IN PREECLAMPSIA, MATERNAL THIRD TRIMESTER SUBCUTANEOUS ADIPOCYTE LIPOLYSIS IS MORE RESISTANT TO SUPPRESSION BY INSULIN THAN IN HEALTHY PREGNANCY

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Abstract

Obesity increases preeclampsia risk and maternal dyslipidaemia may result from exaggerated adipocyte lipolysis. We compared adipocyte function in preeclampsia with healthy pregnancy to establish if 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 (200nM) and insulin (10nM) 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 towards 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.

Key words: adipocyte, lipolysis, pregnancy, preeclampsia

Introduction

Maternal metabolism during pregnancy adapts to support fetal growth and development. All women increase maternal fat stores in early pregnancy in order to meet the feto-placental 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[3-5]. In women of normal weight, the majority of fat is accumulated centrally 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 pre-peritoneal fat (visceral) and the ratio of pre-peritoneal to subcutaneous fat as measured by ultrasound[8]. This pattern may be related to the increasing gestational insulin resistance and hyperlipidaema. Hepatic fat is an important mediator of gestational insulin resistance in the rat[9] and has also been suggested to be important in humans[10]. Visceral adiposity correlates with metabolic risk factors[11] and adverse metabolic outcomes in pregnancy including gestational diabetes mellitus, and preeclampsia (PE)[12-14].

PE occurs in 2-4% 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 PE[15]. There is a marked increase in plasma triglyceride concentration and an early rise in plasma non-esterified fatty acids (NEFA), independent of adiposity, suggesting exaggerated adipocyte lipolysis[15]. Adipocyte lipolysis in the non-pregnant is under adrenergic control with ADRB1, ADRB2 and ADRB3 receptors stimulating catecholamine-mediated lipolysis and ADRA2A mediating its inhibition. Fatty acids are released from cellular triglyceride droplets by the sequential action of adipose tissue triglyceride lipase (ATGL) and hormone sensitive lipase (HSL). This latter enzyme is sensitive to insulin and is the mechanism by which insulin

suppresses lipolysis. Elevated plasma NEFA levels and/or increased flux can contribute to insulin resistance, endothelial dysfunction and inflammation, all key features of PE[10]. 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 has regulating other metabolic and inflammatory pathways. It interacts with leptin receptors which 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 PE 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 PE. The expression of genes relevant to lipolysis and its regulation were also studied in tissue biopsies.

Methods

Subject recruitment

Non-labouring healthy women at term (n=31) and women with PE 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 Data Supplement.

Plasma metabolites

Plasma total cholesterol, triglyceride and high density lipoprotein (HDL) cholesterol[17], glucose and high sensitivity C-reactive protein (CRP) assays[18] were performed by the Department of Clinical Biochemistry, Glasgow Royal Infirmary by routine methods. Other analytes were assayed using commercially available kits (online Data Supplement). HOMA was calculated as follows: [fasting insulin (mU/L) x fasting glucose (mmol/L)]/22.5.

Adipocyte preparation, sizing and DNA content

Adipocyte preparation used a modification Rodbell *et al*[19], and detail is provided in the online Data Supplement. Adipocytes were resuspended at approximately 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^{TM} 6100 Nucleic Acid PrepStation (Applied Biosystems). The concentration of DNA was quantified using a Nanodrop® ND 100.

Adipose tissue lipolysis assay

Adipocyte cell suspension (100ul) was assayed in a final volume of 1 mL as described in the online Data Supplement. The following conditions were included: basal lipolysis (no reagent), isoproterenol (200nM), insulin (Human Actrapid® Novo Nordisk, 10nM), and combined isoproterenol (200nM) and insulin (10nM). Glycerol and NEFA concentrations were quantitated by colorimetric assay (Randox Laboratories Ltd, Co Antrim, UK and Wako NEFA-C; Alpha laboratories, Eastleigh, Hampshire, UK respectively). Adipocyte cell number was measured indirectly by quantifying the DNA content in a known volume of adipocyte suspension and expressing lipolysis rates as umol/L NEFA or glycerol released per ug of DNA. The degree of stimulation by isoproterenol and the degree of suppression by insulin was 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 quantitation

Total RNA was isolated from adipose tissue and cDNA synthesized. Target gene expression was quantitated relative to a control gene by Taqman real time PCR using commercial primer probes sets (Applied Biosystems), see online Data Supplement.

Statistical Analysis

Data was assessed for normal distribution using a Ryan-Joiner test and transformed to achieve a normal distribution where necessary. Means with standard deviation (SD) are presented. Comparison within individuals was by paired t test (SAT vs VAT) and between PE and controls by two sample t-test. Mann-Whitney and Chi-squared tests were used to test between differences in semi-categorical and categorical variables respectively. $P < 0.05$ was considered significant. Cell diameters were divided into tertiles and differences in distribution

among tertiles between control and PE were tested by Chi-squared test. Pearson's correlation coefficients were calculated to assess associations between variables and r value and *P*-value stated (*P* d0.010 was considered significant due to multiple testing). The data was adjusted for potential cofounders using the General Linear Model. Regression modelling 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 carried out in Minitab (version 16).

Results

Maternal demographic and biochemical profile

PE 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 bith weight centile (BWC) (Table 1). PE mothers had higher plasma triglyceride and NEFA and lower placental lactogen concentrations than matched controls.

SAT and VAT cell size in healthy and PE pregnancy

SAT adipocytes were 23um 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 PE 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 PE group (control 1.32 vs PE 1.26 um, 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 PE and controls (Figure S1), it was seen that diameter distribution was shifted toward smaller diameter for SAT (P=0.004) and VAT (P=0.019) in PE adipocyte preparations. In PE 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 PE.

SAT and VAT lipolytic function in healthy pregnancy

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*d0.001). 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/or insulin in SAT but not 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 BWC. 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 *ADRB3* 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 carried out to identify whether the expression of enzymes or receptors was associated with SAT FCISI in controls. 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 were negatively associated with total lipolysis FCISI (r^2 adjusted 46%), independent of cell diameter.

Adipose tissue lipolytic function, receptor and enzyme expression in PE

There were no differences in SAT total or net lipolysis rates between women with PE and age- and BMI-matched controls (Table 3). In PE, 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 PE (-118%) and controls (37%), *P*=0.012. There were no differences in VAT lipolytic function between PE and control women apart from a lower NEFA/glycerol ratio in the presence of isoproterenol and insulin in PE (Figure S3). There were no differences in mRNA expression of any of the measured receptors or enzymes in SAT, although there was a trend towards a 154% higher *LIPE* expression in PE (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 carried out to determine the contribution of cell size, plasma estradiol, placental lactogen, HOMA, leptin, adiponectin and tumour necrosis factor (TNF) ± on basal lipolysis and FCISI in SAT and VAT from healthy pregnancy (Table S1). In initial modelling progesterone was not associated with lipolytic function. SAT basal net and total lipolysis was significantly associated with TNF±, whereas in VAT net lipolysis was associated with cell diameter and HOMA with placental lactogen a non-significant contributor to the model. In healthy SAT a number of variables contributed to FCISI. Estradiol was negatively associated with total FCISI, and cell diameter negatively and leptin and TNF± positively associated with net FCISI. In VAT estradiol (negatively) and placental lactogen were associated with FCISI with HOMA making an additional negative contribution to net FCISI.

Discussion

In PE, SAT adipocyte insulin sensitivity was lower than in matched controls. This is the first direct evidence that PE is associated with increased lipolysis at maternal adipocytes and could explain the exaggerated gestational triglyceridaemia and increased NEFA flux associated with this disease. It is interesting to note that lipolysis rates *per se* were not altered in PE and underlines the importance of insulin resistance in the etiology of the disease. There was borderline higher *HSL* expression in PE 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[20]. Our data also show that maternal booking BMI is not an important determinant of late gestation *ex vivo* adipocyte lipolytic function. In PE, SAT adipocyte diameter was inversely associated with insulin sensitivity but there was no significant difference in adipocyte diameters between PE and controls. However, there was a difference in size distribution between adipocyte populations from healthy and PE pregnancy with PE having a significantly larger proportion of small adipocytes. This is consistent with observations in the non-pregnant that insulin resistance is associated with a greater proportion of small adipose cells[21]. It has previously been observed that adipocyte diameter is higher in late gestation compared to early gestation[22]. Our data might suggest that there may be a failure in the adipocyte hypertrophic response to pregnancy in PE which could result in alterations of cell function and reduced capacity to store NEFA in PE pregnancy. There was a lower placental lactogen concentration in PE in the current study in contrast to an earlier report[23]. This discrepancy could be due to 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 suggesting that placental lactogen can directly stimulate adipose tissue lipolysis[24] and may induce leptin resistance[25] in pregnancy makes this hormone an unlikely candidate for mediating the increase in SAT insulin resistance in

PE. TNF± has been suggested to attenuate the anti-lipolytic effect of insulin via ceramide signalling[26] and TNF± has been shown to be a predictor of insulin resistance in pregnancy[27]. However, regression modelling found no evidence that maternal plasma TNF± levels were associated with either VAT or SAT insulin resistance in healthy pregnancy. TNF± was positively associated with SAT basal lipolysis, but this may be a marker of more "active" inflamed adipose tissue rather than a direct indication of regulation of lipolysis.

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 non-pregnant individuals where VAT lipolysis is greater than SAT[28]. 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[5]. 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 non-pregnant SAT[29]. 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 towards 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[30]. 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 towards 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 while 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 non-pregnant VAT[31, 32]. Visceral 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 (VLDL)[33]. The lower *ADRB3* mRNA expression in VAT may render adipocytes less responsive to ² -adrenergic stimulated lipolysis than SAT. These data contrast with previous reports of enhanced ² 3-adrenergic function in VAT in the non-pregnant[28] 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 recognised as being regulated post-translationally[34] 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 signalling may play a larger role in VAT than 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, PE and control groups were matched for BMI to ascertain obesity-independent effects and all women were non-labouring. There were limitations to our study. The PE sample size was small and we are unable to comment on adipocyte function at early gestations. Furthermore PE adipose biopsies were collected at 3 weeks earlier gestation than controls. Isoproterenol is a non-selective 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 non-pregnant 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 hypertriglyceridaemia of pregnancy. In PE, 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 PE. While pharmacological agents such as thiazolidenediones are inappropriate for use in pregnancy, plant extracts can increase insulin sensitivity of 3T3L1 adipocytes in culture[35] and there is future potential for mimicking effects of adiponectin in adipocytes[36].

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Figure 1. Maternal adipose tissue gene expression in the healthy cohort. Subcutaneous adipose tissue (SAT, n=26) and visceral adipose tissue (VAT, n=23) gene expression relative to a control gene (*PPIA*). Expression of adrenoceptors A2A, B1, B2 and B3 (*ADRA2A, ADRB1, ADRB2, ABRB3*), hormone sensitive lipase (*LIPE*), adipose triglyceride lipase (*PNPLA2*), lipoprotein lipase (*LPL*), insulin receptor (*INSR*), leptin (*LEP*) and leptin receptor short (*LEPR*) and long (*LEPR*(long)) is shown. *Significant differences between SAT and VAT by paired t test on square root transformed data.

Figure 2. Maternal adipose tissue gene expression in women with preeclampsia and matched controls. A) Subcutaneous adipose tissue (SAT) and B) visceral adipose tissue (VAT) from PE (n=13) and control (n=26) pregnancies gene expression relative to a control gene (*PPIA*). Expression of adrenoceptors A2A, B1, B2 and B3 (*ADRA2A, ADRB1, ADRB2, ABRB3*), hormone sensitive lipase (*LIPE*), adipose triglyceride lipase (*PNPLA2*), lipoprotein lipase (*LPL*), insulin receptor (*INSR*), leptin (*LEP*) and leptin receptor short (*LEPR*) and long (*LEPR*(long)) is shown. *Significant differences between PE and control by two sample t test on square root transformed data.

Table 1. Characteristics of healthy cohort, PE cases and matched controls. Blood pressure refers to booking values. All continuous values are expressed as mean and standard deviation (*median and interquartile range) and categorical variables as number (percent). Comparisons between PE and control women were carried out by two sample t test, on †log or ‡square root transformed data if necessary, except *Mann-Whitney and **chi-squared test.

	Healthy cohort (n=31)	Controls (n=26)	PE (n=13)	*P* value PE vs Controls
Demographic data				
Age (years)	30.9 (5.4)	30.7 (5.3)	31.1 (6.3)	0.84
BMI (kg/m^2)†	28.9 (5.2)	29.6 (5.4)	31.1 (8.3)	0.72
Smokers, number (%)**	3 (10.7)	2 (7.6)	2 (15.4)	0.40
DEPCAT*	6 (4-7)	5 (4-6)	6 (4-7)	0.18
Gestation at delivery (weeks)	38.9 (1.3)	38.8 (1.3)	35.6 (3.2)	0.004
Primigravidae, number (%)**	6 (19.4)	5 (19.2)	7 (53.8)	0.029
Systolic pressure (mmHg)	115 (14)	116 (15)	127 (14)	0.029
Diastolic pressure (mmHg)	70 (8)	70 (9)	78 (9)	0.010
Birthweight (g)	3485 (546)	3525 (554)	2330 (926)	0.001
Birthweight centile	60 (28)	62 (29)	26 (33)	0.004
Biochemical data				
Total cholesterol (mmol/L)	6.32 (1.08)	6.24 (1.01)	6.41 (1.45)	0.71
Triglyceride (mmol/L)†	2.63 (0.59)	2.56 (0.59)	3.73 (2.36)	0.035
HDL cholesterol (mmol/L)	1.84 (0.34)	1.84 (0.36)	1.71 (0.42)	0.33
NEFA (mmol/L)‡	0.42 (0.21)	0.39 (0.19)	0.58 (0.24)	0.011
Glucose (mmol/L)	4.74 (0.73)	4.72 (0.42)	5.31 (1.24)	0.12
Insulin (mU/L)†	13 (14)	11 (7)	17 (13)	0.21
HOMA†	3.1 (4.9)	2.4 (1.9)	4.3 (3.8)	0.16
Leptin (mg/mL)†	46 (22)	50 (20)	85 (42)	0.070
Adiponectin (ug/mL)	9.5 (3.8)	9.1 (3.6)	9.0 (4.8)	0.94
IL-6 (pg/mL)†	3.2 (2.1)	3.4 (2.3)	3.8 (3.2)	0.76
TNF alpha (pg/mL)†	1.2 (0.6)	1.2 (0.6)	1.5 (0.7)	0.23
CRP (mg/L)†	5.5 (4.4)	5.9 (4.7)	23.5 (55.5)	0.81
Progesterone (ng/mL)†	266 (86)	247 (85)	409 (391)	0.33
Estradiol (ng/mL)	18.6 (8.5)	17.6 (6.9)	11.7 (2.6)	0.058
Placental lactogen (ug/mL)	11.8 (2.5)	11.7 (2.4)	8.3 (4.8)	0.030
SAT cell size (um)	109 (11)	113 (7)	110 (11)	0.44
VAT cell size (um)	86 (14)	90 (11)	87 (16)	0.64

Table 2. Lipolysis in subcutaneous and visceral adipose tissue (SAT and VAT) from healthy pregnancy. Isoproterenol and insulin were at 200nM and 10nM respectively. Lipolysis rates were corrected for cell number and expressed as mmol/L/ug 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. Fat cell insulin sensitivity index (FCISI) was calculated as the percentage inhibition of isoproterenol-stimulated lipolysis by insulin. Means (standard deviations) are shown. Differences between SAT and VAT were tested by paired t-test on †log or ‡square root transformed data if necessary.

Treatment	Total Lipolysis (Glycerol release)			Net Lipolysis (NEFA release)		
	SAT (n=31)	VAT (n=31)	*P*	SAT (n=31)	VAT (n=31)	*P*
Lipolysis rates (mmol/L/ug DNA)						
Basal	0.12 (0.09)	0.08 (0.08)	0.005†	0.40 (0.28)	0.25 (0.29)	0.026‡
Isoproterenol	0.29 (0.11)	0.19 (0.10)	<0.001†	0.80 (0.42)	0.62 (0.60)	0.080‡
Insulin	0.13 (0.10)	0.08 (0.07)	0.001†	0.25 (0.14)	0.20 (0.24)	0.14‡
Isoproterenol plus insulin	0.26 (0.15)	0.17 (0.11)	0.003†	0.60 (0.33)	0.52 (0.50)	0.22‡
Relative lipolysis (%)						
Stimulation of basal by isoproterenol	240 (216)	337 (479)	0.94†	152 (121)	237 (184)	0.048‡
Inhibition of basal by insulin	-28 (93)	-40 (168)	0.41†	26 (27)	9 (44)	0.086
FCISI	21 (66)	88 (552)	0.68†	88 (135)	10 (153)	0.046

Table 3. Lipolysis in subcutaneous and visceral adipose tissue (SAT and VAT) from women with preeclampsia and BMI-matched controls. Isoproterenol and insulin were at 200nM and 10nM respectively. Lipolysis rates were corrected for cell number and expressed as mmol/L/ug 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. Fat cell insulin sensitivity index (FCISI) was calculated as the percentage inhibition of isoproterenol-stimulated lipolysis by insulin. Means (standard deviations) are shown. Differences between SAT and VAT were tested by two sample t-test on ¶untransformed †log or ‡square root transformed data if necessary (total stated before net lipolysis).

Treatment	SAT Total Lipolysis (Glycerol release)			SAT Net Lipolysis (NEFA release)		
	Control (n= 26)	PE (n=13)	*P*	Control (n=26)	PE (n=13)	*P*
Lipolysis rates (umol/L/ug DNA)						
Basal†,‡	119 (98)	168 (154)	0.67	397 (306)	494 (407)	0.45
Isoproterenol†,‡	270 (111)	330 (216)	0.75	779 (430)	961 (730)	0.50
Insulin†,‡	108 (62)	170 (159)	0.41	229 (144)	372 (420)	0.22
Isoproterenol plus insulin†,‡	231 (114)	331 (231)	0.33	595 (333)	849 (672)	0.27
Relative lipolysis (%)						
Stimulation of basal by isoproterenol†,‡	251 (225)	249 (321)	0.70	151 (119)	136 (146)	0.69
Inhibition of basal by insulin¶,‡	-19 (57)	-40 (111)	0.48	30 (26)	20 (29)	0.31
FCISI¶,‡	25 (44)	-43 (140)	0.23	65 (102)	13 (48)	0.036

Treatment	VAT Total Lipolysis (Glycerol release)			VAT Net Lipolysis (NEFA release)		
	Control (n=25)	PE (n=13)	*P*	Control (n=25)	PE (n=13)	*P*
Lipolysis rates (umol/L/ug DNA)						
Basal†,‡	78 (72)	103 (77)	0.33	267 (311)	226 (186)	0.79
Isoproterenol†,‡	205 (101)	190 (119)	0.53	709 (636)	442 (312)	0.10
Insulin†,‡	77 (63)	109 (80)	0.19	227 (263)	193 (162)	0.83
Isoproterenol†,‡ plus insulin	189 (109)	205 (104)	0.56	602 (524)	405 (260)	0.18

Relative lipolysis (%)						
Stimulation of basal by isoproterenol[†,‡]	393 (517)	390 (1129)	0.15	256 (179)	155 (184)	0.092
Inhibition of basal by insulin [¶,‡]	-45 (187)	-29 (93)	0.41	5 (40)	9 (19)	0.68
FCISI [¶,‡]	1 (59)	183 (607)	0.35	33 (76)	-2 (53)	0.11

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