Gestational Diabetes Induces Placental Genes for Chronic Stress and Inflammatory Pathways

Tatjana Radaelli, Ali Varastehpour, Patrick Catalano, and Sylvie Hauguel-de Mouzon

A physiological state of insulin resistance is required to preferentially direct maternal nutrients toward the feto-placental unit, allowing adequate growth of the fetus. When women develop gestational diabetes mellitus (GDM), insulin resistance is more severe and disrupts the intrauterine milieu, resulting in accelerated fetal development with increased risk of macrosomia. As a natural interface between mother and fetus, the placenta is the obligatory target of such environmental changes. However, the molecular basis for the imbalance that leads to fetal, neonatal, and adult metabolic compromises is not well understood. We report that GDM elicits major changes in the expression profile of placental genes with a prominent increase in markers and mediators of inflammation. Within the 435 transcripts reproducibly modified, genes for stress-activated and inflammatory responses represented the largest functional cluster (18.5% of regulated genes). Upregulation of interleukins, leptin, and tumor necrosis factor-α receptors and their downstream molecular adaptors indicated an activation of pathways recruiting stress-activated protein/c-Jun NH_2-terminal kinases. Transcriptional activation of extracellular matrix components and angiogenic activators pointed to a major structural reorganization of the placenta. Thus, placental transcriptome emerges as a primary target of the altered environment of diabetic pregnancy. The genes identified provide the basis to elucidate links between inflammatory pathways and GDM-associated insulin resistance. *Diabetes* 52:2951–2958, 2003

Gestational diabetes mellitus (GDM) is a common metabolic disease of pregnancy that shares many features of type 2 diabetes, including glucose intolerance and insulin resistance (1,2). The maternal hormonal and metabolic alterations associated with GDM profoundly modify the in utero environment, leading to an abnormal pattern of fetal growth (3). Impaired fetal development has severe metabolic consequences with increased risk to develop glucose intolerance and obesity in adolescence and later life (4,5). The links between GDM-associated insulin resistance and altered fetal growth are not clearly understood. Insulin resistance and obesity are linked to aberrant whole-body homeostasis with a tight control through adipocyte-secreted factors, such as tumor necrosis factor-α (TNF-α) and leptin. We have shown that leptin and TNF-α are the strongest predictors of pregnancy-associated insulin resistance, far greater than previously suggested for gestational hormones, including human placental lactogen and steroids (6,7). Because the placenta is at the same time producer and natural target of both leptin and TNF-α, we asked whether molecular adaptations are elicited by GDM-induced insulin resistance. The remarkable structural diversity of the placenta resting on multiple cell types devoted to nutrient transport, and energy metabolism, as well as endocrine, immunological, and vascular functions, offers a unique opportunity to survey genes responsible for a large variety of biological processes (8).

The purpose of this study was to examine profiles of gene expression in human placenta obtained from normal and GDM pregnancies to determine whether insulin resistance modifies the pattern of placental transcriptome.

From the Department of Reproductive Biology, Schwartz Center for Metabolism and Nutrition, University School of Medicine at MetroHealth Medical Center, Case Western Reserve University, Cleveland, Ohio.

Address correspondence and reprint requests to Dr. Sylvie Hauguel-de Mouzon, Department of Obstetrics, 2500 MetroHealth Dr., Cleveland, OH 44109. E-mail: sdemouzon@aol.com.

Received for publication 28 April 2003 and accepted in revised form 15 September 2003.

ECM, extracellular matrix; GDM, gestational diabetes mellitus; IL, interleukin; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α.

© 2003 by the American Diabetes Association.

RESEARCH DESIGN AND METHODS

The protocol was approved by the institutional review board of Case Western Reserve University. Volunteers gave informed written consent in accordance with the MetroHealth Medical Center guidelines. GDM was defined as an abnormal glucose tolerance during the third trimester according to the criteria defined by Carpenter and Coustan (9). All GDM subjects required chronic insulin therapy for glucose control. Maternal and cord blood samples were obtained at delivery. Neonatal anthropometric measurements were performed within 48 h of delivery. Neonatal body composition was estimated using Total Body Electrical Conductivity (EM-Scan HP-2 TOBEC, Springfield, IL).

Analysis of glucose and insulin. Plasma glucose was measured by the glucose oxidase method with a glucose analyser (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin was assayed by radioimmunoassay as described (1). We estimated insulin sensitivity using a prediction model based on a significant correlation between the 1-h glucose screen and insulin sensitivity measured with the hyperinsulinemic-euglycemic clamp (2).

RNA processing and microarrays. Placental biopsies (~1 cm^3) were randomly obtained from six intact cotyledons after dissecting out the basal and the chorial plates and snap-freezing them in liquid nitrogen. Total RNA was prepared from whole-villous tissue using CsCl gradient (10). RNA samples were electrophoresed to verify integrity, pooled, and reversed transcribed using Superscript first-strand synthesis (Invitrogen, Carlsbad, CA). This pooling strategy was used to minimize variations in structural content of the biopsies due to placental heterogeneity. cDNA served as a template to generate biotinylated cRNA by in vitro transcription (ENZO kit; Affymetrix). The quality of each fragmented cRNA evaluated with test microarrays (Test-3array; Affymetrix) was considered satisfactory when bioC, bioD, and cre were present and the 3′/5′ ratio of the polyA control subjects was <3. The 3′/5′ ratios for β-actin and GAPDH were 1.4 ± 0.2 and 1.2 ± 0.2, respectively. Fragmented cRNAs were spotted onto eight human U133 Affymetrix microarrays. Hybridization was performed according to the manufacturer's instructions. Signal scanning and analysis were performed with Affymetrix equipment (Fluidics station, HPgene array scanner, and MAS 5.0 microarray suite).

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024

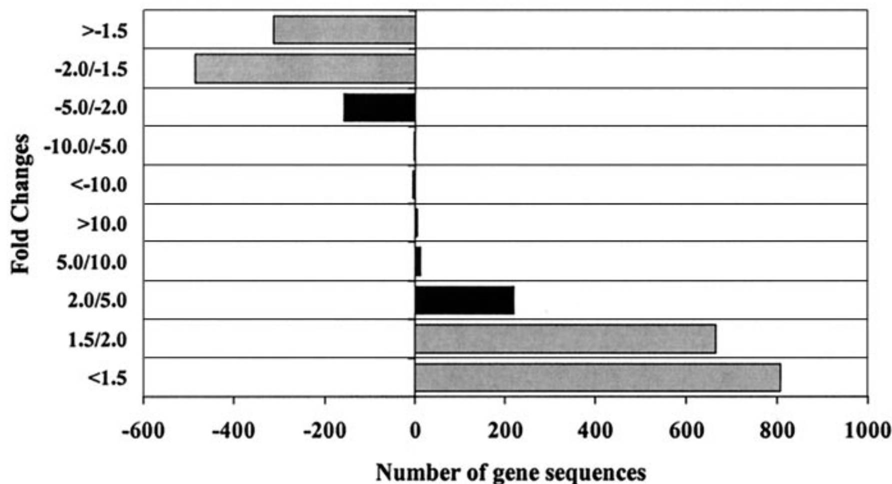

FIG. 1. Stepwise selection analysis of placental gene profiling. The first three consecutive steps of the analysis identified a total of 2,682 genes that were eligible for further comparisons. The fourth filtering step of our analytical strategy allowed us to select 435 genes significantly regulated (black bars) and to reject 2,247 genes (gray bars). Bars to the right correspond to upregulated genes, and bars to the left represent the downregulated genes. The number of gene sequences analyzed is expressed as a function of their relative fold changes.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024

Data analysis. To correct for hybridization efficiency, results were scaled to an average signal intensity of 1500. A serial four-step analysis was completed to select the significantly modified transcripts while minimizing false-positive genes. The first step excluded all probe sets with signal below the probe pair threshold (nc). Average background (67.8 ± 0.80) and scaled noise (3.7 ± 0.3) were calculated for each array and entered in the subsequent analysis. *1*) Genes showing an absolute call of present (P-calls) according to MAS 5 algorithm. The average percentage of P-calls was 39.1 ± 2.8. *2*) Genes showing a difference call of increased "I" or decreased "D." *3*) Genes with a difference in signal detection of at least 4.5 times the average background minus the scaled noise. The genes having satisfied these criteria were included in the fourth level of selection based on a fold change ≥2 or ≤−2 and consistent in at least two comparisons (Fig. 1). Functional clustering of the up- and downregulated genes was based on public databases according to the biological functions of their putative encoded proteins.

Quantitative real-time PCR. RT-PCR analysis was performed using a fluorescence temperature cycler (Lightcycler; Roche Molecular Diagnosis, Indianapolis, IN). Specific primers were designed within the 3′ coding region of the genes (sequences available upon request). Real-time reactions were carried out in duplicate, and amplicons were analyzed by generating melting curves with continuous measurement of fluorescence. The PCR products were separated on 1.5% agarose gel. Results were calculated as relative differences in target Ct values normalized to β-actin.

Statistical analysis. All data are presented as mean ± SE. Significance for statistical differences was calculated using a unpaired Student's *t* test.

RESULTS

A total of 16 women with either normal glucose tolerance (control subjects) or GDM, who were recruited for the study, were matched for BMI and gestational age (Table 1). Insulin resistance was documented by a higher 1-h postchallenge blood and lower predicted insulin sensitivity compared with control subjects. This was observed despite satisfactory glycemic control with normal HbA_{1c} (5.4 vs. 5.1%) and normal fasting blood glucose (90.7 ± 11 vs. 86.5 ± 4.5 mg/dl). Higher plasma insulin levels in the GDM women reflect chronic exogenous insulin therapy. Plasma leptin and TNF-α levels were higher in women with GDM. Birth weights were similar in the two groups; however, placental weights were increased with GDM (585 ± 64 vs. 434 ± 20 g, $P < 0.001$), and neonates of GDM mothers were fatter based on percent fat mass (15.1 ± 1.2 vs. 12.6 ± 1.3, $P < 0.001$) and ponderal index (2.9 ± 0.2 vs. 2.7 ± 0.1 g/cm^3, $P < 0.001$). Plasma insulin levels were higher in neonates of GDM mothers (33.3 ± 9.6 vs. 12.5 ± 1.6 μU/ml, $P < 0.01$).

To characterize gene targets that determine altered placental functions, the global pattern of gene expression was analyzed in placenta from matched control subjects and GDM pregnancies. Of 22,823 gene sequences surveyed, 8,627 ± 172 were present in control subjects and 9,378 ± 165 in GDM, representing placental transcriptome. The consecutive screening filters that we applied allowed us to narrow these numbers to 2,682 genes eligible for analysis. The average fold change value was then calculated for all pairwise comparisons, resulting in the final selection of 435 genes significantly modified in GDM.

The genes included in the final selection represented 2.0% of the total gene sequences analyzed and 5.6% of the placental transcriptome. The 435 genes identified as significantly modified refer to genes whose transcriptional pattern was altered in GDM with 254 upregulated and 181 downregulated genes. Functional clustering of the 435 genes followed by quantitative ranking identified six major subgroups of genes (Fig. 2). They included genes associated with *1*) stress-activated and inflammatory responses (n = 79), *2*) endothelial structure and differentiation (n = 41), *3*) substrate metabolism (n = 39), *4*) transport and trafficking (n = 34), *5*) translation (n = 33), and *6*) signal transduction (n = 23). Genes related to inflammatory responses represented the largest cluster, accounting for up to 18% of the modified genes. Additional single clusters, each accounting for <5% of the modified genes, were those associated with transcription and early genes (n =

TABLE 1
Clinical and metabolic characteristics of study subjects

Subjects	n	Gestational age (weeks)	BMI (kg/m^2)	HbA_{1c} (%)	Fasting glucose (mg/dl)	Insulin (μU/ml)	1-h glucose (mg/dl)	IS (mg · min^{-1} · kg^{-1})	Leptin (ng/ml)	TNF-α (pg/ml)
Control	8	38.9 ± 0.4	34.0 ± 3.3	5.3 ± 0.1	84.0 ± 2.9	36.9 ± 5.8	100 ± 10.1	6.0 ± 0.2	28.3 ± 5.5	1.5 ± 0.4
GDM	7	38.5 ± 0.5	39.7 ± 2.6	5.7 ± 0.3	94.7 ± 8.2	58.7 ± 15.5*	173.2 ± 12.4†	4.3 ± 0.7†	45.0 ± 3.5*	2.3 ± 0.2*

Data are means ± SE. *$P < 0.01$; †$P < 0.001$. IS, glucose disposal rate in mg · min^{-1} · kg fat-free mass^{-1}

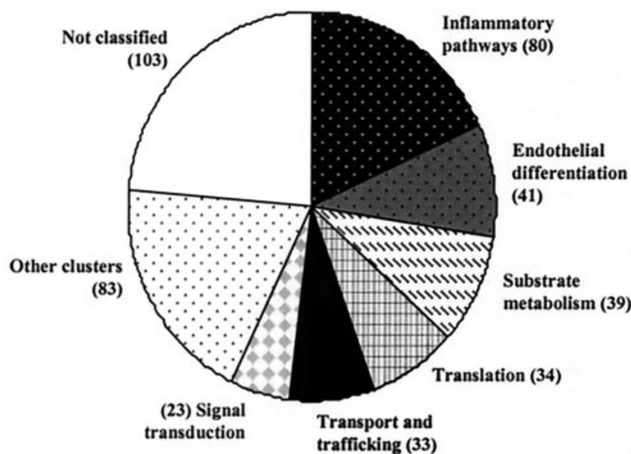

FIG. 2. Functional clustering of the regulated genes. Quantitative ranking of the six main functional categories of the 435 upregulated and downregulated genes revealed genes for inflammatory responses to represent the largest single cluster (18.5% of all the genes significantly modified in response to GDM). Genes assigned to clusters that accounted for <5% were included within "other clusters." There were 103 miscellaneous genes not assigned to any of the functional clusters.

17), immunity and cell surface antigens (n = 19), hormones (n = 15), growth factors (n = 13), cell growth and maintenance (n = 9), and mitochondrial (n = 7). They represented 19% of the modified genes (Fig. 2, other clusters). The 103 genes with unknown functions were listed under nonclassified genes. The list of the two main clusters is given in Table 2, and the complete listing of the 435 significantly modified genes is available at http://www.ncbi.nlm.nih.gov/geo (accession no. GPL130). Modifications observed with microarrays were validated by real-time PCR analysis of the same RNA samples (Fig. 3). We analyzed mRNA expression for 22 genes of interest and were able to confirm 21 modifications with either increased or decreased expression (Fig. 3).

DISCUSSION

Expression profiling of the placenta revealed that genes regulating inflammatory responses and endothelial reorganization represent the two main functional clusters altered in GDM. With a total of 110 genes, they account for one-third of the modified genes, reflecting a state of chronic inflammation with signs of major vascular dysfunction. This is further supported by the findings that most of the genes were upregulated (75 of 110), as compared with a balanced ratio observed in the other gene categories.

The diabetic placentas analyzed in this study were obtained from women with marked glucose intolerance and insulin resistance (Table 1). Growing evidence in the literature suggests that insulin resistance is the result of an inflammatory milieu. Plasma levels of several markers of inflammation, C-reactive protein, lipopolysaccharide (LPS), interleukin (IL)-6, TNF-α, and leptin are elevated in individuals with obesity and type 2 diabetes (11–14). TNF-α has been recognized as the most prominent factor contributing to insulin resistance in obesity and diabetes (15–19). The changes in placental gene expression that we report herein further support our hypothesis that a panel of proinflammatory cytokines and cellular mediators act in concert with TNF-α to either relay or potentiate its action.

IL-1 and TNF-α induce synergistic pleiotropic responses that profoundly affect production of extracellular matrix (ECM) proteins. Increased expression of fibronectin, laminin β-1, and metalloproteinases may induce a fibrotic response and disrupt the structural integrity of placental endothelial cells as in other cell types (20–22). Increase in metalloproteinases may also influence angiogenesis by degrading matrix molecules, loosening the cellular network, and releasing growth factors sequestered in the ECM (23). Several other modified genes are directly modulated by IL-1 and TNF-α. For example, TNF-induced CG12 accompanies inflammatory reactions in atherosclerotic regions (24), and acute-phase reactants, such as LPS, pentaxin-related gene (PTX-3), calgranulin, and thrombospondin, are key components of impaired vascular function (25–28). The increased expression of IL-8 receptor, IL-1 receptor, and the short form of leptin receptor points to an activation of the signaling pathways recruited by these cytokines. The activation of leptin gene expression in diabetic placenta is in keeping with its transcrip-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024

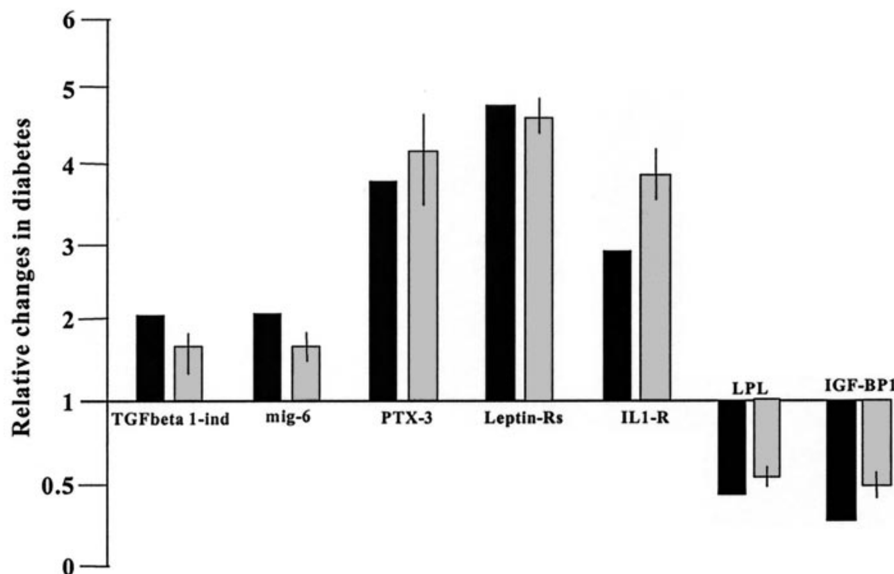

FIG. 3. Real-time PCR analysis of the modified genes. The PCR results expressed are given as the means ± SE of three to four comparisons in GDM versus control subjects (gray bars). Black bars represent the average fold change obtained with microarray analysis. All values are presented as relative fold changes with upregulation >1 and downregulation <1. TGF-beta 1-ind, TGF-β1–induced protein; mig-6, gene 33; PTX-3, pentaxin-related protein; Leptin-Rs, leptin receptor short isoform; IL1-R, IL-1 receptor; LPL, lipoprotein lipase; IGF-BP1, IGF binding protein 1.

TABLE 2
Genes significantly modified in GDM

Accession no.	Gene name	Fold change	Description
Inflammatory pathways			
NM 005345	HSPA1A	2.6	Heat shock 70kDa protein 1A
NM 006948	STCH	2.3	Stress 70 protein chaperone, microsome-associated 60-kDa
NM 006260	DNAJC3	−2	DnaJ (Hsp40) homolog, subfamily C, member 3
NM 006644	HSP105B	2	Heat shock 105kDa
NM 005494	LOC136442	2.1	Heat shock protein J2
NM 012328	DNAJB9	2	DnaJ (Hsp40) homolog, subfamily B, member 9
NM 001423	EMP1	2.3	Epithelial membrane protein 1
NM 005101	ISG15	−2.2	Interferon-stimulated protein, 15 kDa
NM 002053	GBP1	3.5	Guanylate binding protein 1, interferon-inducible, 67kDa
NM 000619	IFNG	−2	Interferon, gamma
NM 006084	ISGF3G	−2.1	Interferon-stimulated transcription factor 3, gamma 48kDa
NM 006332	IFI30	−3	Interferon, gamma-inducible protein 30
NM 001144	AMFR	4.3	Autocrine motility factor receptor
NM 004084	DEFA1	2.9	Defensin, alpha 1, myeloid-related sequence
NM 003246	THBS1	3.3	Thrombospendin 1
NM 004342	CALD1	2.3	Caldesmon 1
NM 006350	FST	−2.6	Follistatin
NM 004079	CTSS	2.7	Cathepsin S
NM 005860	FSTL3	−2	Follistatin-like 3 (secreted glycoprotein)
NM 014795	ZFHX1B	2.3	Zinc finger homeobox 1b
NM 000466	PEX1	2	Peroxisome biogenesis factor 1
NM 015927	TGFB1I1	2	Transforming growth factor beta 1 (TGF beta) induced transcript 1
NM 021073	BMP5	2	Bone morphogenetic protein
NM 003743	NCOA1	6.1	Nuclear receptor coactivator 1
NM 001901	CTGF	2.1	Connective tissue growth factor
NM 005264	GFRA1	−3.7	GDNF family receptor alpha 1
NM 004843	WSX1	2.1	Class I cytokine receptor
NM 002303	LEPR	4.3	Leptin receptor, short isoform
NM 000230	LEP	2.3	Leptin (obesity homolog, mouse)
NM 001243	TNFRSF8	−2	Tumor necrosis factor receptor superfamily, member 8
NM 016442	ARTS-1	18	Type 1 tumor necrosis factor receptor shedding aminopeptidase regulator
NM 030817	DKFZP434F0318	3.2	TNF alpha induced protein, similar to CG-12
NM 005903	MADH5	−2.3	MAD, mothers against decapentaplegic homolog 5
NM 003743	NCOA1	6.1	Nuclear receptor coactivator 1
NM 003489	NRIP1	2.2	Nuclear receptor interacting protein 1
U 59863	TANK	2.3	TRAF family member-associated NFKB activator
NM 018678	LSR68	3.3	Lipopolysaccharide specific response-68 protein
NM 004887	CXCL 14	2.1	Chemokine (C-X-C motif) ligand 14
NM 003856	IL1RL1	3	Interleukin 1 receptor-like 1
NM 001557	IL8RB	5.3	Interleukin 8 receptor, beta
NM 002852	PTX3	5	Pentaxin-related gene, rapidly induced by IL-1 beta
NM 012294	GFR	2.1	Guanine nucleotide exchange factor for Rap1
NM 021955	GNGT1	2	Guanine nucleotide binding protein (G protein)
NM 021183	LOC57826	2.1	Protein similar to small G proteins, especially RAP-2A
NM 012121	CDC42EP4	2.3	CDC42 effector protein (Rho GTPase binding) 4
U 28936	YWHAE	2.5	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide
AL 034417	MIG-6	2	Gene 33/Mig-6
NM 004844	SH3BP5	2.3	SH3-domain binding protein 5 (BTK-associated)
NM 32569	N-PAC	−2.6	Cytokine-like nuclear factor n-pac
NM 030751	TCF8	4	Transcription factor 8 (represses interleukin 2 expression)
NM 001546	ID4	2.3	Inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
NM 004379	CREB1	2.1	cAMP responsive element binding protein 1
NM 014335	CRI1	3	CREBBP/EP300 inhibitory protein 1
NM 002943	RORA	−2.1	RAR-related orphan receptor A
NM 002051	GATA3	2	GATA binding protein 3
NM 000929	PLA2G5	2.6	Phospholipase A2, group V
NM 002415	MIF	−2.1	Macrophage migration inhibitory factor
NM 006039	ENDO180	2.1	Endocytic receptor (macrophage mannose receptor family)
NM 002510	GPNMB	2.4	Glycoprotein (transmembrane)
NM 000362	TIMP3	−2.6	Tissue inhibitor of metalloproteinase 3 (pseudoinflammatory)

Continued

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024

TABLE 2
Continued

NM 002291	LAMB1	2.4	Laminin, beta 1
NM 002727	PRG1	2.2	Proteoglycan 1, secretory granule
NM 000088	COL1A1	2.3	Collagen, type I, alpha 1
NM 004995	MMP14	−2.9	Matrix metalloproteinase 14
NM 002421	MMP1	3.7	Matrix metalloproteinase 1
NM 002426	MMP12	2.2	Matrix metalloproteinase 12
NM 002213	ITGB5	4.8	Integrin, beta 5
NM 004763	ICAP-1A	2.3	Integrin cytoplasmic domain-associated protein 1
NM 000212	ITGB3	−2.5	Integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)
NM 004950	DSPG3	−5.1	Dermatan sulfate proteoglycan 3
NM 001797	CDH11	−2.1	Cadherin 11, type 2, OB-cadherin
NM 014265	ADAM28	−15	A disintegrin and metalloproteinase domain 28
AL 576253	zizimin1	2	Zizimin 1
NM 002026	FN1	−2.5	Fibronectin 1
AI 189753	TM4SF1	2	Transmembrane 4 superfamily member 1
NM 006691	XLKD1	2.1	Extracellular link domain containing 1
NM 012338	NET-2	2.6	Transmembrane 4 superfamily member tetraspan NET-2
NM 006665	HPSE	−2.1	Heparanase
NM 000091	COL4A3	−4	Collagen, type IV, alpha 3
Endothelial differentiation, structural, and contractile proteins			
NM 004986	KTN1	2.1	Kinectin 1 (kinesin receptor)
NM 003373	VCL	2	Vinculin
NM 004343	CALR	2.3	Calreticulin
NM 006136	CAPZA2	2.5	Capping protein (actin filament) muscle Z-line
NM 001839	CNN3	2.4	Calponin 3, acidic
NM 016824	ADD3	2.3	Adducin 3 (gamma)
BF 940043	NID1	2.2	Nidogen (entactin)
NM 001615	ACTG2	2.1	Actin, gamma 2, smooth muscle, enteric
NM 001613	ACTA2	2.4	Actin, alpha 2, smooth muscle, aorta
NM 003072	SMARCA4	2.1	Matrix associated, actin dependent regulator of chromatin
NM 001613	ACTA2	2.4	Actin, alpha 2, smooth muscle, aorta
AI 382123	MYH10	−2	Human nonmuscle myosin heavy chain-B (MYH10)
NM 002465	MYBPC1	−2.6	Myosin binding protein C, slow type
NM 006097	MYL9	2.4	Myosin, light polypeptide 9, regulatory
NM 004093	EFNB2	−2.5	Ephrin-B2
NM 002964	S100A8	10.2	S100 calcium binding protein A8 (calgranulin A)
NM 002961	S100A4	−2	S100 calcium binding protein A4
NM 007269	STXBP3	2.1	Syntaxin binding protein 3
NM 006322	TUBGCP3	4.3	Tubulin, gamma complex associated protein 3
NM 032261	DKFZp434N0650	−2	Tubulin beta5
NM 000227	LAMA3	−2.2	Laminin, alpha 3
NM 000361	THBD	2	Thrombomodulin
NM 001888	CRYM	−4.3	Crystallin, mu
NM 001884	CRTL1	2.8	Cartilage linking protein 1
NM 003186	TAGLN	2.1	Transgelin
NM 002019	FLT1	2	Fms-related tyrosine kinase 1 (VEGF receptor)
NM 003376	VEGF	2.5	Vascular endothelial growth factor
NM 001147	ANGPT2	2.5	Angiopoietin 2
NM 007351	MMRN	2.6	Multimerin
NM 002658	PLAU	−2.1	Plasminogen activator
NM 000128	F11	−2.6	Coagulation factor XI
NM 000300	PLA2G2A	2.4	Phospholipase A2, group IIA
NM 001996	FBLN1	2	Fibulin 1
NM 001102	ACTN1	2.2	Actinin, alpha 1
NM 032261	DKFZp434N0650	−2	Tubulin beta5
NM 005554	KRT6A	−7.8	Keratin 6A
NM 005555	KRT6B	−2.5	Keratin 6B
NM 002397	MEF2C	4	MADS box transcription enhancer factor 2
NM 001730	KLF5	2.5	Kruppel-like factor 5
NM 005139	ANXA3	2.1	Annexin A3
NM 000070	CAPN3	−2.6	Calpain 3, (p94)
NM 003564	TAGLN2	−2.6	Transgelin 2
M 28882	MCAM	2.1	Melanoma cell adhesion molecule
NM 001999	FBN2	2.3	Fibrillin 2

List of the two main functional clusters of up- and downregulated genes in the diabetic placentas. Data indicate the Genbank accession number, the name, the fold change compared with the control subjects (see RESEARCH DESIGN AND METHODS), and the description of each gene or its protein product.

tional regulation (29). The role of placental leptin has raised several hypotheses regarding its autocrine action within the placenta without reaching definite conclusions (30). The increase in short leptin receptor supports the view that this isoform transduces proinflammatory responses in the placenta.

Besides the metabolic control of adipose tissue homeostasis, leptin elicits inflammatory, immunological, and

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024

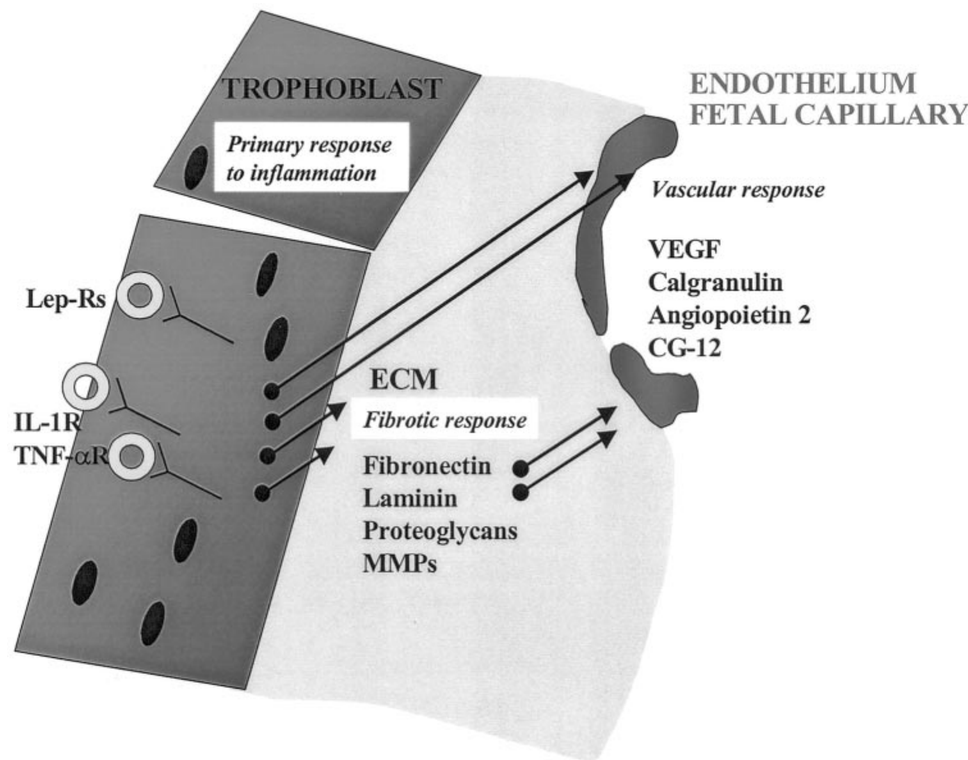

FIG. 4. Placental responses to the diabetic insult. The primary response to diabetes is the recruitment of TNF-α, IL-1, and leptin receptors present on the syncytiotrophoblast facing the maternal blood. Signal transduction through these receptors elicits a cascade of intracellular events leading to excess extracellular matrix components characteristic of a fibrotic response and a vascular reaction. The modifications of ECM gene expression also contribute to enhanced angiogenic activity and endothelial differentiation. The sum of these modifications points to a severe disorganization of placental structure. Lep-Rs, leptin receptor short isoform; IL-1R, IL-1 receptor.

vascular responses (31,32). Administration of IL-6, LPS, and TNF-α to mice increases leptin production, suggesting that they induce concurrent effects in addition to the development of inflammation (33). The coordinated action of ILs, TNF-α, and leptin on placental genes may explain part of a fibrotic response via disruption of extracellular matrix components and vascular architecture (Fig. 4). These transcriptional changes are in line with the modifications of placental morphology and composition documented in diabetes with increased parenchymal tissue cellularity, alterations of surface expression of junctional proteins, and enhanced feto-placental angiogenesis (34–36). The downstream effectors of TNF-α, ILs, and leptin activate p38 MAPK and JNK, which belong to the family of SAPK stress kinases abnormally activated in obesity and insulin resistance (37,38). MAPK and JNK/SAPK pathways cooperate to phosphorylate ras GTPases of the rho family, such as mig-6, a molecular adaptor triggering cellular hypertrophy in diabetic nephropathy (39,40). The upregulation of placental mig-6 expression, makes this gene a particularly attractive candidate as a mediator of placental overgrowth of diabetes.

The array of genes activated by TNF-α, leptin, and ILs also include several transcription factors, GATA, CREB-binding protein, CEBP-α, and AP-1, all of which are involved in the regulation of inflammatory processes (41). Upon activation, they are able to turn on genes involved in signal transduction related to stress and chronic inflammation (Fig. 5). Modifications of the expression of genes assigned to other functional clusters, signal transduction, growth factors, and cytoskeleton reorganization are also likely to contribute to global placental dysfunction. This may be particularly relevant for genes encoding mediators of the insulin signaling cascade, such as the insulin receptor itself, p 110 phosphatidylinositol-kinase, several small G proteins of the ras, rab, and rho families possibly recruited as a result of high insulin levels in maternal and fetal blood.

The best representation of subclasses of genes involved in inflammatory pathways represents the first characterization of an inflammatory response induced through a chronic diabetic insult. No such responses were reported in skeletal muscle of type 2 diabetic patients (42,43), and changes in diabetic mice were related to substrate and energy metabolism (44). These differences could result from cell type specificities or from a greater insulin resistance, as demonstrated by the need for insulin therapy to achieve adequate glucose control in GDM patients. Glucose intolerance and hyperinsulinemia are significant before conception in women who go on to develop GDM (45). A causal role of hyperglycemia has been suggested in the immune activation of diabetes (46). Therefore, the early onset of insulin resistance may initiate an endocrine feedback between mother and placenta with enhanced susceptibility to cytokine that culminate in the inflammation milieu that we describe.

This study provides the first molecular basis linking GDM to modifications of placental transcriptome. It suggests a postreceptor convergence of multiple signal transduction pathways mediated through TNF-α, IL-1, and leptin. Therefore, the fetus of diabetic mothers develops in an inflammatory milieu. We speculate that changes in

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024

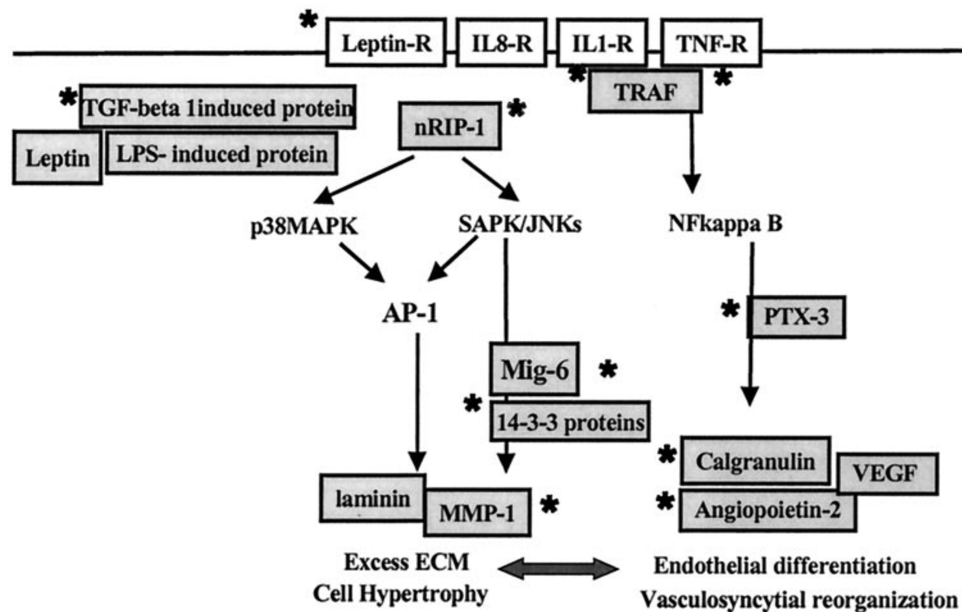

FIG. 5. Model for placental inflammatory pathways recruited in GDM. This scheme includes some of the placental genes participating in the diabetic response as an example of cooperation between proinflammatory cytokines. The array of genes induced in relation to inflammation suggests that concurrent signaling pathways are recruited through TNF-α, IL, and leptin stimulation. Stress kinases (SAPK/JNK) and NF-κB are key mediators of cross-talks linking inflammation to diabetes and insulin resistance. We propose that this cascade of events leads to cell hypertrophy and vasculosyncytial dysfunction. Details are given in the DISCUSSION section. Genes whose expression is modified in microarray analysis are boxed, and the asterisk represents genes for which changes in expression have been further validated by real-time PCR.

expression of specific placental genes may be a leading cause to adverse fetal programming.

ACKNOWLEDGMENTS

T.R. is a recipient of a fetal diagnosis fellowship, University of Milan, Italy. This study is supported by grants from the National Institutes of Health (RO-1 HD22965 to P.C.) and Alfediam Roche Pharma (to S.H.-D.M).

We thank Patrick Leahy and the staff of the Gene Array facility at Case Western Reserve University for advice and excellent technical support.

REFERENCES

1. Catalano PM, Tyzbi ED, Wolfe RR, Roman NM, Amini SB, Sims EA: Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. *Am J Obstet Gynecol* 167:913–919, 1992
2. Friedman JE, Ishizuka T, Shao J, Huston L, Highman T, Catalano P: Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes. *Diabetes* 48:1807–1814, 1999
3. Catalano P, Huston L, Amini S, Kalhan S: Longitudinal changes in glucose metabolism iduring pregnancy in obese women with normal glucose tollerance and GDM. *Am J Obstet Gynecol* 180:903–916, 1999
4. Silverman BL, Rizzo TA, Cho NH, Metzger BE: Long-term effects of the intrauterine environment. *Diabetes Care* 21:142–149, 1998
5. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, Roumain J, Bennett PH, Knowler WC: Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes* 49:2208–2211, 2000
6. Kirwan JP, Hauguel-de Mouzon S, Lepercq J, Challier JC, Huston-Presley L, Friedman JE, Kalhan SC, Catalano PM: TNF-α is a primary mediator of insulin resistance in human pregnancy. *Diabetes* 51:2207–2213, 2002
7. Lepercq J, Caüzac M, Lahlou N, Timsit J, Girard J, Auwerx J, Hauguel-de Mouzon S: Overexpression of placental leptin in diabetic pregnancies: a critical role for insulin. *Diabetes* 47:847–850, 1998
8. Desoye G, Shafrir E: Placental metabolism and its regulation in health and diabetes. *Mol Aspects Med* 15:502–682, 1994
9. Carpenter M, D Coustan: Criteria for screening tests for gestational diabetes. *Am J Obstet Gynecol* 144:768–773, 1982
10. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 18:5294–5299, 1979
11. Ramsay JE, Ferrell WR, Crawford L, Wallace AM, Greer IA, Sattar N: Maternal obesity is associated with dysregulation of metabolic, vascular, and inflammatory pathways. *J Endocrinol Metab* 87:4231–4237, 2002
12. Temelkova-Kurktschiev T, Siegert G, Bergmann S, Henkel E, Koehler C, Jaross W, Hanefeld M: Subclinical inflammation is strongly related to insulin resistance but not to impaired insulin secretion in a high risk population for diabetes. *Metabolism* 51:743–749, 2002
13. Katsuki A, Sumida Y, Murashima S, Murata K, Takarada Y, Ito K, Fujii M, Tsuchihashi K, Goto H, Nakatani K, Yano Y: Serum levels of tumor necrosis factor-alpha are increased in obese patients with noninsulin-dependent diabetes mellitus. *J Endocrinol Metab* 83:859–862, 1998
14. Mantzoros CS, Moschos S, Avramopoulos I, Kaklamani V, Liolios A, Doulgerakis D, Griveas I, Katsilambros N, Flier JS: Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. *J Endocrinol Metab* 82:3408–3413, 1997
15. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 259:87–91, 1993
16. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 95:2409–2415, 1995
17. Miles PD, Romeo OM, Higo K, Cohen A, Rafaat K, Olefsky J: TNF-α induced insulin resistance in vivo and its prevention by troglitazone. *Diabetes* 46:1678–1683, 1997
18. Uysal K, Wiesbrock SM, Marino MW, Hotamisligil GS: Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 389:610–614, 1997
19. Uysal K, Wiesbrock SM, Hotamisligil GS: Functional analysis of tumor necrosis factor (TNF) receptors in TNF-alpha-mediated insulin resistance in genetic obesity. *Endocrinology* 139:4832–4838, 1998
20. Ruan H, Miles PD, Ladd CM, Ross K, Golub TR, Olefsky JM, Lodish HF: Profiling gene transcription in vivo reveals adipose tissue as an immediate target of tumor necrosis factor-α: implications for insulin resistance. *Diabetes* 51:3176–3188, 2002
21. Richardson CA, Gordon KL, Couser WG, Bomsztyk K: IL-1 beta increases laminin B2 chain mRNA levels and activates NF-kappa B in rat glomerular epithelial cells. *Am J Physiol* 268 (Suppl. 2):F273–F278, 1995
22. Esteve PO, Chicoine E, Robledo O, Aoudjit F, Descoteaux A, Potworowski EF, St-Pierre Y: Protein kinase C-zeta regulates transcription of the matrix

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024

metalloproteinase-9 gene induced by IL-1 and TNF-alpha in glioma cells via NF-kappa B. *J Biol Chem.* 277:35150–35155, 2002.
23. Coussens LM, Shapiro SD, Soloway PD, Werb Z: Models for gain-of-function and loss-of-function of MMPs: transgenic and gene targeted mice. *Methods Mol Biol* 151:149–179, 2001
24. Xu K, Geczy CL: IFN-gamma and TNF regulate macrophage expression of the chemotactic S100 protein S100A8. *J Immunol* 164:4916–4923, 2000
25. Bannerman D, Tupper J, Kelly J, Winn R, Harlan J: The Fas-associated death domain protein suppresses activation of NF-kappa B by LPS and IL-1 beta. *J Clin Invest* 109:419–429, 2002
26. Basile A, Sica A, d'Aniello E, Breviario F, Garrido G, Castellano M, Mantovani A, Introna M: Characterization of the promoter for the human long pentraxin PTX3: role of NF-kappaB in tumor necrosis factor-alpha and interleukin-1beta regulation. *J Biol Chem* 272:8172–8178, 1997
27. Morandi V, Cherradi SE, Lambert S, Fauvel-Lefeve F, Legrand YJ, Legrand C: Proinflammatory cytokines (interleukin-1 beta and tumor necrosis factor-alpha) down regulate synthesis and secretion of thrombospondin by human endothelial cells. *J Cell Physiol* 160:367–377, 1994
28. Altmeyer A, Klampfer L, Goodman AR, Vilcek J: Promoter structure and transcriptional activation of the murine TSG-14 gene encoding a tumor necrosis factor/interleukin-1-inducible pentraxin protein. *J Biol Chem* 270:25584–25590, 1995
29. Grosfeld A, André J, Hauguel-de Mouzon S, Berra E, Pouyssegur J, Guerre-Millo M: Hypoxia-inducible factor 1 (HIF-1) transactivates the human leptin gene promoter. *J Biol Chem* 277:42953–42957, 2002
30. Lepercq J, Challier JC, Guerre-Millo M, Vidal H, Hauguel-de Mouzon S: Prenatal leptin production: evidence that fetal adipose tissue produces leptin. *J Clin Endocrinol Metab* 86:2409–2413, 2001
31. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Rivero JR: Biological action of leptin as an angiogenic factor. *Science* 11:1683–1686, 1998
32. Cohen B, Barkan D, Levy Y, Goldberg I, Fridman E, Kopolovic J, Rubinstein M: Leptin induces angiopoietin-2 expression in adipose tissues. *J Biol Chem* 76:7697–7700, 2001
33. Faggioni R, Fantuzzi G, Fuller J, Dinarello CA, Feingold KR, Grunfeld C: IL-1 beta mediates leptin induction during inflammation. *Am J Physiol* 274 (Suppl. 1):R204–R208, 1998
34. Winick M, Noble A: cellular growth in human placenta II diabetes mellitus. *J Pediatr* 71:216–219, 1967
35. Babawale MO, Lovat S, Mayhew TM, Lammiman MJ, James DK, Leach L: Effects of gestational diabetes on junctional adhesion molecules in human term placental vasculature. *Diabetologia* 43:1185–1196, 2000
36. Mayhew TM: Enhanced fetoplacental angiogenesis in pre-gestational diabetes mellitus: the extra growth is exclusively longitudinal and not accompanied by microvascular remodelling. *Diabetologia* 45:1434–1439, 2002
37. Yuasa T, Ohno S, Kehrl JH, Kyriakis JM: Tumor necrosis factor signaling to stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) and p38: germinal center kinase couples TRAF2 to mitogen-activated protein kinase/ERK kinase kinase 1 and SAPK while receptor interacting protein associates with a mitogen-activated protein kinase kinase kinase upstream of MKK6 and p38. *J Biol Chem* 273:22681–22692, 1998
38. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil G: A central role for JNK in obesity and insulin resistance. *Nature* 420:333–336, 2002
39. Choukroun G, Hajjar R, Kyriakis JM, Bonventre JV, Rosenzweig A, Force T: Role of the stress-activated protein kinases in endothelin-induced cardiomyocyte hypertrophy. *J Clin Invest* 102:1311–1320, 1998
40. Makkinje A, Quinn DA, Chen A, Cadilla CL, Force T, Bonventre JV, Kyriakis JM: Gene 33/Mig-6, a transcriptionally inducible adapter protein that binds GTP-Cdc42 and activates SAPK/JNK: a potential marker transcript for chronic pathologic conditions, such as diabetic nephropathy: possible role in the response to persistent stress. *J Biol Chem* 275:17838–17847, 2000
41. Vanden Berghe W, De Bosscher K, Boone E, Plaisance S, Haegeman G: The nuclear factor-kappaB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter. *J Biol Chem* 274:32091–32098, 1999
42. Sreekumar R, Halvatsiotis P, Schimke JC, Nair KS: Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment. *Diabetes* 51:1913–1920, 2002
43. Yang X, Pratley S, Tokraks S, Bogardus C, Permana PA: Microarray profiling of skeletal muscle tissues from equally obese, non diabetic insulin-sensitive and insulin-resistant Pima indians. *Diabetologia* 45:1584–1593, 2002
44. Yechoor V, Patti ME, Saccone R, Kahn CR: Coordinated patterns of gene expression for substrate and energy metabolism in skeletal muscle of diabetic mice. *Proc Natl Acad Sci U S A* 99:10587–10592, 2002
45. Catalano PM, Huston L, Amini SB, Kalhan SC: Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes. *Am J Obstet Gynecol* 180:903–916, 1999
46. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Guigliano D: Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 106:2067–2072, 2002

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/52/12/2951/372042/db1203002951.pdf by guest on 14 July 2024