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Release of Proinflammatory Cytokines and 8-Isoprostane from Placenta, Adipose Tissue, and Skeletal Muscle from Normal Pregnant Women and Women with Gestational Diabetes Mellitus

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The aim of this study was to 1) profile the basal release of TNF-α, IL-6, IL-8, and 8-isoprostane (a marker of oxidative stress); and 2) investigate the effect of stimulation on the release of cytokines from sc adipose tissue and skeletal muscle from normal pregnant women and women with gestational diabetes mellitus (GDM). Placenta, sc adipose tissue, and skeletal muscle were incubated in the absence (control) or presence of lipopolysaccharide (LPS; 10 μg/ml), TNF-α (10 ng/ml), IL-6 (10 ng/ml), or IL-8 (10 ng/ml). After an 18-h incubation, the medium was collected, and the release of TNF-α, IL-6, IL-8, and 8-isoprostane was quantified by ELISA. In all three tissues, 8-isoprostane release was greater in women with GDM, and stimulation with LPS increased 8-isoprostane release from adipose and skeletal muscle, but not placenta, obtained from women with GDM. However, in tissues obtained from normal pregnant women, LPS stimulation increased 8-isoprostane release in placenta and had no effect in adipose tissue and skeletal muscle. Their was no difference in the release of TNF-α, IL-6, and IL-8 from placenta, adipose tissue, and skeletal muscle obtained from normal pregnant women and women with GDM. Stimulation of placenta, adipose tissue, and skeletal muscle with LPS and TNF-α resulted in greater release of IL-6 and IL-8, whereas only LPS increased TNF-α release from all three tissues. The data presented in this study demonstrate that there is a differential release of 8-isoprostane from fetal (placenta) and maternal (adipose tissue and skeletal muscle) tissues obtained from normal pregnant women and women with GDM. These data are consistent with the hypothesis that oxidative stress may be involved in the progression and/or pathogenesis of GDM. (*J Clin Endocrinol Metab* 89: 5627–5633, 2004)

GESTATIONAL DIABETES MELLITUS (GDM) is a glucose intolerance of varying severity, with onset or first recognition during pregnancy, that affects 5–8% of pregnancies in Australia (1). Most women with GDM return to normal glucose tolerance after delivery, but have an increased risk of developing diabetes [mainly type 2 diabetes mellitus (DM)] later in life. As such, GDM is considered a prediabetic state, offering the opportunity to study abnormalities that may appear very early in type 2 DM (2). The offspring of women with GDM are prone to adverse side-effects such as macrosomia, which is strongly associated with fetal death, prematurity, birth trauma and respiratory distress syndrome, and, more importantly, have a higher risk of developing obesity, impaired glucose tolerance, and type 2 DM (3).

Cytokines, including TNF-α, IL-6, and IL-8, through their ability to interfere with insulin signaling, have been implicated in insulin resistance in type 2 DM (reviewed in Refs. 4–7). TNF-α, for example, has been shown to inhibit tyrosine kinase activity of the insulin receptor in adipocytes, reducing the phosphorylation and activation of insulin receptor substrate-1 (IRS-1), and so inhibiting the insulin signaling pathway (reviewed in Ref. 4). Given that type 2 DM is associated with overexpression of TNF-α, this suggests that TNF-α impairment of IRS-mediated insulin signaling may be responsible, at least in part, for insulin resistance.

IL-6 and IL-8 may also be involved in the pathogenesis of insulin resistance, type 2 DM, abnormal adiposity, or lipid disorders. The observation that 10–35% of the body's basal circulating IL-6 is derived from adipose tissue has stimulated interest in this cytokine as a possible mediator of metabolic processes (8). Furthermore, the correlation of circulating concentrations of IL-6 with adiposity appear to be stronger than those of TNF-α (8), there is a direct correlation between insulin resistance and circulating IL-6 levels (9, 10), and IL-6 is elevated in the plasma of patients with type 2 DM (11, 12). High glucose and TNF-α increase the release of IL-8 from endothelial cells (13) and adipose tissue (14, 15), respectively.

Many immunological regulators are known to affect cytokine production; for example, lipopolysaccharide (LPS) induces TNF-α mRNA expression and protein synthesis from placenta, adipose tissue, and skeletal muscle (16–18). Like many cytokines, IL-6 and IL-8 are stimulated by many physiological and pathological stressors, including LPS and TNF-α (19). Given the multiplicity of effects attributed to cytokines, they may play an important integrative function in placenta, adipose tissue, and skeletal muscle. For example, adipose tissue secretes many factors, which, by exerting

Abbreviations: DM, Diabetes mellitus; GDM, gestational DM; HOMA-IR, homeostasis model assessment for insulin resistance; IRS-1, insulin receptor substrate-1; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; OGTT, oral glucose tolerance test.

paracrine or endocrine actions, regulate both adipose development and whole body metabolism. TNF-α production in adipose tissue can potentially have effects on distant tissues, either directly by TNF-α acting as a hormone or indirectly by TNF-α affecting other hormones, such as leptin.

There is also evidence that oxidative stress may be involved in the pathogenesis of type 2 DM (reviewed in Ref. 20). Isoprostanes are prostaglandin-like products derived from free radical-catalyzed, nonenzymatic oxidation of arachidonic acid (21) that are considered to be an accurate marker of oxidative stress and endogenous lipid peroxidation (reviewed in Ref. 22). Although cytokines and oxidative stress have been extensively studied in type 2 DM, there is a paucity of data with regard to GDM.

The biologically active cytokines TNF-α, IL-6, and IL-8 are synthesized and released by placenta, adipose tissue, and skeletal muscle. 8-Isoprostane is also released from placenta (23). Despite the potential importance of cytokines and oxidative stress as putative mediators of type 2 DM, relatively little is known about their profiles in GDM. The aim of this study was to 1) profile the basal release of TNF-α, IL-6, IL-8, and 8-isoprostane; and 2) investigate the effect of LPS and cytokine stimulation on the release of cytokines and 8-isoprostane from fetal (placenta) and maternal (sc adipose tissue and skeletal muscle) tissues obtained from normal pregnant women and women with GDM.

Materials and Methods

Reagents

All chemicals were purchased from BDH Chemicals Australia (Melbourne, Australia) unless otherwise stated. RPMI 1640 (glucose free) was obtained from Invitrogen Life Technologies, Inc. (Grand Island, NY). BSA (RIA grade); human recombinant IL-6, IL-8, and TNF-α; LPS, β-NADH (disodium salt); 3,3′,5,5′-tetramethylbenzidine; and pyruvic acid (dimer free) were supplied by Sigma-Aldrich Corp. (St. Louis, MO). The IL-6, IL-8, and TNF-α kits were supplied by BioSource International (Camarillo, CA). The insulin ELISA was purchased from Diagnostic Systems Laboratories (Webster, TX).

Tissue collection and preparation

Human placenta, sc adipose tissue (from the anterior abdominal wall), and skeletal muscle (pyramidalis) were obtained from 22 pregnant women (10 normal subjects and 12 with GDM) who delivered healthy, singleton infants at term (≥37 wk gestation) by elective cesarean section (indications for cesarean section were breech presentation and/or previous cesarean section). Fasting maternal plasma samples were collected from antenatal patients undergoing an oral glucose tolerance test (OGTT) at approximately 28 wk gestation. Women with GDM were diagnosed according to the criteria of the Australian Diabetes Society (ADS) by either a fasting venous plasma glucose level of 5.5 mmol/liter glucose or more and/or 8.0 mmol/liter glucose or higher 2 h after a 75-g oral glucose load. The clinical characteristics of the subjects are collated in Table 1. 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.

Tissues were obtained within 10 min of delivery, and dissected fragments were placed in ice-cold RPMI. Tissue were dissected to remove visible connective tissue, vessels, and/or calcium deposits. Tissue fragments (100–200 mg, wet weight) were placed in RPMI containing 5 mM glucose at 37 C in a humidified atmosphere of carbogen gas (95% O_2 and 5% CO_2) for 1 h. Explants were blotted dry on sterile filter paper and transferred to 24-well tissue culture plates (200–250 mg wet weight/well). The explants were incubated, in duplicate, in 2 ml RPMI containing penicillin G (100 U/ml) and streptomycin (100 μg/ml). Tissues were incubated in the absence (control) or presence of 10 μg/ml LPS or 10 ng/ml IL-6, IL-8, or TNF-α. The concentrations used in this study were chosen based on our previous studies (16, 19). After an 18-h incubation, tissues were collected and assayed for total protein, whereas the incubation medium was collected and assayed for IL-6, IL-8, TNF-α, or 8-isoprostane release by ELISA.

Assessment of insulin resistance

Fasting maternal plasma samples for insulin concentrations were centrifuged at 4 C, and the plasma was stored at −80 C for subsequent analysis by ELISA according to the manufacturer's instructions (Diagnostic Systems Laboratories). The intra- and interassay coefficients of variation were 2.1% and 5.7%, respectively, and the minimum detectable limit of the assay was 0.26 μU/ml. Insulin resistance was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) method where HOMA-IR = fasting plasma glucose (mmol/liter) times fasting serum insulin (μU/ml) ÷ 22.5 (24).

Experimental assays

The release of IL-6, IL-8, and TNF-α in the explant incubation medium was performed by sandwich ELISA according to the manufacturer's instructions (BioSource International). The limits of detection of the IL-6, IL-8, and TNF-α assays (defined as 2 SD from the zero standard) were 3, 2.8, and 7.2 pg/ml, respectively. The release of 8-isoprostane into the incubation medium was assayed using a commercially available competitive enzyme immunoassay kit according to the manufacturer's specifications (Cayman Chemical Co., Ann Arbor, MI). The limit of detection of the assay was 5 pg/ml. All data were corrected for total protein and expressed as either picograms or nanograms per milligram of protein. The protein content of tissue homogenates was determined using a bicinchoninic acid protein assay (Pierce Chemical Co., Rockford, IL) with BSA as a reference standard, as previously described (25). To determine the effect of experimental treatment on cell membrane integrity, the release of the intracellular enzyme lactate dehydrogenase

TABLE 1. Characteristics of the study group

	Control patients	GDM patients	*P* value
Maternal age (yr)	31.1 ± 1.0	35.3 ± 1.6	0.04
Maternal BMI (kg/m^2)[a]	28.5 ± 2.0	25.7 ± 1.6	NS
Gestational age at birth (wk)	38.7 ± 0.2	38.8 ± 0.2	NS
Fetal birth weight (g)	3293 ± 108	3380 ± 140	NS
Gravida	2.3 ± 0.3	2.6 ± 0.3	NS
Parity	2.3 ± 0.3	1.8 ± 0.2	NS
Fasting plasma glucose (mmol/liter)	4.3 ± 0.1	4.9 ± 0.2	<0.05
1-h plasma glucose (mmol/liter)	6.3 ± 0.4	9.8 ± 0.3	<0.0001
2-h plasma glucose (mmol/liter)	5.8 ± 0.3	8.7 ± 0.1	<0.0001
Fasting insulin (μU/ml)	8.2 ± 0.7	12.1 ± 1.6	0.003
Maternal serum IL-6 (pg/ml)	11.3 ± 4.3	6.1 ± 2.0	NS
Maternal serum IL-8 (pg/ml)	13.5 ± 4.2	9.1 ± 2.1	NS

Values represent the mean ± SEM. BMI, Body mass index; NS, not significant.
[a] Based on first antenatal visit at approximately 12 wk.

(LDH) into incubation medium was determined as described previously (25). Data are presented as a percentage of the total tissue LDH.

Statistical analysis

Statistical analyses were performed using a commercially available statistical software package (Statgraphics, STSC, Rockville, MD). The homogeneity of the data was assessed by Bartlett's test (26), and when significant, data were logarithmically transformed before additional analysis. Sample comparisons were analyzed either by *t* test or ANOVA. Statistical difference was indicated by $P < 0.05$. Data are expressed as the mean ± SEM.

Results

Participants

Demographic data of all participants involved in the investigation are summarized in Table 1. For all measured experimental parameters, there was no significant difference in the release from placental explants in women with GDM who were managed by dietary modification alone compared with women who were treated with insulin. Fasting, 1 h, and 2 h plasma glucose concentrations and fasting plasma insulin concentrations during the OGTT were significantly greater ($P < 0.05$) in women with GDM than those in healthy pregnant women. The median HOMA-IR (interquartile ranges) was 3.01 (1.75, 3.63) in 12 women with GDM compared with 1.62 (1.19, 1.76) in 10 women without GDM ($P = 0.003$, by Mann-Whitney U test). The HOMA-IR was significantly correlated with fasting glucose ($r = 0.45$; $P < 0.001$) and fasting insulin ($r = 0.96$; $P < 0.001$). There was no correlation between *in vitro* IL-6 and IL-8 release from maternal or gestational tissues and maternal serum levels. No data on serum TNF-α is presented because it was below the sensitivity of the assay used in this study.

Validation of explant cultures and viability

To validate the integrity of explants in the presence of LPS, IL-6, IL-8, and TNF-α, cell viability was investigated using LDH release from explants. LDH release was investigated over the 18-h time course of tissue explants (n = 4). Explants were incubated in either medium alone or medium containing 10 μg/ml LPS or 10 ng/ml IL-6, IL-8, or TNF-α. The effects of treatment on LDH release are detailed in Table 2. Neither *in vitro* incubation nor experimental treatments significantly affected LDH activity in the incubation medium. These data indicate that the concentrations used in this study did not affect cell viability.

Basal cytokine release

The release of IL-6 (Fig. 1A), IL-8 (Fig. 1B), and TNF-α (Fig. 1C) was greatest in placenta compared with that in adipose tissue and skeletal muscle. No differences in IL-6, IL-8, and TNF-α release were observed between normal pregnant women and women with GDM in placenta, adipose tissue, and skeletal muscle. In addition, there was no significant difference in basal cytokine release from placenta, adipose tissue, and skeletal muscle obtained from women with GDM who were managed by dietary modification alone compared with women who were treated with insulin.

TABLE 2. Effect of experimental treatments on the release of LDH (percentage of total tissue LDH activity)

	% Total tissue LDH		
	Placenta	Adipose tissue	Skeletal muscle
Basal	6.5 ± 0.7	4.6 ± 0.9	6.7 ± 2.3
10 μg/ml LPS	6.8 ± 0.7	5.0 ± 1.3	7.0 ± 1.7
10 ng/ml IL-6	6.7 ± 0.5	5.1 ± 1.2	6.7 ± 1.9
10 ng/ml IL-8	6.5 ± 0.9	4.4 ± 1.2	6.9 ± 2.8
10 ng/ml TNF-α	6.7 ± 1.2	5.3 ± 1.3	7.6 ± 1.4

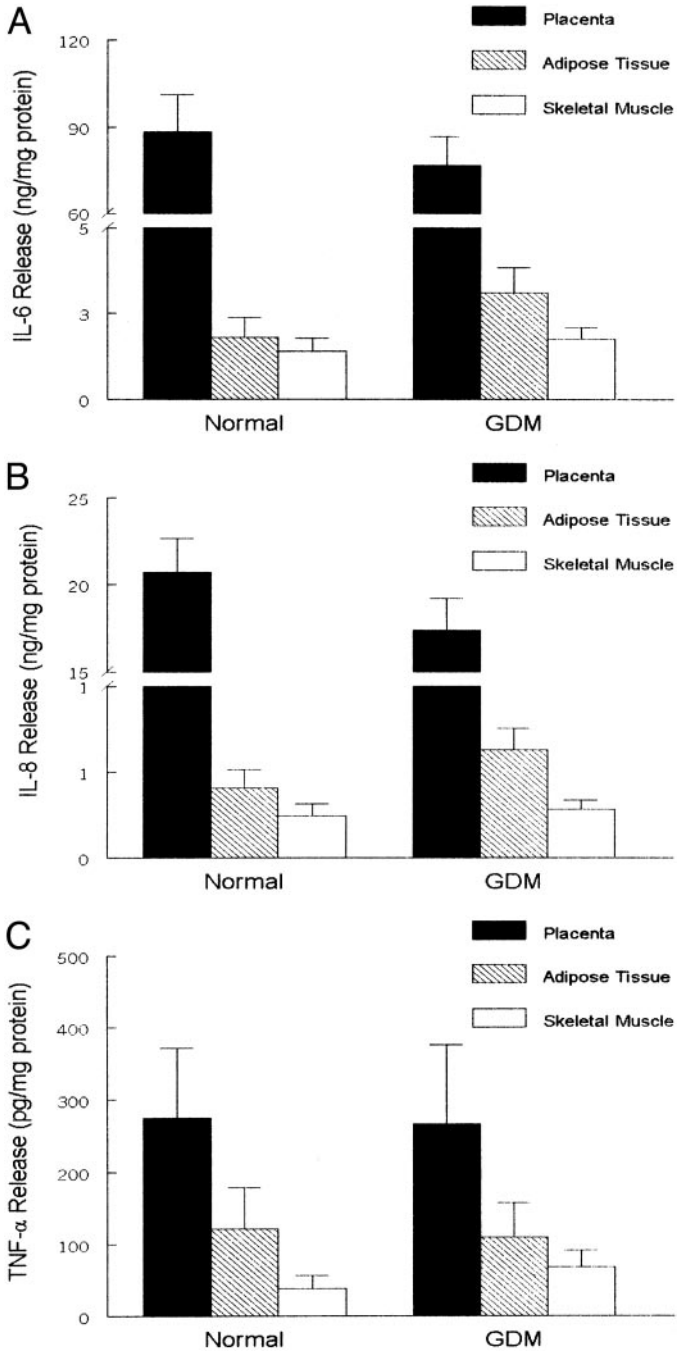

FIG. 1. Basal release of IL-6 (A), IL-8 (B), and TNF-α (C) from human placenta, adipose tissue, and skeletal muscle from normal pregnant women (n = 11) and women with GDM (n = 12). Each *bar* represents the mean ± SEM.

Effect of LPS and cytokines on IL-6 release

For all experimental treatments, there was no significant difference in cytokine release from placenta, adipose tissue, and skeletal muscle obtained from normal pregnant women (n = 5) and women with GDM (n = 5); thus, all data were combined. In placenta, adipose tissue, and skeletal muscle, LPS and TNF-α stimulation resulted in significantly greater release of IL-6 into the incubation medium compared with basal release (Fig. 2; n = 10). No effect of IL-8 was observed on the release of IL-6 from placenta, adipose tissue, and skeletal muscle.

Effect of LPS and cytokines on IL-8 release

In placenta, adipose tissue, and skeletal muscle, LPS and TNF-α stimulation resulted in significantly greater release of IL-8 into the incubation medium compared with basal release (Fig. 3; n = 10), whereas no effect of IL-6 was observed on the release of IL-8 from placenta, adipose tissue, and skeletal muscle.

Effect of LPS and cytokines on TNF-α release

In placenta, adipose tissue, and skeletal muscle, LPS-stimulation significantly increased TNF-α release into the incubation medium (Fig. 4; n = 10); however, there was no effect of IL-6 and IL-8 stimulation on TNF-α release in any of the three tissues.

Release of 8-isoprostane

In placenta, adipose tissue, and skeletal muscle, the release of 8-isoprostane into the incubation medium was significantly greater in women with GDM (n = 8) than in normal (n = 6) pregnant women (Fig. 5). In adipose tissue (Fig. 5B) and skeletal muscle (Fig. 5C) obtained from women with GDM, LPS stimulation significantly increased the release of 8-isoprostane; however, LPS stimulation did not significantly increase 8-isoprostane release in placentas obtained from women with GDM (Fig. 5A). In normal pregnant women, LPS stimulation increased placental 8-isoprostane release (Fig. 5A), whereas LPS stimulation had no effect on 8-isoprostane release from adipose tissue (Fig. 5B) and skeletal muscle (Fig. 5C).

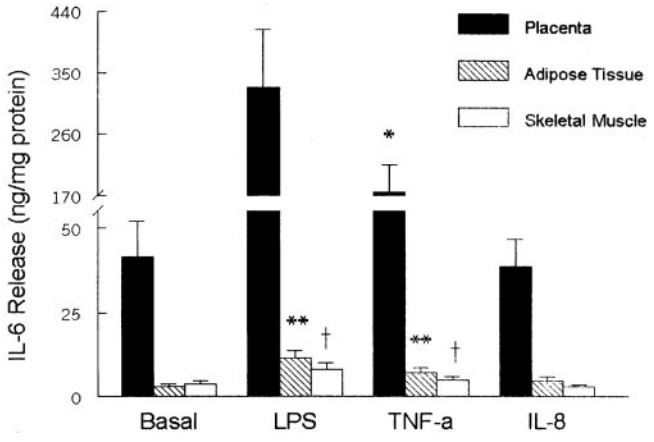

FIG. 2. Release of IL-6 from human placenta, sc adipose tissue, and skeletal muscle (n = 10). Tissue explants were stimulated with 10 μg/ml LPS, 10 ng/ml TNF-α, or 10 ng/ml IL-8 for 18 h. Each *bar* represents the mean ± SEM. *, $P < 0.05$ *vs.* basal placental IL-6 release; **, $P < 0.05$ *vs.* basal adipose tissue IL-6 release; †, $P < 0.05$ *vs.* basal skeletal muscle IL-6 release.

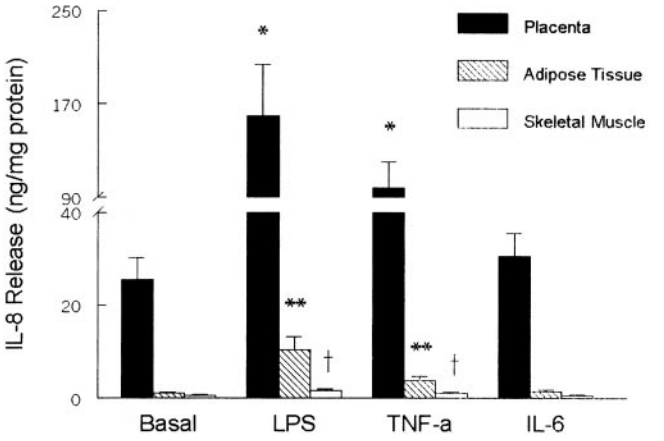

FIG. 3. Release of IL-8 from human placenta, sc adipose tissue, and skeletal muscle (n = 10). Tissue explants were stimulated with 10 μg/ml LPS, 10 ng/ml TNF-α, or 10 ng/ml IL-6 for 18 h. Each *bar* represents the mean ± SEM. *, $P < 0.05$ *vs.* basal placental IL-8 release; **, $P < 0.05$ *vs.* basal adipose tissue IL-8 release; †, $P < 0.05$ *vs.* basal skeletal muscle IL-8 release.

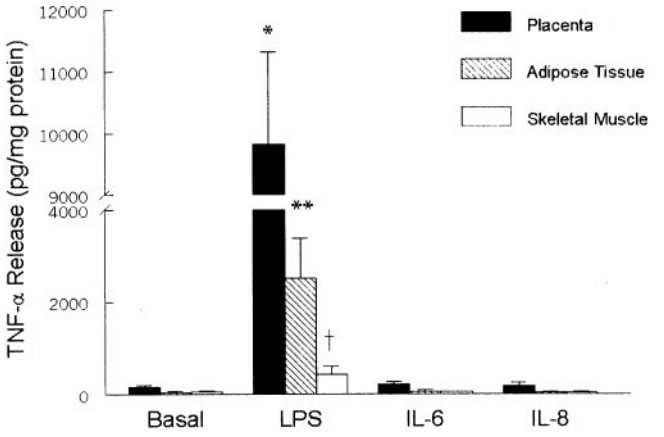

FIG. 4. Release of TNF-α from human placenta, sc adipose tissue, and skeletal muscle (n = 10). Tissue explants were stimulated with 10 μg/ml LPS, 10 ng/ml IL-6, or 10 ng/ml IL-8 for 18 h. Each *bar* represents the mean ± SEM. *, $P < 0.05$ *vs.* basal placental TNF-α release; **, $P < 0.05$ *vs.* basal adipose tissue TNF-α release; †, $P < 0.05$ *vs.* basal skeletal muscle TNF-α release.

Discussion

Studies of type 2 DM have been critical in elucidating the roles of cytokines and oxidative stress in diabetes; however, very limited data are available in relation to GDM. The data presented in this study demonstrate that there is a differential release of IL-6, IL-8, TNF-α, and 8-isoprostane from gestational (placenta) and maternal (adipose tissue and skeletal muscle) tissues obtained from normal pregnant women and women with GDM. Although no difference in cytokine release was observed between normal pregnant women and women with GDM, 8-isoprostane release in all three tissues was higher in women with GDM.

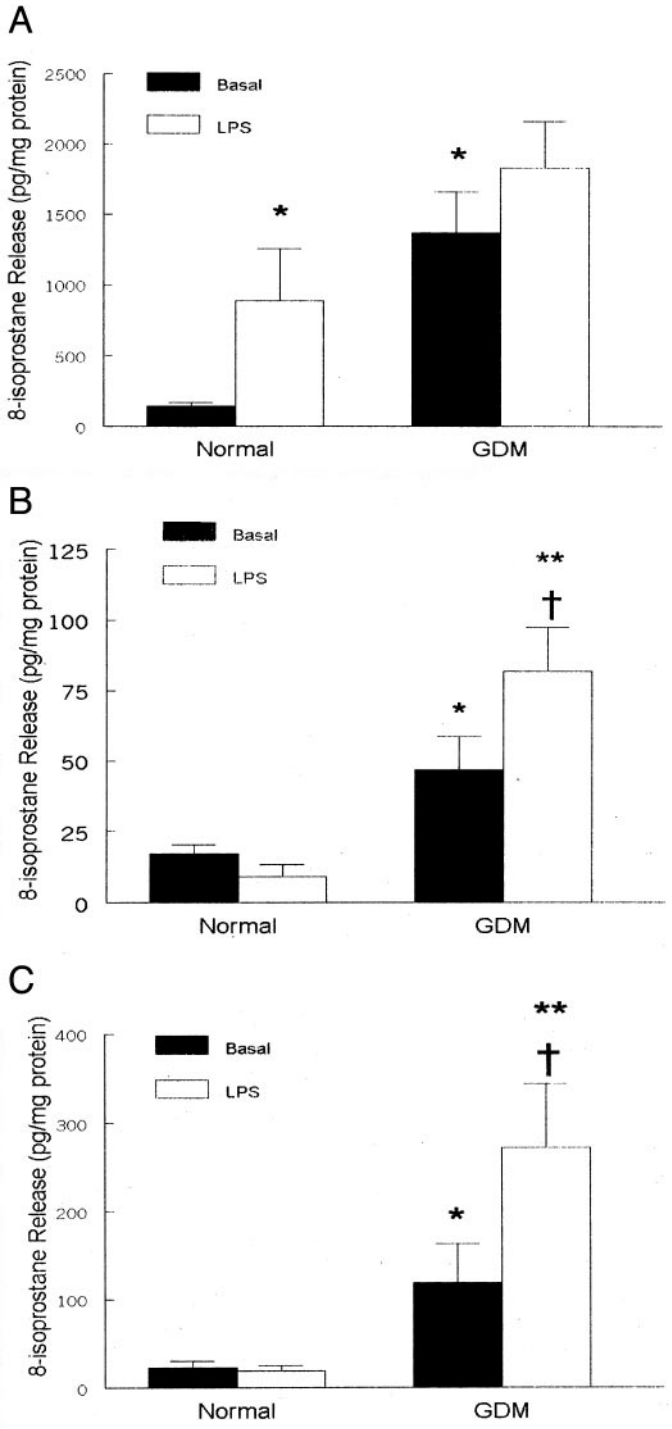

FIG. 5. Effect of LPS on 8-isoprostane release from human placenta (A), sc adipose tissue (B), and skeletal muscle (C) from normal pregnant women (n = 6) and women with GDM (n = 8). Each *bar* represents the mean ± SEM. *, $P < 0.05$ *vs.* basal 8-isoprostane release from normal pregnant women; **, $P < 0.05$ *vs.* LPS-stimulated 8-isoprostane release from normal pregnant women; †, $P < 0.05$ *vs.* basal 8-isoprostane release from women with GDM.

HOMA-IR reference ranges in pregnancy have not, to our knowledge, been established; thus, in our institution, ADS GDM criteria usefully distinguish pregnant women who are more or less insulin resistant. In this study we compared the HOMA-IR levels in women undergoing OGTT at approximately 28 wk gestation. HOMA-IR estimates were statistically different between the groups in whom gestational diabetes was subsequently diagnosed or excluded on ADS criteria. Furthermore, there was a significant correlation between HOMA-IR and both fasting plasma glucose and fasting plasma insulin levels.

Current evidence has demonstrated that TNF-α is the most significant predictor of insulin resistance during pregnancy (27). With respect to GDM, the available data demonstrate that serum concentrations of TNF-α are elevated in women with GDM (28), and placental and sc adipose tissue TNF-α release from women with GDM was lower than that in normal pregnant women (29). However, the data presented in this study fail to collaborate the latter findings, with the release of TNF-α from human placenta, sc adipose tissue, and skeletal muscle not different between normal pregnant women and women with GDM.

In nongestational tissues, the evidence that insulin resistance is linked to TNF-α is well established (reviewed in Ref. 4). The administration of exogenous TNF-α or LPS induced increases in circulating TNF-α that have marked effects on systemic lipid metabolism, and prolonged infusion of TNF-α in animals can induce insulin resistance, whereas neutralization of TNF-α can improve insulin sensitivity. Results from TNF-α knockout mice also support the importance of TNF-α in regulating insulin sensitivity. Furthermore, TNF-α mRNA levels and secretion of TNF-α in human skeletal muscle are elevated in insulin resistance and in patients with type 2 DM (30).

Multiple mechanisms have been suggested to account for the metabolic effects of TNF-α in *in vitro* systems such as isolated adipocytes or cultured 3T3-L1 adipocytes (reviewed in Refs. 4 and 5). These include the down-regulation of genes that are required for normal insulin action (*e.g.* the insulin-responsive glucose transporter), direct effects on components of the insulin signaling (*e.g.* down-regulation of insulin receptor autophosphorylation and IRS-1), and induction of elevated free fatty acids via stimulation of lipolysis.

In contrast to TNF-α, the association between IL-6 and IL-8, and insulin resistance and/or type 2 DM is putative rather than casual. Although type 2 DM is associated with IL-6 polymorphism (31), higher plasma concentrations of IL-6 (11), and IL-6 release from adipose tissue (10), there is no direct evidence for an association between IL-6 expression and insulin resistance, particularly in human skeletal muscle. Circulating IL-8 increases after both an OGTT and a euglycemic hyperinsulinemic clamp (32), there are elevated circulating IL-8 levels in patients with type 2 DM (33), and high glucose stimulates IL-8 production and secretion from cultured endothelial cells (13).

Recent evidence suggests that a rodent rendered insulin resistant in skeletal muscle markedly increases IL-6 gene expression in skeletal muscle (7). The researchers suggest that IL-6 production and subsequent release by skeletal muscle may play a role in the regulation of glucose homeostasis in insulin-sensitive tissue and that IL-6 may be up-regulated in insulin-resistant tissue in an attempt to overcome such a metabolic dysfunction (reviewed in Ref. 7). That is, IL-6 expression may increase glucose transport by up-regulating the

processes involved in the trafficking of glucose transporter-4 from the intracellular pools to the plasma membrane. Stouthard *et al.* (34) demonstrated that IL-6 increased basal and insulin-stimulated glucose uptake in cultured 3T3-L1 adipocytes by increasing glucose transporter intrinsic activity; however, the effect of IL-6 on glucose uptake in skeletal muscle cells has not been investigated. It is feasible that IL-8 may also play a similar role in skeletal muscle; however, additional investigations are required to fully elucidate the roles of IL-6 and IL-8 in skeletal muscle.

Cytokine levels can be increased in a dose-dependent manner under stimulation. Human skeletal muscle cells secrete IL-1β, IL-6, and IL-8 constitutively and under stimulation of a variety of proinflammatory cytokines (reviewed in Ref. 35). In addition, IL-1α, IL-1β, IL-6, and TNF-α stimulate TNF-α (reviewed in Ref. 35). In this study there appears to be a hierarchy of cytokines, so that the higher order cytokine TNF-α orchestrates the synthesis of lower order cytokines IL-6 and IL-8. Autocrine, paracrine, and endocrine effects of cytokines are likely to be critical to their putative roles in the development of insulin resistance. For example, in the case of adipose tissues, its metabolic characteristics vary between anatomical locations, and the site of cytokine production may be important in determining its effects on insulin resistance (reviewed in Ref. 5). Cytokines released from omental fat may act principally on the liver, whereas those from sc fat may affect a range of tissues, including skeletal muscle.

Oxidative stress can result from increased content of reactive oxygen species, and recent studies have linked both reactive oxygen species production and oxidative stress to insulin resistance (36). The data obtained in this study are consistent with increased oxidative stress in women with GDM compared with normal pregnant women, because increased release of 8-isoprostane was observed from placenta, adipose tissue, and skeletal muscle obtained from women with GDM. Furthermore, LPS stimulation increased 8-isoprostane release in normal placentas, but had no effect on GDM placentas. This is in agreement with a recent study that demonstrated an unaltered 8-isoprostane response to oxidative stress and increased superoxide dismutase activity and protein carbonyl content in GDM placentas (37). The researchers suggested that placentas from women with GDM have been previously exposed to oxidative stress *in situ*, and this attenuates their response to *in vitro* oxidative stress. The effect of LPS stimulation observed in placenta is in distinct contrast to the responses observed in maternal tissues. Adipose tissue and skeletal muscle from normal pregnant women showed a reduced capacity to respond to LPS, whereas LPS stimulation induced an increase in 8-isoprostane release in tissues obtained from women with GDM.

The increase in 8-isoprostane in women with GDM suggests that increased lipid peroxidation is a consequence of increased oxidative stress. This is supported by the finding that oxidative stress can give rise to protein carbonyl derivatives, and their increased concentrations in the placentas of women with GDM (37) suggests the presence of oxidative stress in this condition. Furthermore, women with GDM also have an increase in the antioxidant enzyme superoxide dismutase (37), suggesting a compensatory defense mechanism to overcome existing oxidative stress. Oxidative stress can cause vascular dysfunction in the placenta, leading to fetal compromise (38). Elevations in 8-isoprostane secretion from the placenta in women with GDM may induce pathophysiological effects that contribute to adverse pregnancy outcomes.

The data presented in this study have established that there is a differential release of cytokines between maternal and gestational tissues; however, there was no difference between normal pregnant women and women with GDM. However, in terms of oxidative stress, the release of 8-isoprostane from gestational and maternal tissues obtained from women with GDM was greater than that in normal pregnant women. Future studies are required, however, to elucidate the roles of both cytokines and oxidative stress in the pathophysiology of GDM.

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