SUPPLEMENTARY DATA

Supplementary Table 1. Proteomic analysis of normal and GDM HUVEC.

	Protein identity	SWISS PROT Accession No.	Calculated MM Da ($\times 10^3$)	No. of unique peptides	Coverage (%)	Ratio	P
	Stress response						
2	Heat shock cognate 71 kDa protein	HSP7C_HUMAN	70.88	9	14.70	-1.62	0.0061
4	Heat shock protein 75 kDa	TRAP1_HUMAN	80.09	17	26.60	-1.37	0.0029
10	Serpin H1	SERPH_HUMAN	46.42	5	11.20	1.14	0.02
34	Protein S100-A13	S10AD_HUMAN	11.45	3	27.60	1.32	0.016
39	Peptidyl-prolyl cis-trans isomerase FKBP1A	FKB1A_HUMAN	11.93	3	28.70	1.68	0.046
	Redox regulation						
8	Protein disulfide-isomerase A3	PDIA3_HUMAN	56.77	23	51.10	1.12	0.026
30	Glutathione S-transferase kappa 1	GSTK1_HUMAN	25.48	9	47.30	-1.11	0.03
31	Peroxiredoxin-1	PRDX1_HUMAN	22.09	5	27.60	-1.17	0.041
36	Peroxiredoxin-5	PRDX5_HUMAN	22.01	5	21.0	1.15	0.0084
	Apoptosis						
3	Stress-70 protein	GRP75_HUMAN	73.66	7	12.80	-1.62	0.0061
33	Galectin-1	LEG1_HUMAN	14.70	8	67.40	1.29	0.018
29	Voltage-dependent anion-selective channel protein 1	VDAC1_HUMAN	30.76	8	30.70	1.72	0.041
	Cell cycle						
18	Septin-2	SEPT2_HUMAN	41.47	9	24.90	1.11	0.019
38	Histone H4	H4_HUMAN	11.35	5	31.10	1.68	0.046
27	DNA replication GINS protein PSF3	PSF3_HUMAN	24.52	4	24.10	-1.17	0.015

SUPPLEMENTARY DATA

	Protein identity	SWISS PROT Accession No.	Calculated MM Da ($\times 10^3$)	No. of unique peptides	Coverage (%)	Ratio	P
	Glycolysis						
9	Pyruvate kinase isozymes M1/M2	KPYM_HUMAN	57.92	14	30.10	1.35	0.013
28	Malate dehydrogenase	MDHM_HUMAN	35.49	3	11.20	-1.25	0.039
	Mitochondrial ATP generation						
7	ATP synthase subunit beta	ATPB_HUMAN	56.54	6	14.40	-1.17	0.02
11	ATP synthase subunit alpha	ATPA_HUMAN	59.73	6	13.60	1.17	0.0064
	Mitochondrial function						
26	Aspartate aminotransferase	AATM_HUMAN	47.46	12	27.70	1.33	0.00012
25	GrpE protein homolog 1, mitochondrial	GRPE1_HUMAN	24.26	3	14.30	1.16	0.019
	Transcription and translation regulation						
32	Chromobox protein homolog 3	CBX3_HUMAN	20.79	6	30.10	-1.17	0.047
24	Peptidyl-prolyl cis-trans isomerase E	PPIE_HUMAN	33.41	6	15.60	-1.12	0.05
23	Heterogeneous nuclear ribonucleoprotein A1-like protein 2	RA1L2_HUMAN	34.21	5	16.20	-1.15	0.029
22	Protein quaking	QKI_HUMAN	37.65	5	15.80	1.18	0.005
13	Elongation factor 1-delta	EF1D_HUMAN	31.10	7	34.90	-1.17	0.014
15	Eukaryotic translation initiation factor 3 subunit I	EIF3I_HUMAN	36.48	8	27.10	-1.11	0.0017
	Translation machinery						
12	40S ribosomal protein SA	RSSA_HUMAN	32.84	10	36.30	-1.14	0.016

SUPPLEMENTARY DATA

16	60S acidic ribosomal protein P0	RLA0_HUMAN	34.26	7	31.20	-1.24	0.024
	Protein identity	**SWISS PROT Accession No.**	**Calculated MM Da (×10^3)**	**No. of unique peptides**	**Coverage (%)**	**Ratio**	**P**
35	40S ribosomal protein S12	RS12_HUMAN	14.50	4	27.30	-1.1	0.036
37	60S ribosomal protein L23	RL23_HUMAN	14.85	3	22.10	-1.27	0.033
	Protein degradation						
19	3-hydroxyisobutyryl-CoA hydrolase	HIBCH_HUMAN	43.47	2	5.96	-1.27	0.032
20	26S proteasome non-ATPase regulatory subunit 7	PSD7_HUMAN	37.01	4	15.10	-1.27	0.032
	Cytoskeletal proteins						
5	Lamin-A/C	LMNA_HUMAN	74.12	11	19.90	-1.21	0.04
14	Vimentin	VIME_HUMAN	53.63	7	17.20	-1.15	0.019
6	Lamina-associated polypeptide 2, alpha	LAP2A_HUMAN	75.48	6	11.00	-1.21	0.04
	Others						
17	Uroporphyrinogen decarboxylase	DCUP_HUMAN	40.77	5	13.10	-1.15	0.011
21	Methionine adenosyltransferase 2 subunit beta	MAT2B_HUMAN	37.53	3	10.80	-1.27	0.032
1	Annexin A6	ANXA6_HUMAN	75.86	13	20.70	-1.37	0.0041

*A negative or positive ratio indicates a decrease or an increase in GDM HUVEC compared with normal HUVEC, respectively. P values are calculated using unpaired Student's *t*-test.

SUPPLEMENTARY DATA

Supplementary Table 2. Clinical characteristics of normal and gestational diabetic patients for proteomic analysis

	Normal (n=5)	**Gestational diabetes** (n=5)
Maternal age (years)	32 ± 7	34 ± 6
Body mass index	25 ± 4	33 ± 4*
Fasting glucose (mmol/L)	-	8.4 ± 0.7###
Oral glucose tolerance test (75g, 2h, mmol/L)	-	9.0 ± 0.6
HbA_1C (mmol/mol)	-	42 ± 7
Systolic blood pressure (mmHg)	111 ± 12	120 ± 9
Diastolic blood pressure (mmHg)	64 ± 9	70 ± 7
Ethnicity	2 Caucasian 1 Black 0 Asian 2 Other/mixed	2 Caucasian 2 Black 1 Asian 0 Other/mixed
Gestational age (weeks)	39.8 ± 0.4	38.6 ± 0.3
Caesarean delivery Newborn gender (Male:Female) Birth weight (kg)	0/5 3:2 3.6 ± 0.4	4/5 1:4 3.8 ± 1.1

Values denote mean ± S.D, * P<0.05 versus normal subjects, ### P<0.001 versus theoretical mean of 5.1 mmol/L.

Supplementary Table 3. Primer sequences of qRT-PCR

Gene names		**Sequence (5'-3')**
Genes of interest		
NQO1	Sense	GCCCAGATATTGTGGCTGA
	Anti-sense	ACCACTGCAGGGGGAACT
xCT	Sense	GGGAAAGTCTTGGAACTCAGG
	Anti-sense	CCAAGTTAGGGATTTAGCTGGTC
GCLM	Sense	GAAGAAGATATTTTTCCTGTCATTGAT
	Anti-sense	CCATTCATGTATTGAAGAGTGAATTT
Keap1	Sense	ACCACAACAGTGTGGAGAGGT
	Anti-sense	CGATCCTTCGTGTCAGCAT
Bach1	Sense	GCAGCAGTTCCACTCAAG
	Anti-sense	GGTTCAAATCCTTTAACTGTCACC
Nox4	Sense	TCCTCGGTGGAAACTTTTGT
	Anti-sense	CCACAACAGAAAACACCAACTG
Reference genes		
ACTB	Sense	CCAACCGCGAGAAGATGA
	Anti-sense	CCAGAGGCGTACAGGGATAG
SDHA	Sense	AGAAGCCCTTTGAGGAGCA
	Anti-sense	CGATTACGGGTCTATATTCCAGA
RPL13A	Sense	GAGGCCCCTACCACTTCC
	Anti-sense	AACACCTTGAGACGGTCCAG

SUPPLEMENTARY DATA

Supplementary Table 4. Primer sequences for COBRA

Gene		**Sequence (5'-3')**
Nrf2		
Positive strand		
CGI-176-BIS-2	Sense	TTATAAAGTAGGAAAGGGTTAAT
	Anti-sense	CCTTAAACTCTCTTAAAAACCCAAC
CGI-176-BIS-3	Sense	AGTTTAAGGTTGTTAGAGAGTGATT
	Anti-sense	AAAAAATCTAAAAACTAAACCC
CGI-176-BIS-4	Sense	GGTAGTTTTAAGTTTATTATGATGAGTTGT
	Anti-sense	CTACTAAAACCCCAACTAACAATCC
Negative strand		
CGI-176-BIS-1	Sense	TATGTTGTGGTATTATATATTGTTGAAGG
	Anti-sense	ATCTCCATTCTCCTAAACTCAAATC
CGI-176-BIS-2	Sense	ATTTGAGTTTAGGAGAATGGAGATA
	Anti-sense	AAAACCAATAAACCCTACCTAAAAAAA
CGI-176-BIS-3	Sense	GATTTGAGTTTAGGAGAATGGAGATA
	Anti-sense	AAACCAATAAACCCTACCTAAAAAAAA
CGI-176-BIS-4	Sense	TGTTTTAATTGTTTAAATTGTTTTAAAG
	Anti-sense	ACTACCAACTAAAATCCCAACAAAC
CGI-176-BIS-5	Sense	TGAGATAAAAGTAGGGTAAGGTTTTGTA
	Anti-sense	AAACTACCAACTAAAATCCCAACAA
CGI-176-BIS-6	Sense	GGTTTTGGGGGAGAATTTAGT
	Anti-sense	CTTTCAAAAAAACTCAAAACTACCAA

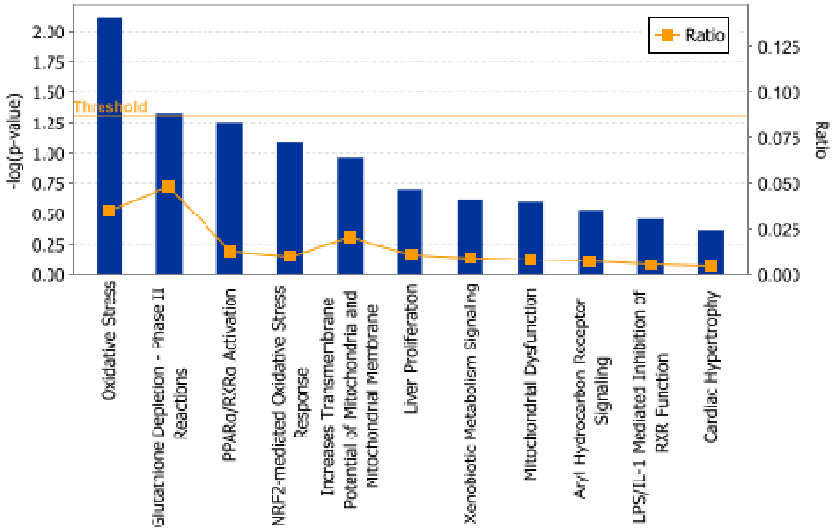
SUPPLEMENTARY DATA

Supplementary Table 5. Primer sequences for HRM

Gene		**Sequence (5'-3')**
Nrf2		
CGI-176-HRM-1	Sense	CGTTTTATATATTTTGGAATTGTAATTT
	Anti-sense	CGATTAACCCTTTCCTACTTTATAA
CGI-176-HRM-2	Sense	CGAGGGTTGTTGTGACGGTTAAT
	Anti-sense	CGAAAACAATAAACTCTAAAA
CGI-176-HRM-3	Sense	GGCGTTACGAGGGGTTAATTT
	Anti-sense	CGCCTTAAATACCCAACGAACTCAAA
CGI-176-HRM-4	Sense	TTTCGTTTCGGGGTTGGGTTTTTA
	Anti-sense	CGCCCCAAAACCTCCTTAAA
CGI-79-HRM-1	Sense	CGTTGGATTTATTAGGTTTAGTAGTATT
	Anti-sense	CGCGCACCTACCAAACAAATAAA
CGI-79-HRM-2	Sense	CGAGGATATTATTTAGGGTTTT
	Anti-sense	CGACGAATACCATACGTCTAAAA
CGI-79-HRM-3	Sense	CGTTTTGAGTGATTTTTTATT
	Anti-sense	CGAAACCGACCCAACAAATAACCAAA
CGI-79-HRM-4	Sense	CGGTTGTAGCGGGTGGGATTGGTTT
	Anti-sense	CGCTACTCACCGCTTCTTTAAA
CGI-79-HRM-5	Sense	CGGGTTTGTGTTGTAGGAGGGTAT
	Anti-sense	CGCCCCCTTCCTAACAAAATCCAAA
CGI-79-HRM-6	Sense	CGGGATGGGATGGTGGATTTTGGTT
	Anti-sense	CGATTTCCTCCCTCAAAATCAA
NQO1		
CGI-19-HRM-1	Sense	CGGTTTTGGTTTTCGTTAAATT
	Anti-sense	CGAACTTCTAAAAAAAATAAAAA
CGI-19-HRM-2	Sense	CGTAAGTTTGATTTGGTTTTT
	Anti-sense	CGAACTCCGAAAAAAAAAAAACTTC
CGI-19-HRM-3	Sense	CGATTAAGGTTTATTTTAGGTTT
	Anti-sense	CGATAAATACTACAAAAAA
CGI-19-HRM-4	Sense	CGGGGCGTTGATTGGTTGGGTT
	Anti-sense	CGCCCTTATAAACTATCCACCTCAAA
CGI-19-HRM-5	Sense	CGGGATTTAACGTTTGAATTTT
	Anti-sense	CGAAAAACTTTTCTTAAC
CGI-19-HRM-7	Sense	CGAAATGGAGTAGAAAAAGAGT
	Anti-sense	CGTCTCCACGAAACATA

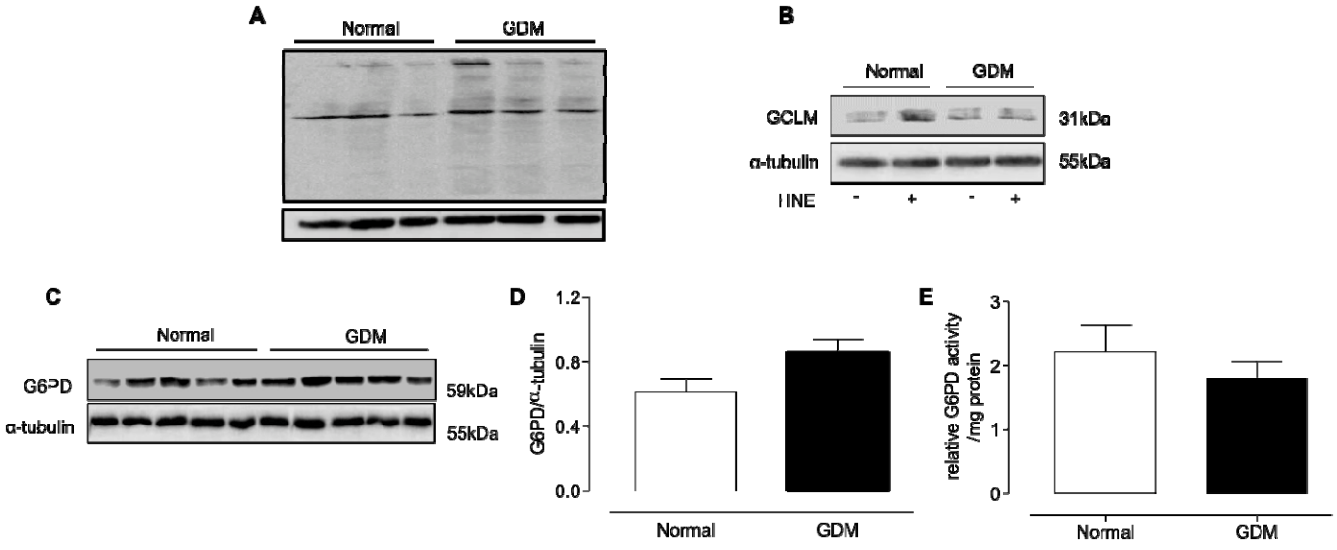
SUPPLEMENTARY DATA

Supplementary Figure 1. Canonical pathway analysis by Ingenuity Pathway Analysis (IPA) of normal and GDM HUVEC under basal conditions. An IPA analysis computed signaling pathways likely to be affected in the GDM proteome. Submission of the 39 altered proteins (see Supplemental Table I) to IPA canonical pathway analysis identified the pathways from the IPA library of canonical pathways that were most significant to the data set. Molecules from the data set that met the Normal/GDM ratio cutoff of >1.1 or <-1.1 and P cutoff of <0.05 and associated with a canonical pathway in the Ingenuity Knowledge Base were considered for analysis. The significance of the association between the data set and the canonical pathway was measured in TWO ways: (i) Ratio (orange square) of the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway is displayed (right-hand Y-axis) and (ii) Fisher's exact test is used to calculate a P value determining the probability that the association between the genes in the data set and the canonical pathway (left-hand Y-axis), with the threshold line displayed to show the level of statistical significance.



SUPPLEMENTARY DATA

Supplementary Figure 2. Comparison of basal protein carbonylation, GCLM protein expression and glucose-6-phosphate dehydrogenase protein expression and enzyme activity in normal and GDM HUVEC. *A*, Basal protein carbonylation was assessed in DNPH-derivatized extracts and expressed relative to α-tubulin in cells from normal (n=3) and GDM (n=3) donors. Representative immunoblot of normal versus GDM lysates run on a single membrane with non-relevant lanes omitted. *B*, Normal and GDM HUVEC were treated with vehicle or HNE (20μmol/L) for 12 h before determining GCLM protein expression relative to α-tubulin in cells from normal (n=4) and GDM (n=4) donors. Representative immunoblot of normal and GDM samples run on the same membrane with non-relevant lanes omitted. *C-D*, G6PD protein expression in cell lysates from 5 normal and 5 GDM donors, with densitometric analysis of basal expression relative to α-tubulin. *E*, G6PD enzyme activity in HUVEC from normal and GDM pregnancies was measured using a previously reported protocol (Afzal-Ahmed, Free Radic. Biol. Med. 2007; 42:1781-1790). Relative G6PD activity was assessed measuring the rate of NADPH generation and expressed per mg protein. Data denote mean ± S.E.M. of measurements in independent cultures derived from 5 normal and 5 GDM donors.



Supplementary Figure 3. Analysis of Nrf2 CpG island methylation in normal and GDM HUVEC by COBRA and HRM. DNA from normal and GDM untreated HUVEC (passage 1 and 3) was extracted (Qiagen #69504) before bisulphite modification. Nrf2 CpG Island 176 methylation was assessed by combined bisulphite restriction analysis (COBRA). PCR products containing 1 or more CpGTaq$^{\alpha}$1 restriction sites were obtained using bisulphite-specific primers and incubated in the presence of Taq$^{\alpha}$1 to cleave methylated DNA in which the Taq$^{\alpha}$1 restriction site remains conserved. *A:* Representative composite gel image of PCR products obtained using CGI-176-BIS-5 primers in normal and GDM HUVEC showing no widespread sample methylation. In each gel, samples were run alongside 50% methylated standards (M50) which show digested products of a lower molecular weight (~150bp) in the Taq$^{\alpha}$1 digested but not undigested control sample. Nrf2 and NQO1 methylation was further critically assessed by high resolution melt (HRM) analysis. *B:* Representative high resolution melt analysis obtained using Nrf2_79_HRM_1 primers. 0% methylated DNA (M0) melts at the lower temperature of 790C compared with the 100% methylated DNA (M100, 81.50C). The 50% methylated DNA (M50) standard melts at both temperatures giving a bimodal peak. Standards were run alongside sample DNA to assess whether GDM was associated with DNA methylation of the Nrf2 and NQO1 (not shown) promoter regions. All primers used are detailed in Supplemental Tables IV and V for COBRA and HRM analysis, respectively.