lipolytic activity.3,4 In women of normal weight, the majority of fat is accumulated in the subcutaneous compartment of the trunk and upper thigh.6,7 In later stages of pregnancy, there is an increase in both the thickness of preperitoneal fat (visceral) and the ratio of preperitoneal to subcutaneous fat as measured by ultrasound.8 This pattern may be related to the increasing gestational insulin resistance and hyperlipidemia. Hepatic fat is an important mediator of gestational insulin resistance in the rat9 and has also been suggested to be important in humans.10 Visceral adiposity correlates with metabolic risk factors11 and adverse metabolic outcomes in pregnancy including gestational diabetes mellitus and preeclampsia.12-14

Preeclampsia occurs in 2% to 4% of all pregnancies and is a leading cause of maternal and neonatal morbidity and mortality. There is considerable evidence that maternal obesity, increased insulin resistance, and aberrant fatty acid metabolism are involved in the pathogenesis of preeclampsia.15 There is a marked increase in plasma triglyceride concentration and an early rise in plasma nonesterified fatty acids (NEFA), independent of adiposity, suggesting exaggerated adipocyte lipolysis.15 Adipocyte lipolysis in the nonpregnant is under adrenergic control with adrenoreceptor B1, B2, and B3 receptors stimulating catecholamine-mediated lipolysis and adrenoreceptor A2A mediating its inhibition. Fatty acids are released from cellular triglyceride droplets by the sequential action of adipose tissue triglyceride lipase and hormone sensitive lipase. This latter enzyme is sensitive to insulin and is the mechanism by which insulin suppresses lipolysis. Elevated plasma NEFA levels and increased flux can contribute to insulin resistance, endothelial dysfunction, and inflammation.
Adipocytes store triglyceride using fatty acids released from plasma lipoproteins by the enzyme lipoprotein lipase (LPL). The hormone leptin is secreted by adipocytes and is involved in regulation of body weight and energy expenditure as well as regulating other metabolic and inflammatory pathways. It interacts with leptin receptors that exist as short and long isoforms.16

Despite the relevance of adipose tissue and adipocyte function to both normal and complicated pregnancy metabolism, there has been little direct study of adipocytes in human pregnancy. We hypothesized that adipocytes in preeclampsia are more susceptible to lipolysis and contribute to the increased NEFA seen in this condition. The aim of this study was to compare the lipolytic function of both visceral adipose tissue (VAT) and upper body subcutaneous adipose tissue (SAT) adipocytes under basal conditions, after isoproterenol stimulation, after inhibition with insulin, and also in the presence of both isoproterenol and insulin to assess the insulin sensitivity of the lipolytic response in healthy human pregnancy and in preeclampsia. The expression of genes relevant to lipolysis and its regulation were also studied in tissue biopsies.

Methods

Subject Recruitment
Nonlaboring healthy women at term (n=31) and women with preeclampsia undergoing caesarean section (n=13) were recruited from the Princess Royal Maternity Hospital, Glasgow. Age- and body mass index (BMI)–matched controls (2 controls per case) were selected from the healthy cohort. The study was approved by the Local Research Ethics Committee, and all women gave written informed consent. Details of selection criteria, patient data collection, and tissue sampling are provided in the online-only Data Supplement.

Plasma Metabolites
Plasma total cholesterol, triglyceride and high-density lipoprotein cholesterol,17 glucose, and high sensitivity C-reactive protein assays18 were performed by the Department of Clinical Biochemistry, Glasgow Royal Infirmary by routine methods. Other analytes were assayed using commercially available kits (online-only Data Supplement). HOMA was calculated as follows: fasting insulin (mU/L)×fasting glucose (mmol/L)/22.5.

Adipocyte Preparation, Sizing, and DNA Content
Adipocyte preparation was performed using a modification from Rodbell et al.19 and detail is provided in the online-only Data Supplement. Adipocytes were resuspended at 90% cytocrit. The diameters of 100 adipocytes were manually measured using a stage micrometer and a mean calculated for each preparation. DNA was isolated from a known volume of adipocytes using the Blood Prep DNA Purification protocol on the ABI Prism 6100 Nucleic Acid PrepStation (Applied Biosystems). The concentration of DNA was quantified using a Nanodrop ND 100.

Adipose Tissue Lipolysis Assay
Adipocyte cell suspension (100 μL) was assayed in a final volume of 1 mL as described in the online-only Data Supplement. The following conditions were included: basal lipolysis (no reagent), isoproterenol (200 nmol/L), insulin (Human Actrapid Novo Nordisk, 10 nmol/L), and combined isoproterenol (200 nmol/L) and insulin (10 nmol/L). Glycerol and NEFA concentrations were quantitated by colorimetric assay (Randox Laboratories Ltd, Co Antrim, United Kingdom and Wako NEFA-C, Alpha laboratories, Eastleigh, Hampshire, United Kingdom, respectively). Adipocyte cell number was measured indirectly by quantifying the DNA content in a known volume of adipocyte suspension and expressing lipolysis rates as μmol/L NEFA or glycerol released per μg of DNA. The degree of stimulation by isoproterenol and the degree of suppression by insulin were calculated as a percentage of basal release of NEFA or glycerol. Fat cell insulin sensitivity index (FCISI) was calculated as the percentage inhibition of isoproterenol-stimulated lipolysis by insulin.

Adipose Tissue mRNA Expression Quantification
Total RNA was isolated from adipose tissue and cDNA synthesized. Target gene expression was quantitated relative to a control gene by Taqman real-time polymerase chain reaction using commercial primer probes sets (Applied Biosystems; see online-only Data Supplement).

Statistical Analysis
Data were assessed for normal distribution using a Ryan–Joiner test and transformed to achieve a normal distribution where necessary. Means with SD are presented. Comparison within individuals was by paired t test (SAT versus VAT) and between preeclampsia and controls by 2 sample t test. Mann–Whitney and χ2 tests were used to test between differences in semicategorical and categorical variables, respectively. P<0.05 was considered significant. Cell diameters were divided into tertiles, and differences in distribution among tertiles were tested by χ2 test. Pearson correlation coefficients were calculated to assess associations between variables and r value and P value stated (P≤0.010 was considered significant because of multiple testing). The data were adjusted for potential confounders using the general linear model. Regression modeling used stepwise regression with P-to-enter and P-to-stay set at 0.15. Best models with significant predictive variables (P<0.05) are shown. All statistical analysis was performed in Minitab (version 16).

Results

Maternal Demographic and Biochemical Profile
Preeclampsia women had similar smoking rates to controls, were more likely to be primiparous, had higher systolic and diastolic blood pressure, delivered at an earlier gestation, and had offspring of lower birth weight centile (Table 1). Preeclampsia mothers had higher plasma triglyceride and NEFA and lower placental lactogen concentrations than matched controls.

SAT and VAT Cell Size in Healthy and Preeclampsia Pregnancy
SAT adipocytes were 23 μm larger in diameter than VAT adipocytes (P<0.001; Table 1). SAT and VAT cell sizes were correlated with each other (r=0.72; P<0.001) and also with maternal BMI (SAT, r=0.46, P=0.010; VAT, r=0.55, P=0.001) and plasma leptin (SAT, r=0.51, P=0.003; VAT, r=0.51, P=0.004). Neither SAT nor VAT cell diameter differed between control and preeclampsia pregnancies (Table 1). A significant difference in the SD of the 100 SAT cell diameters collected for each individual in the control group and the preeclampsia group (control 1.32 versus preeclampsia 1.26 μm; P=0.033) suggested that the distribution of cell size may differ between groups. When cell diameter distribution among tertiles of diameter was compared between preeclampsia and controls (Figure S1 in the online-only Data Supplement), it was seen that diameter distribution was shifted toward smaller diameter for SAT (P=0.004) and VAT (P=0.019) in preeclampsia adipocyte preparations. In preeclampsia, there was a correlation between SAT and VAT diameter (r=0.79; P=0.001). Maternal BMI, but not plasma leptin, was correlated with SAT (r=0.73; P=0.005) and VAT (r=0.84; P<0.001) diameter in preeclampsia.
Total lipolysis rates under all conditions were lower in VAT than SAT (Table 2), and net basal lipolysis rate was lower in VAT. VAT net lipolysis was more responsive to stimulation by isoproterenol than SAT. VAT had lower net lipolysis FCISI than SAT (Table 2). In SAT and VAT, basal total and basal net lipolysis rates were inversely correlated with percent stimulation by isoproterenol ($r=−0.55$ to $−0.79$; $P≤0.001$). In SAT only, net basal lipolysis rate was positively correlated with percent suppression by insulin ($r=0.62$; $P<0.001$). When cell diameter was included as a covariate in a general linear model, adipose tissue depot location was no longer a significant predictor of total or net lipolysis rates while cell diameter was ($P<0.05$), apart from isoproterenol-stimulated lipolysis where neither was associated. Cell diameter was significantly associated with percent stimulation of total basal lipolysis by isoproterenol ($P<0.05$).

The ratio of NEFA to glycerol released during lipolysis significantly decreased on exposure to isoproterenol and insulin in SAT but not in VAT (Figure S2). However, there were no significant differences in the NEFA/glycerol ratio between SAT and VAT under any condition. For VAT, but not SAT, recycling was correlated with cell size under basal ($r=0.46$; $P=0.009$) and isoproterenol-stimulated conditions ($r=0.56$; $P=0.001$).

There was no correlation between any measure of either SAT or VAT lipolytic function and maternal BMI, age, parity, blood pressure, offspring birth weight, and birth weight centile. VAT net lipolysis FCISI was inversely correlated with plasma triglyceride ($r=−0.50$; $P=0.004$) concentrations, but there were no univariate associations of SAT or VAT lipolytic activity with glucose, insulin, HOMA, or leptin.
SAT and VAT Receptor and Enzyme Expression in Healthy Pregnancy

There was no difference in adrenoceptor A2A, B1, and B2 expression, but VAT had 79% ($P=0.036$) lower $ADRB2$ mRNA expression than SAT (Figure 1). $LPL$ mRNA expression was 30% lower ($P=0.010$), and leptin mRNA expression was 57% lower ($P<0.001$) in VAT than SAT. Insulin receptor had 62% ($P=0.027$) higher expression in VAT than SAT. Expression of the long form of the leptin receptor was undetectable in SAT, whereas low levels of expression were seen in VAT. None of the receptor or enzyme expression levels that differed between VAT and SAT was correlated with maternal BMI, plasma leptin, or rates of lipolysis or FCISI.

Stepwise regression was performed to identify whether the expression of enzymes or receptors was associated with SAT and VAT lipolytic function in the healthy pregnancy. Table 2 shows the associations between lipolysis and receptor and enzyme expression. In healthy SAT, neither cell size nor any enzyme or receptor expression was associated with net lipolysis FCISI, whereas $ADRB1$ ($P=0.032$) and $LPL$ ($P=0.043$) expression was negatively associated with total lipolysis FCISI ($r^2$ adjusted 46%), independent of cell diameter.

Adipose Tissue Lipolytic Function, Receptor and Enzyme Expression in Preeclampsia

There were no differences in SAT total or net lipolysis rates between women with preeclampsia and age- and BMI-matched controls (Table 3). In preeclampsia, SAT had a lower net FCISI than controls. In univariate analysis, maternal BMI, SAT cell size, and progesterone were found to have an association with SAT total, but not net, lipolysis FCISI. When these factors were included in a general linear model, SAT total FCISI was now significantly different between preeclampsia (−118%) and controls (37%), $P=0.012$. There were no differences in VAT lipolytic function between preeclampsia and control women (Table 4) apart from a lower NEFA/glycerol ratio in the presence of isoproterenol and insulin in preeclampsia (Figure S3). There were no differences in mRNA expression of any of the measured receptors or enzymes in SAT, although there was a trend toward a 154% higher $LIPE$ expression in preeclampsia ($P=0.059$; Figure 2). In VAT, there was 45% increased expression of $LPL$ ($P=0.022$).

Regulation of SAT and VAT Lipolysis in Healthy Pregnancy

Stepwise multiple regression was performed to determine the contribution of cell size, plasma estradiol, placental lactogen, HOMA, leptin, adiponectin, and tumor necrosis factor-α (TNF-α) on basal lipolysis and FCISI in SAT and VAT from healthy pregnancy (Table S1). In initial modeling, progesterone was not associated with lipolytic function. SAT basal net and total lipolysis were significantly associated with TNF-α, whereas in VAT net lipolysis was associated with cell diameter and HOMA with placental lactogen, a nonsignificant contributor to the model. In healthy SAT, several variables were associated with lipolytic function, including maternal BMI, leptin, and expression of the long form of the leptin receptor.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Lipolysis (Glycerol Release)</th>
<th>Net Lipolysis (NEFA Release)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>SAT (n=31)</td>
<td>VAT (n=31)</td>
</tr>
<tr>
<td>Basal</td>
<td>0.12 (0.09)</td>
<td>0.08 (0.08)</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>0.29 (0.11)</td>
<td>0.19 (0.10)</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.13 (0.10)</td>
<td>0.08 (0.07)</td>
</tr>
<tr>
<td>Isoproterenol plus insulin</td>
<td>0.26 (0.15)</td>
<td>0.17 (0.11)</td>
</tr>
</tbody>
</table>

SAT and VAT lipolysis from healthy pregnancy.
In preeclampsia, SAT adipocyte insulin sensitivity was lower than in matched controls. This is the first direct evidence that in preeclampsia, SAT adipocyte insulin sensitivity was lower than in matched controls. This is consistent with the observation that increased late gestation lipolysis is associated with a high third trimester HSL mRNA expression to LPL activity ratio in rats. Our data also show that maternal booking BMI is not an important determinant of late gestation ex vivo adipocyte lipolytic function. In preeclampsia, SAT adipocyte diameter was inversely associated with insulin sensitivity, but there was no significant difference in adipocyte diameters between preeclampsia and controls. However, there was a difference in size distribution between adipocyte populations from healthy and preeclampsia pregnancy with preeclampsia having a significantly larger proportion of small adipocytes. This is consistent with observations in the nonpregnant that insulin resistance is associated with a greater proportion of small adipocyte cells. It has previously been observed that adipocyte diameter is higher in late

### Discussion

In preeclampsia, SAT adipocyte insulin sensitivity was lower than in matched controls. This is the first direct evidence that preeclampsia is associated with increased lipolysis at maternal adipocytes and could explain the exaggerated gestational triglyceridemia and increased NEFA flux associated with this disease. It is interesting to note that lipolysis rates per se were not altered in preeclampsia and underlines the importance of insulin resistance in the pathogenesis of the disease. There was borderline higher HSL expression in preeclampsia, which is consistent with the observation that increased late gestation lipolysis is associated with a high third trimester HSL mRNA expression to LPL activity ratio in rats. Our data also show that maternal booking BMI is not an important determinant of late gestation ex vivo adipocyte lipolytic function. In preeclampsia, SAT adipocyte diameter was inversely associated with insulin sensitivity, but there was no significant difference in adipocyte diameters between preeclampsia and controls. However, there was a difference in size distribution between adipocyte populations from healthy and preeclampsia pregnancy with preeclampsia having a significantly larger proportion of small adipocytes. This is consistent with observations in the nonpregnant that insulin resistance is associated with a greater proportion of small adipocyte cells. It has previously been observed that adipocyte diameter is higher in late

### Table 3. Lipolysis in SAT From Women With Preeclampsia and BMI-Matched Controls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (n=26)</th>
<th>P</th>
<th>Preeclampsia (n=13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipolysis rates, μmol/L per μg DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal*†</td>
<td>119 (98)</td>
<td>0.67</td>
<td>168 (154)</td>
<td>0.07</td>
</tr>
<tr>
<td>Isoproterenol*†</td>
<td>270 (111)</td>
<td>0.75</td>
<td>330 (216)</td>
<td>0.01</td>
</tr>
<tr>
<td>Insulin*†</td>
<td>108 (62)</td>
<td>0.41</td>
<td>170 (159)</td>
<td>0.03</td>
</tr>
<tr>
<td>Isoproterenol plus insulin*†</td>
<td>231 (114)</td>
<td>0.33</td>
<td>331 (231)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Relative lipolysis, %

| Stimulation of basal by isoproterenol*† | 251 (225) | 0.70 | 249 (321)         | 0.07       |
| Inhibition of basal by insulin††       | −19 (57)  | 0.48 | −40 (111)         | 0.03       |
| FCISI‡†                               | 25 (44)   | 0.23 | −43 (140)         | 0.04       |

Isoproterenol and insulin were at 200 nmol/L and 10 nmol/L, respectively. Lipolysis rates were corrected for cell number and expressed as mmol/L per μg DNA. Relative lipolysis represents the degree of stimulation by isoproterenol and the degree of suppression by insulin and was calculated as a percentage of basal release of NEFA or glycerol. FCISI was calculated as the percentage inhibition of isoproterenol-stimulated lipolysis by insulin. Means (SDs) are shown. Differences between SAT and visceral adipose tissue were tested by 2 sample t test on *log, †square root transformed, or ‡untransformed data if necessary (total stated before net lipolysis). BMI indicates body mass index; FCISI, fat cell insulin sensitivity index; NEFA, nonesterified fatty acid; and SAT, subcutaneous adipose tissue.

### Table 4. Lipolysis in VAT From Women With Preeclampsia and BMI-Matched Controls

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (n=25)</th>
<th>P</th>
<th>Preeclampsia (n=13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipolysis rates, μmol/L per μg DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal*†</td>
<td>78 (72)</td>
<td>0.33</td>
<td>103 (77)</td>
<td>0.55</td>
</tr>
<tr>
<td>Isoproterenol*†</td>
<td>205 (101)</td>
<td>0.53</td>
<td>190 (119)</td>
<td>0.09</td>
</tr>
<tr>
<td>Insulin*†</td>
<td>77 (63)</td>
<td>0.19</td>
<td>109 (80)</td>
<td>0.01</td>
</tr>
<tr>
<td>Isoproterenol*† plus insulin</td>
<td>189 (109)</td>
<td>0.56</td>
<td>205 (104)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Relative lipolysis, %

| Stimulation of basal by isoproterenol*† | 393 (517) | 0.15 | 390 (1129)        | 0.03       |
| Inhibition of basal by insulin††       | −45 (187) | 0.41 | −29 (93)          | 0.09       |
| FCISI‡†                               | 1 (59)     | 0.35 | 183 (667)         | 0.02       |

Isoproterenol and insulin were at 200 nmol/L and 10 nmol/L, respectively. Lipolysis rates were corrected for cell number and expressed as mmol/L per μg DNA. Relative lipolysis represents the degree of stimulation by isoproterenol and the degree of suppression by insulin and was calculated as a percentage of basal release of NEFA or glycerol. FCISI was calculated as the percentage inhibition of isoproterenol-stimulated lipolysis by insulin. Means (SDs) are shown. Differences between subcutaneous adipose tissue and VAT were tested by 2 sample t test on *log, †square root transformed, or ‡untransformed data if necessary (total stated before net lipolysis). BMI indicates body mass index; FCISI, fat cell insulin sensitivity index; NEFA, nonesterified fatty acid; and VAT, visceral adipose tissue.
gestation compared with early gestation. Our data might suggest that there may be a failure in the adipocyte hypertrophic response to pregnancy in preeclampsia, which could result in alterations of cell function and reduced capacity to store NEFA in preeclampsia pregnancy. There was a lower placental lactogen concentration in preeclampsia in the current study in contrast to an earlier report. This discrepancy could be because of the fact that the present study groups were matched for BMI. Placental lactogen was associated with VAT, but not SAT, FCISI. This information along with published data suggests that placental lactogen can stimulate adipose tissue SAT, FCISI. This information along with published data suggests that placental lactogen can stimulate adipose tissue and may induce leptin resistance in pregnancy.

Third trimester healthy control SAT adipocytes had higher lipolysis rates than VAT adipocytes, but this was not independent of larger SAT cell size. These observations are contrary to what is observed in nonpregnant individuals in whom VAT lipolysis is greater than SAT. This suggests that pregnancy is associated with alterations in adipocyte lipolytic function and that SAT is a potential source, especially when total fat depot mass is taken into account, of the increased NEFA flux in healthy pregnancy that is physiologically required to provide fatty acids for placental transport and maternal metabolism. Higher SAT lipolysis rates were more apparent for total rather than net lipolysis measurements, resulting in lower SAT NEFA to glycerol ratios. This suggests that a greater degree of fatty acid reuptake occurs in SAT consistent with observations in nonpregnant SAT. The ability to recycle fatty acids may provide greater flexibility for the SAT adipocyte to balance both its fat accrual and NEFA production functions in pregnancy. In SAT, the lower the basal lipolysis of the adipocyte, the higher the susceptibility to stimulation by catecholamines. This suggests that women with lower lipolysis rates in pregnancy may have an inherent metabolic flexibility toward lipolytic stimuli and respond more effectively to stressful stimuli. We observed that SAT adipocyte total lipolysis insulin sensitivity inversely associated with plasma estradiol. Estradiol (or associated downstream responses) may thus be responsible for at least some of the physiological insulin resistance of healthy pregnancy. Lower expression levels of the insulin receptor in SAT may also play a part in a decreased regulation of lipolytic activity by insulin in pregnancy.

There are regional differences in adipocyte function in pregnancy. VAT adipocytes were smaller, less lipolytic, and had a trend toward lower insulin sensitivity after correction for cell size than SAT. Maternal plasma estradiol was inversely associated with VAT insulin sensitivity. Maternal systemic insulin resistance, assessed by HOMA, was also inversely associated, whereas placental lactogen and leptin were positively associated. Potentially a balance between the pregnancy hormones estradiol and placental lactogen may regulate VAT adipocyte function in pregnancy. VAT, but not SAT, adipocyte insulin sensitivity was inversely associated with maternal triglycerides, thereby linking VAT function with systemic insulin resistance. The relationship between VAT insulin resistance and plasma triglyceride is consistent with observations in nonpregnant VAT. Viseral fat is drained by the portal vein, and increased NEFA have direct effects on liver function including increased triglyceride secretion in very-low-density lipoprotein. The lower ADRB3 mRNA expression in VAT may render adipocytes less responsive to β-adrenergic–stimulated lipolysis than SAT. These data contrast with previous reports of enhanced β-adrenergic function in VAT in the nonpregnant and might suggest a gestational alteration in VAT function. Lower LPL mRNA expression in VAT may reflect diversion of the gestational accrual of fat to SAT. However, LPL is recognized as...
being regulated post-translationally, and changes in mRNA expression should be interpreted with caution. The long form of the leptin receptor was expressed in VAT but not in SAT, suggesting that leptin signaling may play a larger role in VAT than in SAT adipocyte function.

The strength of our data is the direct measurement of adipocyte lipolytic function in combination with plasma hormone measurements. Comparison between adipose depots was strengthened by paired data, preeclampsia and control groups were matched for BMI to ascertain obesity-independent effects, and all women were nonlaboring. There were limitations to our study. The preeclampsia sample size was small, and we are unable to comment on adipocyte function at early gestations. Furthermore, preeclampsia adipose biopsies were collected at 3 weeks earlier gestation than controls. At early gestations. Furthermore, preeclampsia adipose biopsies were collected at 3 weeks earlier gestation than controls. Isoproterenol is a nonselective adrenoceptor agonist, and use of selective agonists may provide further information. It is not clear to what extent our observations in a closed in vitro system reflect in vivo lipolysis by adipocytes, where newly released NEFA may be rapidly removed by the circulation. Comparison of nonpregnant and pregnant SAT and VAT lipolytic activity is required to address which differences reflect adaptation to pregnancy per se.

Perspectives
Adipocyte function adapts to pregnancy and in the third trimester SAT is likely to be the adipose tissue depot that contributes most fatty acids to generate the physiological hypertriglyceridemia of pregnancy. In preeclampsia, SAT insulin sensitivity is lower, and the resulting exaggerated NEFA flux may contribute to ectopic fat accumulation and lipotoxic pathological pathways. Increasing adipocyte insulin sensitivity may be a potential strategy for slowing or reversing the development of preeclampsia. Although pharmacological agents, such as thiazolidenediones, are inappropriate for use in pregnancy, plant extracts can increase insulin sensitivity of 3T3-L1 adipocytes in culture, and there is future potential for mimicking effects of adiponectin in adipocytes.

Sources of Funding
This research was supported by a project grant from the British Heart Foundation (PG/03/147), a Glasgow Royal Infirmary Research Endowments Trust Grant, and a Medical Research Council Studentship (R. Forrest).

Disclosures
None.

References
What Is New?

- Subcutaneous and visceral fat cell mean diameter was similar to healthy pregnancy, but cell distribution was shifted toward smaller cell diameter in preeclampsia.
- Visceral fat cell insulin sensitivity was lower than subcutaneous fat in healthy pregnancy.
- Subcutaneous fat cells had lower insulin sensitivity in preeclampsia.

What Is Relevant?

- Maternal obesity, increased insulin resistance, and aberrant fatty acid metabolism are involved in pathogenesis of preeclampsia.

Novelty and Significance

- This study shows direct evidence of differences in lipolytic function of adipose tissue between healthy pregnancy and preeclampsia, a potential mechanism through which modification of the disease could occur.

Summary

In healthy pregnancy, the larger total mass of subcutaneous fat may release more fatty acids into the circulation than visceral fat. Reduced insulin suppression of subcutaneous adipocyte lipolysis may increase the burden of potentially damaging fatty acids that the mother has to process in preeclampsia.
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Hypertension. 2014;63:1094-1101; originally published online March 3, 2014;
doi: 10.1161/HYPERTENSIONAHA.113.01824

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/63/5/1094

Data Supplement (unedited) at:
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IN PREECLAMPSIA, MATERNAL THIRD TRIMESTER SUBCUTANEOUS ADIPOCYTE LIPOLYSIS IS MORE RESISTANT TO SUPPRESSION BY INSULIN THAN IN HEALTHY PREGNANCY

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Short Title: Adipocyte lipolytic function in preeclampsia

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Methods

Subject recruitment and sample collection
Healthy non-labouring women at term (n=31) and non-labouring women with PE undergoing Caesarean section (n=13) were recruited from the Princess Royal Maternity Hospital, Glasgow. Age- and BMI-matched Controls (2 Controls per case) were selected from the healthy cohort. The study was approved by the Local Research Ethics Committee and all women gave written informed consent. Preeclampsia was defined according to the International Society for the Study of Hypertension in Pregnancy criteria. None of the women had a medical history of cardiovascular or metabolic disease and multiparous pregnancies were excluded. Subject characteristics were recorded at time of sampling. Delivery details were recorded from patient notes. Deprivation category (DEPCAT score), a measure of socioeconomic status, was assigned using the Scottish Area Deprivation Index for Scottish postcode sectors, 1998. Customised birth weight centiles were calculated using the Gestation Network Centile Calculator 5.4.

Plasma metabolites
Plasma non esterified fatty acids (NEFA) were quantitated by colorimetric assay (Wako, Alpha Laboratories, Eastleigh, UK). Insulin (Merckodia, Sweden) and human placental lactogen (Leinco, Universal Biologicals, Cambridge, UK) were performed by ELISA according to the manufacturer’s instructions. Plasma leptin, adiponectin, IL-6 and TNFα were carried out by ELISA (R&D Systems, Abingdon UK). Plasma estradiol and progesterone was estimated using the Immulite semi-automated assay system (Siemens, Erlangen, Germany).

Adipocyte preparation, sizing and DNA content
Temperature was maintained near to 37°C throughout. Adipose tissue was placed immediately in assay buffer (KRH buffer - NaCl 118mM, NaHCO3 5mM, KCl 4.7 mM, KH2PO4 1.2mM, MgSO4 1.2mM, HEPES 25mM, 2.5mM CaCl2, 20g/L bovine serum albumin, pH7.4) containing 3mM glucose. Attached connective or vascular tissue was dissected from the sample prior to digestion in 4ml of 2 mg/ml collagenase (Worthington Type 1, Lorne Laboratories, Twyford, UK) per g of tissue for 30 minutes with agitation. The digestate was then filtered (pore size 600µM), leaving a layer of adipocytes floating on top of the digestion buffer. The adipocytes were washed 4 times in warm KRH buffer and
resuspended at approximately 90% cytocrit. An unfixed fresh cellular suspension of adipocytes was prepared on a glass slide and a digital image captured. Digital images of fresh adipocyte preparation on glass slides were captured at x10 magnification on an Olympus BX50 microscope connected to 3-CCD colour camera (JVC). Computer visualisation of images was using Image-Pro Plus 4.0 and images were analysed with Adobe Photoshop Vs7.0.

**Adipose tissue lipolysis assay**

Adipocyte cell suspension (100ul) was added to 900ul of warm assay buffer and incubated in a 37°C shaking water bath at 91 cycles per minute. Appropriate reagents were added to the relevant tubes and incubated for 120 minutes. All assays were carried out in duplicate. At the end of the assay, aliquots (5ul) were obtained from the buffer layer below the cellular suspension and glycerol and non-esterified fatty acid (NEFA) concentration was quantitated by colorimetric assay using a microplate spectrophotometer (Multiscan EX, Thermo Electron Corporation) at 520nm and 550nm respectively.

**Adipose tissue mRNA expression quantitation**

Total RNA was isolated from adipose tissue using the ABI PRISM 6100 Nucleic Acid Prepstation following manufacturer’s instructions (Applied Biosystems, Warrington, UK). cDNA was reverse transcribed from RNA using a High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Warrington, UK) according to manufacturer’s instructions. Target gene expression was quantitated relative to a control gene\(^2\) (PPIA Hs99999904_m1) using commercial primer probe sets (ADRA2A Hs00265081_s1; ADRB1 Hs02330048_s1; ADRB2 Hs00240532_s1; ADRB3 Hs00609046_m1; LIPE Hs00193510_m1; PNPLA2 Hs00386101_m1; LPL Hs00173425_m1; INSR Hs00961554_m1; LEP Hs00174877_m1; LEPR Hs00900240_m1; LEPR (long) custom primers forward LEPTTRLONG_F TGTCCGAACCCCAAGAATTGTT, reverse LEPTTRLONG_R ATGTCACTGATGCTGTATGCTTGAT, probe LEPTTRLONG_M 6FAM TCTGGCTTCTGAAAATT) in a final volume of 25ul on an 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK) according to manufacturer’s instructions. Quantification analysis was carried out using SDS Version 2.3 software (Applied Biosystems), which calculated the threshold cycle (C\(_T\)) values. The expression of target assays were normalised by subtracting the C\(_T\) value of the endogenous control from the C\(_T\) value of the target assay. The fold increase relative to the control was calculated using the 2\(^{-\Delta C T}\). The expression of the target assay was then expressed as a percentage relative to the endogenous control assay.
References


Table S1. Stepwise regression models of subcutaneous and visceral adipose tissue (SAT and VAT) lipolytic function in healthy pregnancy. Stepwise regression was carried out to determine the contribution of cell size, plasma estradiol, placental lactogen, HOMA, leptin, adiponectin and TNF α to lipolytic function with $P$-to-enter and $P$-to-stay 0.15 in healthy pregnancy (n=31). Best models are shown with regression coefficients and $P$ value given for each contributory variable and $R^2$ adjusted for the model. Fat cell insulin sensitivity index (FCISI) was calculated as the percentage inhibition of isoproterenol-stimulated lipolysis by insulin.

<table>
<thead>
<tr>
<th>Lipolytic function</th>
<th>Variable</th>
<th>Coefficient</th>
<th>$P$</th>
<th>% $r^2$ (adjusted)</th>
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<td><strong>SAT</strong></td>
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<td>Total basal lipolysis</td>
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<td>Net basal lipolysis</td>
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<td>FCISI (total)</td>
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<td>FCISI (Net)</td>
<td>Cell diameter (um)</td>
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<td></td>
<td>TNFα (log pg/mL)</td>
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<td>Total basal lipolysis</td>
<td>HOMA (log)</td>
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<td>Placental lactogen (ug/mL)</td>
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<td>Placental lactogen (ug/mL)</td>
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Figure S1. Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) adipocyte diameter distribution in control and preeclamptic (PE) pregnancy. Adipocyte diameter was measured in n=100 adipocytes from each adipocyte preparation. SAT and VAT adipocyte diameters were divided into tertiles and percent adipocytes within the diameter ranges calculated for healthy and PE samples. Percentage adipocytes in each tertile for the whole control and PE groups are shown.
Figure S2. Maternal nonesterified fatty acid/glycerol ratio in maternal adipose tissue from the healthy cohort. Nonesterified fatty acid (NEFA) to glycerol ratios in subcutaneous adipose tissue (SAT, n=31) and visceral adipose tissue (VAT, n=31) under basal conditions or in the presence of 200nM isoprotenerol, 10nM insulin or combined 200nM isoprotenerol plus 10nM insulin. *Significantly different from basal, P<0.010, using square root transformed data.
Figure S3. Maternal nonesterified fatty acid/glycerol ratio in maternal adipose tissue in women with preeclampsia and healthy age- and BMI-matched controls. Nonesterified fatty acid (NEFA) to glycerol ratios in A) subcutaneous adipose tissue (SAT) and B) visceral adipose tissue (VAT) from PE (n=13) and control (n=26) pregnancies under basal conditions or in the presences of 200nM isoproterenol, 10nM insulin or combined 200nM isoproterenol plus 10nM insulin. *Significantly different between PE and control, using square root transformed data.