

# Release and regulation of leptin, resistin and adiponectin from human placenta, fetal membranes, and maternal adipose tissue and skeletal muscle from normal and gestational diabetes mellitus-complicated pregnancies

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## Abstract

The aim of this study was to determine the release and regulation of leptin, resistin and adiponectin from human placenta and fetal membranes, and maternal subcutaneous adipose tissue and skeletal muscle obtained from normal and gestational diabetes mellitus (GDM)-complicated pregnancies at the time of Cesarean section. Tissue explants were incubated in the absence (basal control) or presence of 10 µg/ml lipopolysaccharide (LPS), 10, 20 or 40 ng/ml tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6 and IL-8, 1 µM phorbol myristate acetate, 10, 20 and 40 mM glucose, 0.1, 1 and 10 µM insulin and 0.1 and 10 µM dexamethasone, progesterone and estrogen. After an 18-h incubation, the medium was collected and the release of leptin, resistin and adiponectin was quantified by ELISA. Human gestational tissues and maternal tissues released immunoreactive leptin, resistin and adiponectin; however, there was no difference in the release of either resistin or adiponectin between normal pregnant women and women with gestational diabetes. The release of leptin was significantly higher in placenta, amnion and choriondecidua obtained from normal pregnant women

compared with women with GDM. However, in maternal tissues, the situation was reversed, with adipose tissue and skeletal muscle obtained from women with GDM releasing significantly greater amounts of leptin. In adipose tissue and skeletal muscle the release of leptin was significantly greater in insulin-controlled GDM compared with diet-controlled GDM, and leptin release from adipose tissue was significantly correlated with maternal body mass index. In all tissues tested, there was no effect of incubation with LPS, IL-6, IL-8 or TNF- $\alpha$  on leptin, resistin or adiponectin release. PMA significantly increased the release of resistin from placenta and adipose tissue. Insulin increased placental resistin release, whereas the hormones dexamethasone, progesterone and estrogen significantly decreased placental resistin release. The data presented in this study demonstrate that dysregulation of leptin metabolism and/or function in the placenta may be implicated in the pathogenesis of GDM. Furthermore, resistin release is greatly affected by a variety of inflammatory mediators and hormones.

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## Introduction

Recent investigations have focused on several new potential mediators of insulin resistance including leptin, resistin and adiponectin. Leptin, resistin and adiponectin are known to be produced within the intrauterine environment (Masuzaki *et al.* 1997, Lea *et al.* 2000, Lepercq *et al.* 2001, Akerman *et al.* 2002, Lindsay *et al.* 2003, Yura *et al.* 2003); however their expression and regulation in gestational tissues and in relation to gestational diabetes mellitus (GDM) remains to be fully elucidated.

Leptin was originally discovered as a protein involved in the development of obesity, and although it is now recognized as a hormone that is produced by several

tissues, adipose tissue is the principal site of leptin production and the major determinant of the level of circulating hormone (Wauters *et al.* 2000). The functions attributed to leptin are extensive, including the regulation of food intake and energy balance through central hypothalamic pathways, acting as a major signal to the reproductive system, inhibition of insulin secretion by pancreatic  $\beta$ -cells, and stimulation of glucose transport (reviewed in Wauters *et al.* 2000). Leptin is produced in the human placenta and is secreted into both the maternal and fetal circulation (Masuzaki *et al.* 1997). Expression of leptin has been identified in placenta, chorionic villi, chorion laeve and amnion (Akerman *et al.* 2002). Human leptin mRNA and protein are also localized to the villous vascular

endothelial cells, which are in direct contact with the fetal blood (Lea *et al.* 2000). Both long and short leptin receptor (Ob-R) isoforms are present in placenta, and are co-localized with leptin to the syncytiotrophoblast at the maternal interface (Bodner *et al.* 1999, Lea *et al.* 2000), implicating a potential autocrine or paracrine effect of leptin on placental function.

Resistin is a recently discovered protein that is secreted by adipocytes, and is thought to impair glucose tolerance (Steppan *et al.* 2001). Resistin induces severe hepatic, but not peripheral, insulin resistance. Furthermore, initial studies demonstrated that resistin is up-regulated in both genetic and diet-induced obesity *in vivo* and down-regulated by the anti-diabetic agents, the thiazolidinediones (TZDs) (Steppan *et al.* 2001). These studies by Steppan and colleagues led to the hypothesis that resistin might be a link between obesity and diabetes, as well as a candidate to explain the anti-diabetic effects of TZDs. However, subsequent studies have failed to show an association with resistin and diabetes. In particular, it is not clear whether human adipocytes express substantial amounts of resistin mRNA and protein, and studies on the regulation of resistin are contradictory (reviewed in Fasshauer & Paschke 2003).

Adiponectin is another protein that is secreted by adipocytes, and is postulated to play a role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues in both humans and animals. In humans, plasma adiponectin concentrations exceed those of any other hormone by a thousand times; they decrease in insulin-resistant states including type II diabetes mellitus, and are positively associated with whole-body insulin sensitivity (Hu *et al.* 1996, Weyer *et al.* 2001).

Leptin and adiponectin levels are dysregulated in pathological conditions such as GDM, pre-eclampsia and intra-uterine growth restriction, representing an effect or a cause of disturbances in the fetoplacental/maternal unit (Festa *et al.* 1999, Lea *et al.* 2000, Lepercq *et al.* 2001, Chan *et al.* 2003, Pighetti *et al.* 2003, Ranheim *et al.* 2004, Williams *et al.* 2004). Furthermore, a variety of hormones, cytokines and inflammatory stimuli have also been shown to regulate leptin, resistin and adiponectin expression and release. Therefore, we hypothesize that the release of leptin, resistin and adiponectin may be affected by a number of factors, including GDM, glucose, and hormones and inflammatory stimuli known to affect insulin sensitivity. Therefore, the main purpose of this study was to investigate the release and regulation of leptin, resistin and adiponectin from placenta, fetal membranes, adipose tissue and skeletal muscle. A well characterized tissue explant system is used to determine the effect of cytokine stimulation (tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6 and IL-8), glucose treatment, hormone stimulation (insulin, progesterone, dexamethasone and estrogen) and phorbol myristate acetate (PMA) induction on leptin, resistin and adiponectin release from placenta and fetal

membranes (amnion and choriodecidua), and maternal subcutaneous adipose tissue and skeletal muscle tissue from normal pregnant women and women with GDM.

## Materials and Methods

### Reagents

All chemicals were purchased from BDH Chemicals Australia (Melbourne, Victoria, Australia) unless otherwise stated. RPMI 1640 (glucose free) was obtained from Gibco Laboratories (Grand Island, NY, USA). BSA (RIA grade), dexamethasone (water soluble), estrogen (water soluble), human recombinant IL-6, IL-8 and TNF- $\alpha$ , insulin (from bovine pancreas), lipopolysaccharide (LPS),  $\beta$ -NADH (disodium salt), 3,3',5,5'-tetramethylbenzidine (TMB), progesterone (water soluble) and pyruvic acid (dimer free) were supplied by Sigma (St Louis, MO, USA). PMA and 4 $\alpha$ -PMA were purchased from Tocris (Ellisville, MO, USA). The leptin kit was supplied by Biosource International (Camarillo, CA, USA). The resistin and adiponectin ELISA kits were supplied by CytoLab (Rehovot, Israel) and R&D Systems (Minneapolis, MN, USA) respectively.

### Patients and samples

Human placenta with attached fetal membranes, subcutaneous adipose tissue (from the anterior abdominal wall), skeletal muscle (pyramidalis, small muscle of the anterior abdomen wall), and maternal blood were obtained from a total of 30 pregnant women (15 normal and 15 with GDM) who delivered healthy, singleton infants at term (>37 weeks gestation) undergoing elective Cesarean section (indications for Cesarean section were breech presentation and/or previous Cesarean section). Women with GDM were diagnosed according to the criteria of the Australasian Diabetes in Pregnancy Society by either a fasting venous plasma glucose level of  $\geq 5.5$  mmol/l glucose, and/or  $\geq 8.0$  mmol/l glucose 2 h after a 75 g oral glucose load. Approval for this study was obtained from the Mercy Hospital for Women's Research and Ethics Committee and informed consent was obtained from all participating subjects.

### Tissue explants

Tissues were obtained within 10 min of delivery and dissected fragments were placed in ice-cold PBS. Tissues were dissected to remove visible connective tissue, vessels and/or calcium deposits. Tissue fragments were placed in RPMI (gestational tissues) or DMEM (maternal tissues) containing 5 mM glucose at 37 °C in a humidified atmosphere of carbogen gas (95% O<sub>2</sub> and 5% CO<sub>2</sub>) for 1 h. Explants were blotted dry on sterile filter paper and

transferred to 24-well tissue culture plates (100–200 mg wet weight/well). The explants were incubated, in duplicate, in 2 ml media containing penicillin G (100 U/ml) and streptomycin (100 µg/ml), in the absence (basal release) or presence of 10 µg/ml LPS, 10, 20 and 40 ng/ml IL-6, IL-8 and TNF- $\alpha$ , 1 µM PMA or 1 µM 4 $\alpha$ -PMA (inactive PMA analog), 10, 20 and 40 µM glucose and 0.1, 10 and 10 µM insulin, dexamethasone, progesterone and estrogen. Time course experiments showed that an 18-h incubation was optimal for hormone release. Following an 18-h incubation, tissues were collected and assayed for total protein, while the incubation media were collected and assayed for leptin, resistin and adiponectin release by ELISA.

#### Validation of explant cultures and viability

To determine the effect of experimental treatment on cell membrane integrity, the release of the intracellular enzyme lactate dehydrogenase (LDH) into the incubation medium was determined as described previously (Lappas *et al.* 2004). Data are presented as a percentage of total tissue LDH. LDH release was investigated over the 18-h time course of tissue explants ( $n=3$ ). *In vitro* incubation did not significantly affect LDH activity in the incubation media with none of these measurements exceeding 7% of total activity present in the tissue (data not shown). These data indicate that the concentrations used in this study did not affect cell viability.

#### Experimental assays

The release of leptin (Biosource International), resistin (CytosLab) and adiponectin (R&D Systems) into the explant incubation medium was determined by sandwich ELISA according to the manufacturers' instructions. The intra- and interassay coefficients of variation for the leptin ELISA were 3.4% and 4.5% respectively, and the minimum detectable limit of the assay was 7.2 pg/ml. The intra- and interassay coefficients of variation for the resistin ELISA were 3.4% and 4.5% respectively, and the minimum detectable limit of the assay was 16 pg/ml. The intra- and interassay coefficients of variation for the adiponectin ELISA were 4.2% and 4.7% respectively, and the minimum detectable limit of the assay was 32 pg/ml. All tissue release data were corrected for total protein and expressed as pg per mg protein for leptin and resistin or ng per mg protein for adiponectin. The protein content of tissue homogenates was determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA), using BSA as a reference standard, as previously described (Lappas *et al.* 2004).

#### Statistical analysis

Statistical analyses were performed using a commercially available statistical software package (Statgraphics, Stat

Point Inc., Herndon, VA, USA). Homogeneity of data was assessed by Bartlett's test, and when significant, data were logarithmically transformed before further analysis by one-way analysis of variance (ANOVA). Two sample comparisons were analyzed using Student's *t*-test. Linear regression analyses were used to evaluate the relationship among the variables of interest. Statistical difference was indicated by a *P* value of less than 0.05. Data are expressed as means  $\pm$  standard error of the mean (S.E.M.).

## Results

### Participants

Demographic data of the participants involved in the investigation are summarized in Table 1. The characteristics of this study group relate to the data obtained in part 1 of this study. There were no significant differences in maternal age, maternal body mass index (BMI), fetal birth weight and gestational age between normal pregnant women and women with GDM. Fasting, one-hour and two-hour plasma glucose concentrations after an oral glucose tolerance test were significantly greater in women with GDM compared with healthy pregnant women. Significantly greater maternal plasma insulin levels and significantly lower serum adiponectin levels were observed in women with GDM. Maternal serum leptin and resistin levels were not significantly different between normal pregnant women and women with GDM.

### Part 1: basal hormone release from gestational and maternal tissues

**Basal leptin release from gestational and maternal tissues** With respect to human gestational tissues, placental tissues released the greatest amount of leptin compared with amnion and choriondecidua (Fig. 1). In all three tissues, the basal release of leptin was significantly greater in women with normal pregnancies ( $n=7$ ), compared with women with GDM ( $n=11$ ).

Figure 2a demonstrates that leptin release from both adipose tissue and skeletal muscle collected from women with GDM ( $n=9$ ) was significantly greater than the release from normal pregnant women ( $n=9$ ). Furthermore, as demonstrated in Fig. 2b, there was significantly more leptin released from tissue explants in women with GDM who were treated with insulin ( $n=5$ ) compared with women who were managed by dietary modification alone ( $n=4$ ). In regression analysis (Fig. 3), the release of leptin from adipose tissue was significantly correlated with maternal BMI from normal pregnant women and women with GDM combined ( $n=18$ ;  $r=0.71$ ,  $P=0.02$ ).

**Basal resistin release from gestational and maternal tissues** Immunoreactive resistin was detected in all gestational and maternal tissue samples assayed; however, there

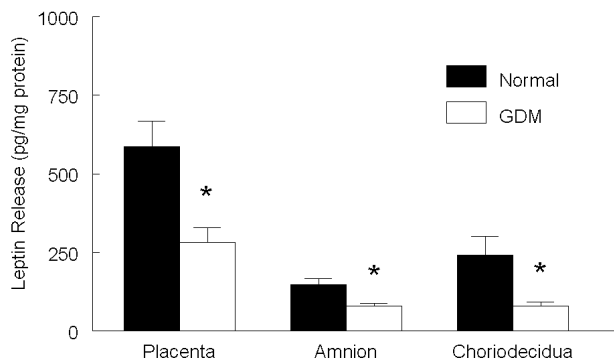
**Table 1** Characteristics of the study group. Values represent means  $\pm$  S.E.M.

	Control patients (n=9)	GDM patients* (n=11)	P values§
Maternal age (years)	31.7 $\pm$ 1.3	35.9 $\pm$ 1.7	NS
Maternal BMI† (kg/m <sup>2</sup> )	28.5 $\pm$ 2.6	24.8 $\pm$ 1.5	NS
Gestational age at birth (weeks)	38.7 $\pm$ 0.3	38.8 $\pm$ 0.4	NS
Fetal birth weight (g)	3287 $\pm$ 160	3402 $\pm$ 165	NS
Male	4/9	6/11	
Female	5/9	5/11	
Fasting plasma glucose (mmol/l)	4.5 $\pm$ 0.2	5.0 $\pm$ 0.2	<0.05
1 hour plasma glucose (mmol/l)	6.8 $\pm$ 0.7	9.8 $\pm$ 0.3	<0.05
2 hour plasma glucose (mmol/l)	5.8 $\pm$ 0.4	8.6 $\pm$ 0.1	<0.05
Maternal serum insulin ( $\mu$ U/ml)	15.3 $\pm$ 2.4	25.6 $\pm$ 2.9	<0.05
Maternal serum leptin (ng/ml)	35.5 $\pm$ 10.8	24.6 $\pm$ 5.5	NS
Maternal serum resistin (ng/ml)	3.4 $\pm$ 0.8	2.3 $\pm$ 0.2	NS
Maternal serum adiponectin ( $\mu$ g/ml)	4.7 $\pm$ 0.5	3.2 $\pm$ 0.4	<0.05

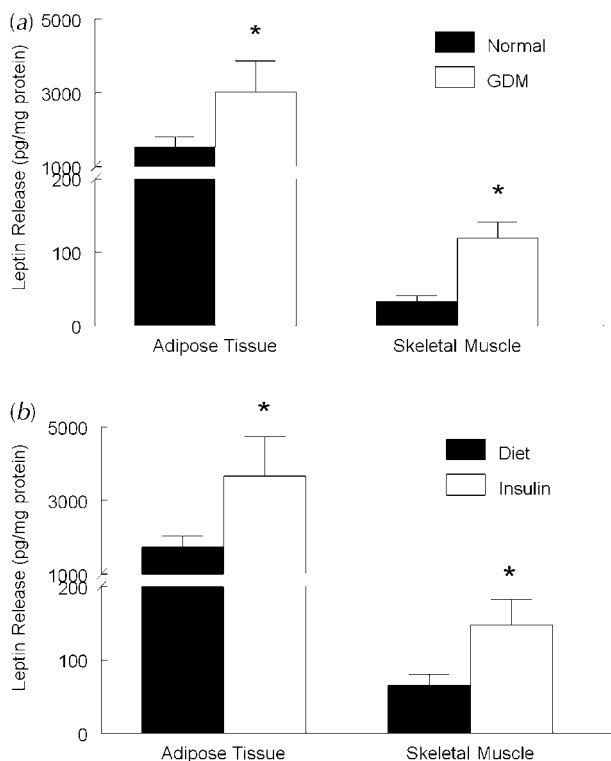
\*Eight diet controlled GDM and seven insulin-controlled GDM patients; §Student's *t*-test; †based on first antenatal visit at approximately 12 weeks. NS, not significant.

was no significant difference in the release of resistin from tissues obtained from normal pregnant women and from women with GDM (data not shown). When the data from normal pregnant women and women with GDM were combined, basal resistin release from adipose tissue (1396.1  $\pm$  325.1 ng/ml) was significantly greater than that from skeletal muscle (665.3  $\pm$  107.9 ng/ml). With respect to intrauterine tissues, placenta (407.9  $\pm$  36.0 ng/ml) and choriodecidua (437.1  $\pm$  43.3 ng/ml) released significantly greater amounts of resistin compared with amnion (203.2  $\pm$  41.8 ng/ml).

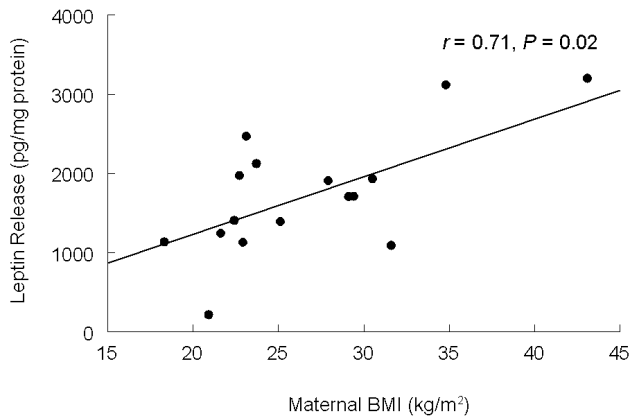
**Basal adiponectin release from gestational and maternal tissues** Immunoreactive adiponectin was detected in all gestational and maternal tissue samples assayed. There was no difference in adiponectin released from tissues obtained from normal pregnant women and from women with GDM (data not shown). When the data



**Figure 1** Release of leptin from human placenta, amnion and choriodecidua obtained from normal pregnant women ( $n=7$ ) and women with GDM ( $n=11$ ). Each bar represents the mean  $\pm$  S.E.M. \* $P<0.05$  vs leptin release from normal women.



**Figure 2** (a) Release of leptin from human subcutaneous adipose tissue and skeletal muscle obtained from normal pregnant women ( $n=9$ ) and women with GDM ( $n=9$ ). Each bar represents the mean  $\pm$  S.E.M. \* $P<0.05$  vs leptin release from normal pregnant women. (b) Release of leptin from human subcutaneous adipose tissue and skeletal muscle obtained from women with insulin-controlled GDM ( $n=5$ ) and diet-controlled GDM ( $n=4$ ). Each bar represents the mean  $\pm$  S.E.M. \* $P<0.05$  vs leptin release from diet-controlled GDM.



**Figure 3** Correlation between maternal BMI and leptin released from adipose tissue. Data are shown for 9 normal pregnant women and 9 women with GDM ( $r=0.71$ ,  $P=0.02$ ).

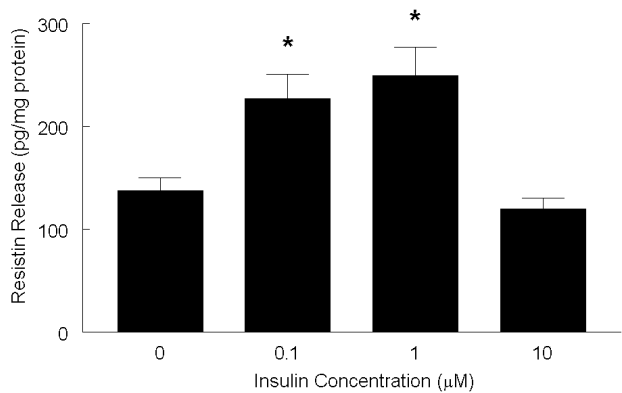
from normal pregnant women and women with GDM were combined, basal release of adiponectin from adipose tissue ( $26.7 \pm 3.1 \mu\text{g/ml}$ ) was significantly greater than that from skeletal muscle ( $17.9 \pm 2.2 \mu\text{g/ml}$ ). Placental adiponectin release ( $19.3 \pm 1.4 \mu\text{g/ml}$ ) was significantly greater than that from amnion ( $6.3 \pm 0.9 \mu\text{g/ml}$ ) and choriondecidua ( $6.8 \pm 0.8 \mu\text{g/ml}$ ).

#### Part 2: regulation of hormone release from gestational and maternal tissues

**Effect of LPS and cytokine stimulation on leptin, resistin and adiponectin release** Gestational tissues (placenta, amnion, and choriondecidua) were incubated in the presence of  $10 \mu\text{g/ml}$  LPS, and increasing concentrations of TNF- $\alpha$ , IL-6 and IL-8 (10, 20 and  $40 \text{ ng/ml}$ ). Due to the small sample size of maternal tissue that was available, adipose tissue and skeletal muscle were incubated in the presence of  $10 \mu\text{g/ml}$  LPS, and  $10 \text{ ng/ml}$  TNF- $\alpha$ , IL-6 and IL-8. At the concentrations tested in this study, neither LPS nor cytokine stimulation had any effect on leptin, resistin or adiponectin release from gestational and maternal tissues obtained from normal pregnant women and from women with GDM (data not shown).

**Effect of high glucose on leptin, resistin and adiponectin release** Placenta and adipose tissue obtained from normal pregnant women ( $n=5$ ) were incubated in the presence of increasing concentrations of glucose (10, 20 and  $40 \text{ mM}$ ). There was no effect of glucose on the release of leptin, resistin and adiponectin from placenta or adipose tissue (data not shown).

**Effect of insulin on leptin, resistin and adiponectin release** Human placentas obtained from normal pregnant women ( $n=5$ ) were incubated in the presence of increasing concentrations of insulin (0.1, 1 and  $10 \mu\text{M}$ ). There

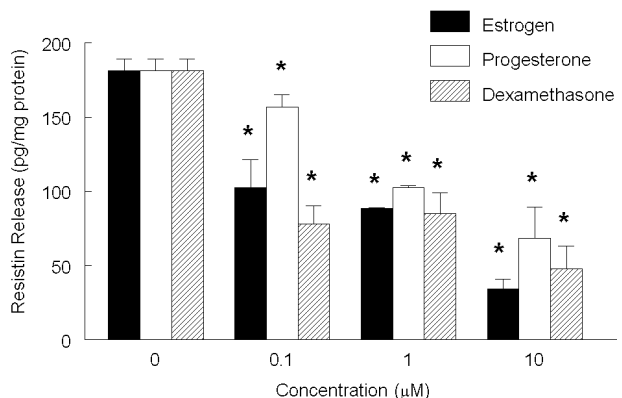


**Figure 4** Effect of 0.1, 1 and  $10 \mu\text{M}$  insulin on the release of resistin from placenta obtained from normal pregnant women ( $n=5$ ). Each bar represents the mean  $\pm$  S.E.M. \* $P<0.05$  vs basal resistin release.

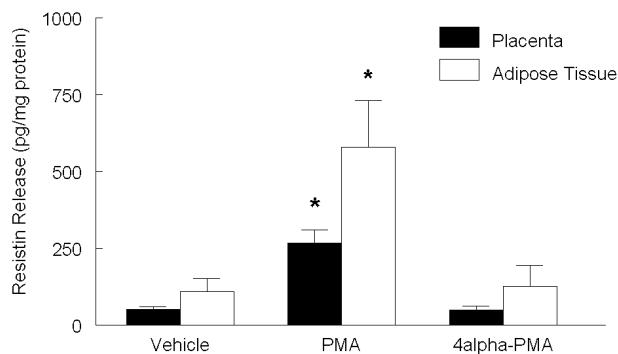
was no effect of insulin on the release of leptin and adiponectin (data not shown). There was a biphasic effect of insulin on the release of resistin from human placenta. Insulin at 0.1 and  $1 \mu\text{M}$  significantly increased resistin release from human placenta (Fig. 4), whereas at  $10 \mu\text{M}$  insulin, resistin release returned to basal levels.

**Effect of dexamethasone, progesterone and estrogen on leptin, resistin and adiponectin release** Human placentas obtained from normal pregnant women ( $n=5$ ) were incubated in the presence of increasing concentrations of dexamethasone, progesterone or estrogen (0.1, 1 and  $10 \mu\text{M}$ ). Progesterone, dexamethasone and estrogen, at all concentrations tested, decreased the release of resistin from placenta (Fig. 5), however no effect was observed on the release of leptin and adiponectin (data not shown).

**Effect of PMA on leptin, resistin and adiponectin release** Human placenta and adipose tissue obtained from normal pregnant women ( $n=5$ ) were incubated in the



**Figure 5** Effect of 0.1, 1 and  $10 \mu\text{M}$  dexamethasone, progesterone and estrogen treatment on the release of resistin from placenta obtained from normal pregnant women ( $n=5$ ). Each bar represents the mean  $\pm$  S.E.M. \* $P<0.05$  vs basal resistin release.



**Figure 6** Effect of 1  $\mu$ M PMA and 1  $\mu$ M 4 $\alpha$ -PMA on the release of resistin from placenta, and adipose tissue obtained from normal pregnant women ( $n=5$ ). Each bar represents the mean  $\pm$  S.E.M. \* $P<0.05$  vs basal resistin release.

presence of 1  $\mu$ M PMA, and 1  $\mu$ M of the PMA analog 4 $\alpha$ -PMA. There was no effect of PMA on the release of leptin and adiponectin from these tissues (data not shown). However, in the presence of 1  $\mu$ M PMA, resistin release was increased from both placenta and adipose tissue (Fig. 6). Furthermore, there was no effect on resistin release upon treatment with 1  $\mu$ M 4 $\alpha$ -PMA.

## Discussion

In this study, a well characterized explant system was used to determine the release and regulation of leptin, resistin and adiponectin from human gestational and maternal tissues. The data presented in this study demonstrate that leptin, resistin and adiponectin are released from human placenta and fetal membranes, and from maternal subcutaneous adipose tissues and skeletal muscle obtained from pregnant women. There were no differences in the release of resistin and adiponectin between normal pregnant women and women with GDM; however there is a differential release of leptin from gestational (placenta, amnion and choriodecidua) and maternal (adipose tissue and skeletal muscle) tissues obtained from normal and GDM-complicated pregnancies. In particular, adipose tissue and skeletal muscle from women with GDM produced greater amounts of leptin than normal pregnant women. This is in marked contrast to the situation in gestational tissues, where leptin release was greater in placenta, amnion and choriodecidua obtained from normal pregnant women.

The role of leptin synthesized from the placenta is not clear, although it may have both autocrine and paracrine activities. Although human placental leptin is identical to that of adipose origin on the basis of size, charge and immunoreactivity, and it has the same promoter, it has a placenta-specific upstream enhancer (Bi *et al.* 1997). This suggests that the placental and adipose leptin gene expres-

sions are regulated differently, and therefore placental leptin may play a distinct role in pregnancy. As both the short and long splice variants of the leptin receptor have been localized in human placenta and fetal membranes (Hoggard *et al.* 1997, Ebenbichler *et al.* 2002), it suggests a possible role for leptin in fetal growth and development, transport of maternal leptin to the fetus or the removal of leptin from the maternal-fetal circulation (reviewed in Ashworth *et al.* 2000, Henson & Castracane 2000).

Previous studies have demonstrated that leptin gene and protein expression are markedly elevated in placentas of women with GDM (Lea *et al.* 2000, Lepercq *et al.* 2001). The authors concluded that this may represent a protective response to counter the effects of an imbalance of pro-inflammatory cytokines which is characteristic of many pathological pregnancies, including GDM. This is, however, in contrast to our findings of decreased leptin release from placenta and fetal membranes from GDM-complicated pregnancies, and those reported by Festa *et al.* (1999) who demonstrated that women with mild GDM had lower leptin levels compared with women with normal glucose tolerance. In this study also, maternal serum leptin levels at the time of Cesarean section were lower in women with GDM, although this did not reach significance. There are many possible reasons for the conflicting results between our studies and previous studies with respect to leptin levels and GDM. Previous studies have looked at the mRNA or protein expression of leptin in placentas of women with GDM (Lea *et al.* 2000, Lepercq *et al.* 2001), whereas in this study, we investigated the release from placental explants. Tissue explants are not comprised of just one cell type and paracrine mediators produced by, for example, non-trophoblast cells present in placenta (such as immunocytes and vascular cells) may be having an effect on leptin release. Furthermore, several hormonal and metabolic changes that occur in insulin-resistant women could contribute to the regulation of placental leptin expression.

The expression of leptin receptors may signal differently in pregnancies of normal and diabetic women. Challier *et al.* (2003) reported that, in contrast with the transmembrane leptin receptor, expression of the soluble receptor is increased in placentas of women with GDM. Soluble leptin receptor is capable of binding leptin with a high affinity. Therefore, it is feasible that this increased expression of soluble leptin receptor may act as a leptin binder, therefore limiting its accessibility to the transmembrane receptor. This, in turn, may modulate the release of leptin from GDM placentas, therefore accounting for the decreased release of leptin observed in this study.

Correlations have been found among plasma leptin level, BMI and adipose tissue mass (particularly subcutaneous fat) in both animals and non-pregnant adults (Hamilton *et al.* 1995, Lonnqvist *et al.* 1995, Cseh *et al.* 2002). Similarly, in this study, the release of leptin from adipose tissue strongly correlated with maternal

body weight and BMI. As leptin circulates at levels proportionate to body adiposity, it has been postulated that insulin may also regulate adipose tissue leptin secretion. Insulin regulation of leptin mRNA expression has been observed in adipose tissue of rodents and humans (MacDougald *et al.* 1995, Malmstrom *et al.* 1995, Saladin *et al.* 1995), although some studies have failed to find an association between body adiposity and circulating levels of insulin and leptin (Schwartz *et al.* 1997). In this study, there was a significant difference in leptin release from adipose tissue and skeletal muscle explants in women with GDM who were treated with insulin compared with women who were managed by dietary modification alone. The finding that there was no difference in placenta and fetal membranes suggests that maternal, and not fetal, hyperinsulinemia may be responsible for this regulation. This is in contrast to Lepercq *et al.* (1998) who demonstrated that placental leptin mRNA and protein contents were increased in women requiring chronic insulin therapy during pregnancy.

Reduced plasma concentrations of adiponectin have been reported in patients with type II diabetes mellitus, and more recently GDM (Ranheim *et al.* 2004, Williams *et al.* 2004). In this study, although maternal serum adiponectin levels at the time of Cesarean section were significantly lower in the GDM group, there was no significant difference in the release of adiponectin from gestational and maternal tissues obtained from normal pregnant women and women with GDM. Adiponectin was released from maternal skeletal muscle in significant amounts (approximately 70% of that secreted by adipose tissue), and it has been suggested that this may represent adiponectin secreted from adipocytes within the skeletal muscle itself. However, these same skeletal muscle samples released very minimal levels of leptin, whereas in adipose tissue the release of leptin was approximately 10-fold.

Leptin, resistin and adiponectin expression and release are known to be affected by a number of inflammatory mediators known to affect insulin sensitivity, including LPS and TNF- $\alpha$ . Placental leptin mRNA production is up-regulated by TNF- $\alpha$  and IL-6 (Chardonens *et al.* 1999, Soh *et al.* 2000, Cameo *et al.* 2003, Nuamah *et al.* 2004), and although there is little information available on the regulation of resistin and adiponectin within intrauterine tissues, resistin expression is up-regulated by LPS and suppressed by TNF- $\alpha$  (Lu *et al.* 2002, Shojima *et al.* 2002, Kaser *et al.* 2003), and adiponectin gene expression and secretion is inhibited by TNF- $\alpha$  (Kappes & Loffler 2000, Halleux *et al.* 2001) and IL-6 (Fasshauer *et al.* 2003). In this study, there was no effect of LPS or pro-inflammatory cytokines on the release of leptin, resistin and adiponectin from gestational and maternal tissues. Similarly, Soh *et al.* (2000) demonstrated that leptin production from placenta, amnion and choriodecidua was unaffected by treatment with IL-1 $\beta$ , TNF- $\alpha$  and LPS. There are a number of possible reasons for the lack of a

response by leptin, resistin and adiponectin to LPS and pro-inflammatory cytokines in this study. (1) It may suggest that the normal regulatory mechanisms are no longer in place; however, we have previously demonstrated that under the same tissue explant conditions these tissues are in fact viably active as they do respond to these stimuli by increasing the release of TNF- $\alpha$ , IL-6 and/or IL-8 (Lappas *et al.* 2004). (2) The explant system and the preservation of the extracellular matrix may provide a more natural environment that allows placental cells and adipocytes to respond to stimuli as they would *in vivo*. (3) Greater concentrations of these inflammatory stimuli, or shorter or longer periods of stimulation, may be required to elicit a response. Lu *et al.* (2002) demonstrated that the concentration of LPS required to increase resistin gene expression in 3T3-L1 adipocytes was significantly greater than that required to stimulate resistin gene expression in isolated human peripheral blood mononuclear cells (75  $\mu$ g/ml compared with 10 ng/ml LPS). Furthermore, concentrations of TNF- $\alpha$  as high as 500 ng/ml have been used to observe increased resistin mRNA expression (Kaser *et al.* 2003).

Resistin release from placental and adipose tissue was up-regulated by PMA. PMA, a potent activator of protein kinase C (PKC), increases the release of several cytokines and other cellular mediators such as TNF- $\alpha$  and IL-6 via activation of mitogen-activated protein kinase and nuclear factor-kappa B, (Voon *et al.* 2004, Wang *et al.* 2004, Eligini *et al.* 2005, Kostadinova *et al.* 2005). It is therefore possible that PMA induces resistin release in placenta and adipose tissue via one of these pathways.

Normal pregnancy, which is associated with high circulating levels of both estrogen and progesterone, is also associated with decreased insulin sensitivity. Dexamethasone, progesterone and estrogen have been proposed to cause insulin resistance by reducing the cellular content of insulin receptor substrate proteins which, in turn, results in a reduction in proximal insulin-stimulated signaling cascades (Collison *et al.* 2000, Buren *et al.* 2002, Garcia-Arencibia *et al.* 2005). Several studies have provided evidence that glucocorticoids may play an important role in the physiological modulation of adipocytokines, and thus insulin resistance. Both *in vitro* and *in vivo* studies have demonstrated that hormone treatment results in either stimulation (Machinal *et al.* 1999, Shojima *et al.* 2002) or inhibition (Combs *et al.* 2003, Huang *et al.* 2005) of leptin, resistin and/or adiponectin secretion, and mRNA and protein expression. In this study, dexamethasone, progesterone and estrogen decreased placental resistin release, but had no effect on leptin and adiponectin release. The present findings suggest that glucocorticoid-induced down-regulation of resistin release may not contribute to insulin resistance in human placenta.

A number of studies have found insulin to be a key regulator of resistin gene expression, although both inhibitory and stimulatory effects have been reported. Insulin

inhibits resistin gene expression in 3T3-L1 and mouse adipocytes (Haugen *et al.* 2001, Shojima *et al.* 2002), whereas in streptozotocin-diabetic mice (Kim *et al.* 2001) and in Zucker diabetic fatty rats (Way *et al.* 2001), insulin stimulates resistin gene expression. Likewise, in this study, insulin stimulated the release of resistin from human placenta. Normal pregnancy is considered a state of hyperinsulinemia and insulin resistance, and our finding of increased resistin secretion by insulin is consistent with a role for resistin in the induction of insulin resistance during pregnancy.

The data presented in this study have established that there is a differential release of leptin from fetal and maternal tissues obtained from women with GDM and from normal pregnant women, and dysregulation of leptin metabolism and/or function in the placenta may be implicated in the pathogenesis of GDM. Resistin release is greatly affected by a variety of inflammatory mediators and hormones, but future studies are warranted to elucidate fully the function of leptin, resistin and adiponectin in normal and diabetic pregnancies.

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