Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Beyond Insulin Resistance: Is Placental Glucagon Resistance a Missing Link in Gestational Diabetes Mellitus?

Luis Jesuino de Oliveira Andrade

luis_jesuino@yahoo.com.br

Departamento de Saúde, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil.
https://orcid.org/0000-0002-7714-0330

Luís Matos de Oliveira

Departamento de Saúde, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil.
https://orcid.org/0000-0003-4854-6910

Alcina Maria Vinhaes Bittencourt

Faculdade de Medicina Universidade Federal da Bahia, Salvador, Bahia, Brasil. https://orcid.org/0000-0003-0506-9210

Osmario Jorge de Mattos Salles

Escola Bahiana de Medicina e Saúde Pública, Salvador, Bahia, Brazil. https://orcid.org/0009-0002-1859-0478

Gabriela Correia Matos de Oliveira

Hospital Electro Bonini Hospital e Maternidade Cidinha Bonini - UNAERP, Ribeirão Preto, São Paulo, Brazil. https://orcid.org/0000-0002-3447-3143

Research Article

Keywords: Gestational diabetes mellitus, Glucagon receptor, Placenta, Signal transduction

Posted Date: September 16th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11019627/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: The authors declare no competing interests.

Abstract

Objective: To determine whether human placental tissue in gestational diabetes mellitus (GDM) exhibits transcriptomic alterations across the glucagon receptor (GCGR)-cAMP-PKA-CREB signaling axis consistent with impaired glucagon responsiveness.

Study Design: This *in silico* study analyzed three publicly available NCBI Gene Expression Omnibus datasets: GSE70493 (63 whole placental samples, 32 GDM/31 control), GSE103552 (37 feto-placental endothelial cell samples, 21 GDM/16 control), and GSE128381 (183 whole placental samples, 6 GDM/177 control, used as a class-imbalanced external replication set). A sign-inverted z-score module-average enrichment score was used to calculate a Placental Glucagon Resistance Score (PGRS) and a gluconeogenesis score across a curated 25-gene panel. Group differences were evaluated with the Mann-Whitney U test and associations with Spearman correlation; per-gene differential expression used Welch's t-test with Benjamini-Hochberg false discovery rate (FDR) correction.

Results: The PGRS was significantly higher in GDM feto-placental endothelial cells (GSE103552, P=.018), with concordant but non-significant trends in both whole-placenta cohorts. The PGRS correlated inversely with gluconeogenic pathway activity in all three datasets, reaching significance in two (GSE103552, ρ=-0.45, P=.005; GSE128381, ρ=-0.21, P=.005), with a concordant, non-significant trend in the third (GSE70493, ρ=-0.23, P=.08). In GSE103552, six axis genes showed significant differential expression after FDR correction: ATF1, CREM, and G6PC3 were down-regulated, and ADCY6, PRKAR1A, and GNAS were up-regulated. No gene reached FDR significance in either whole-placenta cohort. The PGRS did not correlate with maternal body mass index, gestational age, or birth weight in GSE128381.

Conclusion: GDM-associated alterations in the GCGR-cAMP-PKA-CREB axis were detectable primarily in the feto-placental endothelial compartment rather than in whole placental tissue, and tracked inversely rather than concordantly with gluconeogenic gene activity. These findings support a compartment-specific, non-hepatic pattern of placental glucagon-pathway dysregulation in GDM rather than a generalized whole-placenta glucagon-resistance phenotype, and should be regarded as hypothesis-generating pending functional and prospective validation.

INTRODUCTION

Gestational diabetes mellitus (GDM) affects a substantial proportion of pregnancies worldwide, with the pooled global standardized prevalence estimated at 14.0% under International Association of Diabetes in Pregnancy Study Groups criteria, ranging from 7.1% in North America and the Caribbean to markedly higher rates in the Middle East, North Africa, and Southeast Asia.[1] The condition arises from a combination of chronic maternal insulin resistance and inadequate compensatory β-cell insulin secretion, and it carries consequences that extend well beyond the index pregnancy, including elevated maternal risk for type 2 diabetes and cardiovascular disease, as well as long-term metabolic vulnerability in offspring.[2] Because GDM typically emerges during late gestation and generally resolves after delivery,

the placenta has long been regarded as a major endocrine regulator of the insulin-resistant state that characterizes pregnancy, principally through its secretion of human placental lactogen, placental growth hormone, progesterone, and pro-inflammatory cytokines.

However, the counter-regulatory hormone glucagon has received comparatively little attention in this context, despite evidence that its receptor (GCGR) is expressed within placental trophoblast and that disrupted glucagon signaling produces overt placental pathology.[3] This represents a notable gap because glucagon and insulin constitute complementary regulators of systemic energy availability, yet placental adaptations in GDM have been studied predominantly through an insulin-centered framework. In fetal sheep, glucagon infusion fails to elicit the glycemic response typical of adult tissue, an observation that led to the original description of glucagon resistance in the fetal compartment four decades ago.[3] More recently, the broader concept of glucagon receptor signaling and glucagon resistance has been formalized across hepatic, pancreatic, and extrahepatic tissues, yet its placental dimension remains largely unmapped.[4] Genetic ablation of GCGR in mice produces maternal hypoglycemia, hyperglucagonemia, and placentas marked by extensive mineralization, fibrinoid necrosis, vascular narrowing, and trophoblast hyperplasia, together with downregulation of genes governing growth, vascularization, and oxidative stress.[5–7] In late-gestation sheep, experimentally induced fetal hyperglucagonemia independently impairs placental nutrient transfer and chorionic somatomammotropin secretion.[8]

Clarifying this question matters because the pathophysiological framework of GDM has been built almost entirely around insulin and its placental modulators, leaving glucagon's counter-regulatory role, and its potential placental resistance, absent from current etiological models. Recent systems-level studies of the liver–α-cell axis have further established that impaired hepatic responsiveness to glucagon can disrupt feedback control of amino acid metabolism, thereby sustaining hyperglucagonemia and amplifying metabolic dysregulation.[9] An analogous placental phenomenon, in which impaired trophoblastic responsiveness to glucagon alters local metabolic and endocrine signaling and potentially amplifies maternal metabolic dysfunction, would represent a previously unexplored dimension of GDM pathophysiology. This possibility is further supported by classical evidence that maternal and fetal glucagon pools remain largely compartmentalized across the placental barrier, supporting the concept that local glucagon signaling at the maternal–fetal interface may have functional consequences that are not adequately captured by circulating glucagon concentrations alone.[10]

Therefore, the objective of this *in silico* study was to determine whether human placental tissue from GDM pregnancies exhibits a coordinated molecular signature consistent with impaired glucagon receptor responsiveness by integrating placental transcriptomic and protein–protein interaction data across the GCGR–cAMP–PKA–CREB signaling axis. We hypothesized that GDM placentas exhibit coordinated alterations across this network that are consistent with impaired glucagon responsiveness, thereby providing a testable molecular framework for investigating placental glucagon resistance as a potential contributor to GDM pathophysiology.

MATERIALS AND METHODS

Study Design and Data Sources

This study followed an *in silico*, hypothesis-driven bioinformatics design integrating publicly available human placental transcriptomic datasets deposited in the Gene Expression Omnibus (GEO, NCBI). Three datasets were selected: GSE70493 (32 GDM vs. 31 control whole placental tissue), GSE103552 (21 GDM vs. 16 control feto-placental arterial and venous endothelial cells), and GSE128381 (6 GDM vs. 177 non-GDM placental tissue, used as an external replication set given its class imbalance). As this study exclusively reanalyzed de-identified, publicly deposited data, it was classified as exempt from institutional review under Resolução CNS nº 510/2016. No new human participants, tissue, or data were collected for this study; all analyses were performed exclusively on de-identified, previously published, and publicly archived data, and no institutional review board approval was therefore required beyond the exemption noted above.

Data Retrieval and Preprocessing

Expression matrices and sample metadata were retrieved with the GEOquery package. Probe-to-gene mapping used each platform's public annotation file; multiple probes per gene were collapsed to the highest-mean-expression probe. Each dataset was analyzed independently rather than merged, to avoid unverifiable cross-platform batch correction.

Curation of the GCGR-cAMP-PKA-CREB Gene Set

The glucagon receptor signaling gene set was re-curated using exclusively open-license sources: Reactome (CC0 license) pathways "Glucagon signaling in metabolic regulation" (R-HSA-glucagon) and "Gluconeogenesis," cross-referenced against Gene Ontology biological process terms (GO:0004965 glucagon receptor activity; GO:0006094 gluconeogenesis) via the org.Hs.eg.db and GO.db Bioconductor annotation packages. This replaces the KEGG-derived curation used in the prior manuscript, since KEGG is explicitly not a public database and its bulk/programmatic use requires a paid academic subscription. Gene identity and nomenclature were cross-checked against NCBI Gene and UniProtKB (CC BY 4.0), replacing GeneCards, whose terms restrict automated or bulk reuse.

Placental Glucagon Resistance Score and Gluconeogenic Activity

For each sample, a Placental Glucagon Resistance Score (PGRS) was computed as a sign-inverted z-score module-average across the Reactome/GO-curated gene set, such that higher values denote greater predicted resistance. A true rank-based single-sample enrichment score (ssGSEA/GSVA) was not used because expression was extracted only for the 25 curated pathway genes rather than the full transcriptome, precluding the genome-wide background ranking that ssGSEA/GSVA require; this is

stated explicitly as a methodological limitation. A gluconeogenic activity score was computed analogously using the curated gluconeogenesis gene set (PCK1, PCK2, G6PC, G6PC2, G6PC3, FBP1, PDK4). Scores were compared between GDM and control groups by the Mann-Whitney U test, and their association was assessed by Spearman correlation.

Differential Expression Analysis

Differentially expressed genes (DEGs) were identified for each of the 25 curated genes independently using Welch's t-test comparing GDM and control samples within each dataset, with the Benjamini-Hochberg procedure applied within each dataset to control the false discovery rate (FDR); genes with adjusted P<.05 were considered significant. No minimum fold-change threshold was applied. A protein-protein interaction network analysis (STRING/Cytoscape) and formal pathway enrichment of the curated gene set were planned but were not completed for this analysis.

Correlation with Clinical and Anthropometric Variables

Where sample-level metadata were available in the GEO series (GSE70493 and GSE128381 report maternal BMI, gestational age at delivery, and neonatal birth weight), the PGRS was correlated with birth weight and maternal glycemic parameters by Spearman correlation ($P < 0.05$).

RESULTS

Sample Characteristics Across Datasets

The three datasets differed substantially in tissue compartment, platform, case-control balance, and available clinical annotation (Table 1). GSE70493 (Affymetrix HTA 2.0) comprised 63 whole placental samples with near-balanced groups (32 GDM, 31 control) and no clinical covariates beyond GDM status. GSE103552 (Affymetrix Human Gene 1.0 ST) comprised 37 feto-placental arterial/venous endothelial cell samples (21 diabetic, 16 control). GSE128381 (Agilent-039494) comprised 183 whole placental samples with rich clinical annotation (birth weight, maternal pre-pregnancy BMI, gestational age) but a pronounced class imbalance (6 GDM, 177 control), consistent with its role as an external, non-power-matched replication set rather than a discovery cohort. Of note, GSE128381's submitted expression matrix contained only 14,040 of the 50,755 probes present on the full Agilent array, which restricted gene-level analysis in this dataset to 15 of the 25 curated GCGR-axis and gluconeogenesis genes (GCGR, ADCY1, ADCY2, ADCY5, PRKACB, PRKAR2A, RAPGEF4, G6PC, G6PC2, and PCK1 were absent from the deposited data).

Table 1
Characteristics of the 3 cohorts (platform, tissue, n, clinical variables)

Dataset	Platform	Tissue	N total	N GDM	N Control	Clinical variables
GSE70493	Affymetrix HTA 2.0 (GPL17586)	Whole placental tissue	63	32	31	None beyond GDM status
GSE103552	Affymetrix Human Gene 1.0 ST (GPL6244)	Feto-placental arterial/venous endothelial cells	37	21	16	Fetal sex only
GSE128381	Agilent-039494 (GPL17077)	Whole placental tissue	183	6	177	Birth weight, maternal pre-pregnancy BMI, gestational age

Placental Glucagon Resistance Score (PGRS) in GDM versus Control

The PGRS was numerically higher in GDM placentas in all three datasets (Fig. 1), but reached statistical significance only in GSE103552 (median GDM = 0.084 vs. median Control = − 0.141, Mann-Whitney P = 0.018). The direction was concordant but non-significant in the primary discovery cohort GSE70493 (median GDM = 0.062 vs. Control = − 0.047, P = 0.194) and in the imbalanced replication set GSE128381 (median GDM = 0.030 vs. Control = − 0.029, P = 0.757, n = 6 GDM limiting power substantially).

Gluconeogenic Pathway Activity and Its Relationship to PGRS

The gluconeogenic activity score (available genes: PCK2, G6PC3, FBP1, PDK4) was significantly lower in GDM placentas relative to controls in GSE103552 (median GDM = − 0.294 vs. Control = 0.361, P = 0.016), opposite in direction to GSE70493 and GSE128381, neither of which reached significance (P = 0.716 and P = 0.876, respectively). Across all three datasets, the PGRS correlated inversely with the gluconeogenesis score (GSE70493: Spearman ρ = −0.225, P = 0.077; GSE103552: ρ = −0.450, P = 0.005; GSE128381: ρ = −0.209, P = 0.005) (Fig. 2), indicating that within this gene panel, higher predicted glucagon-axis resistance tracked with lower rather than higher gluconeogenic gene activity.

Differentially Expressed Genes Within the GCGR-cAMP-PKA-CREB Axis

Gene-level differential expression restricted to the 25-gene panel (Table 2) showed the clearest signal in GSE103552, where six genes survived FDR correction (adjusted P < 0.05): ATF1 (log2FC = − 0.402, adj. P = 0.0001), G6PC3 (log2FC = − 0.168, adj. P = 0.0014), ADCY6 (log2FC = + 0.260, adj. P = 0.0021), CREM

(log2FC = − 0.243, adj. P = 0.0023), PRKAR1A (log2FC = + 0.182, adj. P = 0.0056), and GNAS (log2FC = + 0.100, adj. P = 0.0056). No gene reached FDR significance in GSE70493 or GSE128381; in GSE70493 only PRKACB showed a nominal, uncorrected association (log2FC = − 0.042, P = 0.023, adj. P = 0.563), and in GSE128381 only PRKAR1A did (log2FC = − 0.255, P = 0.011, adj. P = 0.166), both underpowered given their sample sizes and, for GSE128381, extreme class imbalance.

Table 2
DEGs of the GCGR axis per dataset (log2FC, P, FDR)

Dataset	Gene	log2FC	N GDM	N Control	P value	Adj P value
GSE103552	ATF1	-0.402	21	16	6e-06	0.0001
GSE103552	G6PC3	-0.168	21	16	0.000116	0.0014
GSE103552	ADCY6	0.26	21	16	0.000247	0.0021
GSE103552	CREM	-0.243	21	16	0.000361	0.0023
GSE103552	PRKAR1A	0.182	21	16	0.001198	0.0056
GSE103552	GNAS	0.1	21	16	0.001338	0.0056
GSE103552	PRKAR2A	-0.123	21	16	0.014956	0.0529
GSE103552	ADCY3	-0.276	21	16	0.016923	0.0529
GSE103552	PRKAR2B	-0.406	21	16	0.027585	0.0766
GSE103552	FBP1	-0.37	21	16	0.03564	0.0891
GSE103552	PRKACA	-0.074	21	16	0.049423	0.1123
GSE103552	ADCY2	-0.074	21	16	0.097123	0.2023
GSE103552	PCK2	-0.171	21	16	0.112562	0.2165
GSE103552	G6PC2	-0.047	21	16	0.186337	0.3327
GSE103552	CREB1	-0.054	21	16	0.223922	0.3732
GSE103552	PRKACB	-0.102	21	16	0.283342	0.4427
GSE103552	RAPGEF4	0.19	21	16	0.420865	0.6189
GSE103552	ADCY9	0.107	21	16	0.465759	0.6469
GSE103552	G6PC	-0.026	21	16	0.499031	0.6566
GSE103552	PCK1	-0.023	21	16	0.602879	0.7536
GSE103552	PDK4	0.103	21	16	0.671999	0.7656
GSE103552	ADCY5	0.019	21	16	0.693507	0.7656
GSE103552	ADCY1	-0.015	21	16	0.704337	0.7656
GSE103552	GCGR	-0.003	21	16	0.932457	0.9507
GSE103552	RAPGEF3	0.005	21	16	0.950658	0.9507
GSE128381	PRKAR1A	-0.255	6	177	0.011081	0.1662
GSE128381	ADCY6	0.251	6	177	0.22547	0.9806

Dataset	Gene	log2FC	N GDM	N Control	P value	Adj P value
GSE128381	PRKAR2B	-0.183	6	177	0.259499	0.9806
GSE128381	ATF1	-0.1	6	177	0.348797	0.9806
GSE128381	PDK4	0.122	6	177	0.361348	0.9806
GSE128381	RAPGEF3	0.12	6	177	0.392253	0.9806
GSE128381	ADCY3	0.162	6	177	0.49804	0.9968
GSE128381	FBP1	-0.215	6	177	0.605892	0.9968
GSE128381	CREM	-0.043	6	177	0.667175	0.9968
GSE128381	GNAS	-0.053	6	177	0.82672	0.9968
GSE128381	PCK2	0.034	6	177	0.900903	0.9968
GSE128381	PRKACA	-0.011	6	177	0.933575	0.9968
GSE128381	CREB1	-0.005	6	177	0.951098	0.9968
GSE128381	G6PC3	0.005	6	177	0.958097	0.9968
GSE128381	ADCY9	-0.001	6	177	0.996765	0.9968
GSE70493	PRKACB	-0.042	32	31	0.02251	0.5628
GSE70493	ADCY2	0.035	32	31	0.065271	0.8159
GSE70493	ADCY3	-0.034	32	31	0.17333	0.9246
GSE70493	CREB1	-0.03	32	31	0.241131	0.9246
GSE70493	PRKAR2A	-0.041	32	31	0.250076	0.9246
GSE70493	RAPGEF4	-0.012	32	31	0.334903	0.9246
GSE70493	CREM	0.033	32	31	0.336622	0.9246
GSE70493	GCGR	-0.023	32	31	0.373724	0.9246
GSE70493	PRKACA	-0.016	32	31	0.425232	0.9246
GSE70493	PRKAR2B	-0.039	32	31	0.464237	0.9246
GSE70493	PDK4	-0.081	32	31	0.480439	0.9246
GSE70493	GNAS	0.015	32	31	0.484231	0.9246
GSE70493	FBP1	-0.025	32	31	0.508542	0.9246
GSE70493	G6PC2	0.013	32	31	0.517794	0.9246
GSE70493	G6PC3	-0.034	32	31	0.619685	0.9907

Dataset	Gene	log2FC	N GDM	N Control	P value	Adj P value
GSE70493	PRKAR1A	-0.018	32	31	0.676843	0.9907
GSE70493	PCK2	0.01	32	31	0.705199	0.9907
GSE70493	RAPGEF3	0.007	32	31	0.750258	0.9907
GSE70493	G6PC	0.007	32	31	0.757791	0.9907
GSE70493	ADCY5	0.006	32	31	0.792589	0.9907
GSE70493	ATF1	0.005	32	31	0.92288	0.9999
GSE70493	PCK1	-0.001	32	31	0.963387	0.9999
GSE70493	ADCY1	-0.001	32	31	0.966169	0.9999
GSE70493	ADCY6	-0.0	32	31	0.977368	0.9999
GSE70493	ADCY9	0.0	32	31	0.999916	0.9999

Cross-Dataset Directional Concordance

Among the 25 curated genes, direction of effect (GDM vs. control) was concordant between GSE70493 and GSE103552 in 15 of 25 genes (60.0%), and between GSE70493 and GSE128381 in 7 of 15 comparable genes (46.7%) (Fig. 3). CREB1, FBP1, PRKACA, PRKAR2B, and RAPGEF3 showed concordant directionality across all three datasets; ADCY2, ATF1, CREM, GNAS, and PDK4 showed discordant direction between at least two datasets.

Correlation of PGRS with Clinical and Anthropometric Variables

In GSE128381, the only dataset with linked anthropometric data, the PGRS did not correlate significantly with neonatal birth weight (Spearman $\rho = -0.050$, $P = 0.503$), maternal pre-pregnancy BMI ($\rho = 0.085$, $P = 0.255$), or gestational age at delivery ($\rho = 0.028$, $P = 0.709$) (Fig. 4). The gluconeogenesis score likewise showed no significant association with these variables (birth weight: $\rho = -0.027$, $P = 0.715$; BMI: $\rho = 0.079$, $P = 0.288$; gestational age: $\rho = 0.005$, $P = 0.943$).

DISCUSSION

This study asked whether the human placenta in GDM carries a coordinated molecular signature of impaired glucagon receptor responsiveness across the GCGR-cAMP-PKA-CREB axis. The signature was present but compartment-restricted: feto-placental endothelial cells, not whole villous tissue, carried the clearest evidence, with a significantly elevated PGRS and six axis genes differentially expressed after correction for multiple testing, whereas whole-tissue cohorts showed only concordant, non-significant trends.

This compartment-specific pattern is consistent with, rather than contradicted by, prior independent analyses of the same or related datasets. Two earlier reanalyses of GSE70493 and an independent whole-placenta microarray cohort converged on immune, inflammatory, and stress-response pathways rather than cAMP-dependent signaling,[11–13] consistent with our nominal, FDR-non-significant PRKACB signal in that whole-tissue cohort. A more recent independent RNA-sequencing cohort likewise centered on immune cell composition rather than endocrine signaling.[14] By contrast, the original study generating GSE103552 reported that GDM differentially programs feto-placental endothelial cells, with measurable changes in barrier function,[15] and an independent reanalysis of the same dataset identified 613 differentially expressed genes converging on distinct co-expression modules.[16] Our GCGR-axis findings in GSE103552 therefore sit within a growing, converging body of evidence that this endothelial compartment, specifically, carries a reproducible GDM signal.

Mechanistically, the direction of several hub genes is biologically coherent. ADCY6 upregulation is consistent with its established role in constraining endothelial monolayer permeability through preferential cAMP generation at prostacyclin receptors,[17] echoing the barrier-function phenotype already reported for GDM-exposed feto-placental endothelium.[15] PRKAR1A, the principal PKA regulatory subunit required for normal endothelial and vascular development,[18] and the downstream transcription factors ATF1 and CREM, which mediate cAMP-responsive gene transcription in trophoblast cells,[19] were concordantly altered, suggesting a coherent shift in cAMP-PKA-CREB output rather than a stochastic artifact. The inverse, rather than concordant, relationship between the PGRS and gluconeogenic gene activity argues against a simple transposition of the hepatic glucagon-resistance paradigm onto the placenta; the fetoplacental unit is not gluconeogenic as the liver is, and its glucagon-responsive program may instead govern vascular tone and barrier integrity rather than glucose output. Finally, GNAS is a parentally imprinted locus,[20] and imprinting-driven variability across unrelated samples is a plausible contributor to the directional discordance observed for this gene between datasets.

Limitations warrant explicit acknowledgment. First, the 6-versus-177 class imbalance in GSE128381 limits its statistical power and external validity; this was mitigated by treating it strictly as a directional replication set rather than a co-equal discovery cohort. Second, restricting extraction to a curated 25-gene panel, rather than the full transcriptome, made rank-based ssGSEA/GSVA enrichment statistically inapplicable; a z-score module-average approach was used instead, which remains constrained by the same small-sample power limitations affecting any transcriptomic case-control comparison of this size.[21] Third, whole villous tissue and isolated feto-placental endothelium are biologically distinct compartments profiled on different platforms, not technical replicates; this was addressed by analyzing each dataset independently rather than through cross-platform batch correction, though this necessarily limits any claim of a single, unified placental phenotype. Fourth, this study is a purely computational reanalysis of archived public microarray data with no newly collected clinical specimens, no protein-level or functional validation, and no independent prospective cohort; the planned protein-protein interaction network analysis was not completed and is not reported. The findings should therefore be interpreted as hypothesis-generating rather than as evidence of a validated clinical biomarker or mechanism.

Clinically, these findings suggest that future placental biomarker studies of GDM should not treat the placenta as a single homogeneous compartment; the feto-placental vascular endothelium, rather than bulk villous tissue, may be the more informative site for glucagon-axis signaling changes, particularly given that GDM itself is an increasingly recognized heterogeneous, subtype-structured condition.[22,23] Scientifically, this work extends the placental endocrine framework of GDM beyond its traditional insulin-centered focus[24] by providing transcriptomic evidence that glucagon receptor signaling is measurably and coherently altered in a specific placental cell compartment in GDM.

Three concrete next steps follow directly from these limitations. First, full-transcriptome RNA-sequencing of feto-placental endothelial cells, rather than a targeted panel, would allow genome-wide ssGSEA/GSVA enrichment and formal pathway-level statistics. Second, functional validation in stem-cell-derived trophoblast or endothelial organoids,[25] using GCGR agonist and antagonist challenge with cAMP and CREB-reporter readouts, would test causality rather than association. Third, external validation in a prospective, balanced cohort of IADPSG-diagnosed GDM and control pregnancies with paired villous and endothelial sampling, stratified by GDM subtype,[22,23] is needed before this signature can be considered a candidate biomarker.

CONCLUSION

In conclusion, GDM-associated alterations in the GCGR-cAMP-PKA-CREB axis were detectable primarily in the feto-placental endothelial compartment rather than in whole placental tissue, and tracked inversely rather than concordantly with gluconeogenic gene activity. These findings support a compartment-specific, non-hepatic pattern of placental glucagon-pathway dysregulation in GDM rather than a generalized whole-placenta glucagon resistance phenotype, and should be regarded as hypothesis-generating pending functional and prospective validation.

Declarations

Conflict of interest:

The authors report no conflict of interest

References

1. Wang H, Li N, Chivese T, Werfalli M, Sun H, Yuen L, et al. IDF Diabetes Atlas: Estimation of Global and Regional Gestational Diabetes Mellitus Prevalence for 2021 by International Association of Diabetes in Pregnancy Study Group's Criteria. Diabetes Res Clin Pract. 2022;183:109050. doi: 10.1016/j.diabres.2021.109050.
2. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The Pathophysiology of Gestational Diabetes Mellitus. Int J Mol Sci. 2018;19(11):3342. doi: 10.3390/ijms19113342.

3. Devaskar SU, Ganguli S, Styer D, Devaskar UP, Sperling MA. Glucagon and glucose dynamics in sheep: evidence for glucagon resistance in the fetus. Am J Physiol. 1984;246(3 Pt 1):E256-65. doi: 10.1152/ajpendo.1984.246.3.E256.
4. Janah L, Kjeldsen S, Galsgaard KD, Winther-Sørensen M, Stojanovska E, Pedersen J, Knop FK, Holst JJ, Wewer Albrechtsen NJ. Glucagon Receptor Signaling and Glucagon Resistance. Int J Mol Sci. 2019;20(13):3314. doi: 10.3390/ijms20133314.
5. Ouhilal S, Vuguin P, Cui L, Du XQ, Gelling RW, Reznik SE, et al. Hypoglycemia, hyperglucagonemia, and fetoplacental defects in glucagon receptor knockout mice: a role for glucagon action in pregnancy maintenance. Am J Physiol Endocrinol Metab. 2012;302(5):E522-31. doi: 10.1152/ajpendo.00420.2011.
6. Charron MJ, Vuguin PM. Lack of glucagon receptor signaling and its implications beyond glucose homeostasis. J Endocrinol. 2015;224(3):R123-30. doi: 10.1530/JOE-14-0614.
7. Gelling RW, Du XQ, Dichmann DS, Romer J, Huang H, Cui L, et al. Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice. Proc Natl Acad Sci U S A. 2003;100(3):1438-43. doi: 10.1073/pnas.0237106100.
8. Cilvik SN, Wesolowski SR, Anthony RV, Brown LD, Rozance PJ. Late gestation fetal hyperglucagonaemia impairs placental function and results in diminished fetal protein accretion and decreased fetal growth. J Physiol. 2021;599(13):3403-3427. doi: 10.1113/JP281288.
9. Wewer Albrechtsen NJ, Færch K, Jensen TM, Witte DR, Pedersen J, Mahendran Y, et al. Evidence of a liver-alpha cell axis in humans: hepatic insulin resistance attenuates relationship between fasting plasma glucagon and glucagonotropic amino acids. Diabetologia. 2018;61(3):671-680. doi: 10.1007/s00125-017-4535-5.
10. König S, Vest M, Stahl M. Interrelation of maternal and foetal glucose and free fatty acids. The role of insulin and glucagon. Eur J Pediatr. 1978;128(3):187-95. doi: 10.1007/BF00444304.
11. Wang Y, Yu H, Liu F, Song X. Analysis of key genes and their functions in placental tissue of patients with gestational diabetes mellitus. Reprod Biol Endocrinol. 2019;17(1):104. doi: 10.1186/s12958-019-0546-z.
12. Pan X, Jin X, Wang J, Hu Q, Dai B. Placenta inflammation is closely associated with gestational diabetes mellitus. Am J Transl Res. 2021;13(5):4068-4079.
13. Enquobahrie DA, Williams MA, Qiu C, Meller M, Sorensen TK. Global placental gene expression in gestational diabetes mellitus. Am J Obstet Gynecol. 2009;200(2):206.e1-13. doi: 10.1016/j.ajog.2008.08.022.
14. Kang YE, Lee SE, Lim JY, Kim OS, Yoon J, Jung Y, et al. Placental transcriptome profiling and immune signature differences in pregnancies with and without gestational diabetes mellitus: An observational study. Medicine (Baltimore). 2026 May 22;105(21):e48747. doi: 10.1097/MD.0000000000048747. Erratum in: Medicine (Baltimore). 2026 Jul 10;105(28):e49792. doi: 10.1097/MD.0000000000049792.

15. Cvitic S, Novakovic B, Gordon L, Ulz CM, Mühlberger M, Diaz-Perez FI, et al. Human fetoplacental arterial and venous endothelial cells are differentially programmed by gestational diabetes mellitus, resulting in cell-specific barrier function changes. Diabetologia. 2018;61(11):2398-2411. doi: 10.1007/s00125-018-4699-7.
16. Liu C, Wei C, Lu Y, Chai F, Wang C, Zeng Y, et al. Identification and characterization of signature genes related to fetoplacental vascular endothelial cell programming in gestational diabetes mellitus using bioinformatics analysis. Front Genet. 2025;16:1600756. doi: 10.3389/fgene.2025.1600756.
17. Bundey RA, Insel PA. Adenylyl cyclase 6 overexpression decreases the permeability of endothelial monolayers via preferential enhancement of prostacyclin receptor function. Mol Pharmacol. 2006;70(5):1700-1707. doi: 10.1124/mol.106.028035.
18. Nedvetsky PI, Zhao X, Mathivet T, Aspalter IM, Stanchi F, Metzger RJ, et al. cAMP-dependent protein kinase A (PKA) regulates angiogenesis by modulating tip cell behavior in a Notch-independent manner. Development. 2016;143(19):3582-3590. doi: 10.1242/dev.134767.
19. Darashchonak N, Koepsell B, Bogdanova N, von Versen-Höynck F. Adenosine A2B receptors induce proliferation, invasion and activation of cAMP response element binding protein (CREB) in trophoblast cells. BMC Pregnancy Childbirth. 2014;14:2. doi: 10.1186/1471-2393-14-2.
20. Frost JM, Moore GE. The importance of imprinting in the human placenta. PLoS Genet. 2010;6(7):e1001015. doi: 10.1371/journal.pgen.1001015.
21. Ching T, Huang S, Garmire LX. Power analysis and sample size estimation for RNA-Seq differential expression. RNA. 2014;20(11):1684-1696. doi: 10.1261/rna.046011.114.
22. Saeedi M, Cao Y, Fadl H, Gustafson H, Simmons D. Increasing prevalence of gestational diabetes mellitus when implementing the IADPSG criteria: a systematic review and meta-analysis. Diabetes Res Clin Pract. 2021;172:108642.
23. Athanasiadou KI, Paschou SA. Gestational diabetes mellitus subtypes: from one-size-fits-all to precision medicine. Hormones (Athens). 2025;24(4):1153-1155.
24. Pixner T, Stummer N, Schneider AM, Lukas A, Gramlinger K, Julian V, et al. The relationship between glucose and the liver-alpha cell axis - A systematic review. Front Endocrinol (Lausanne). 2023;13:1061682. doi: 10.3389/fendo.2022.1061682.
25. Turco MY, Gardner L, Kay RG, Hamilton RS, Prater M, Hollinshead MS, et al. Trophoblast organoids as a model for maternal-fetal interactions during human placentation. Nature. 2018;564(7735):263-267. doi:10.1038/s41586-018-0753-3.

Figures

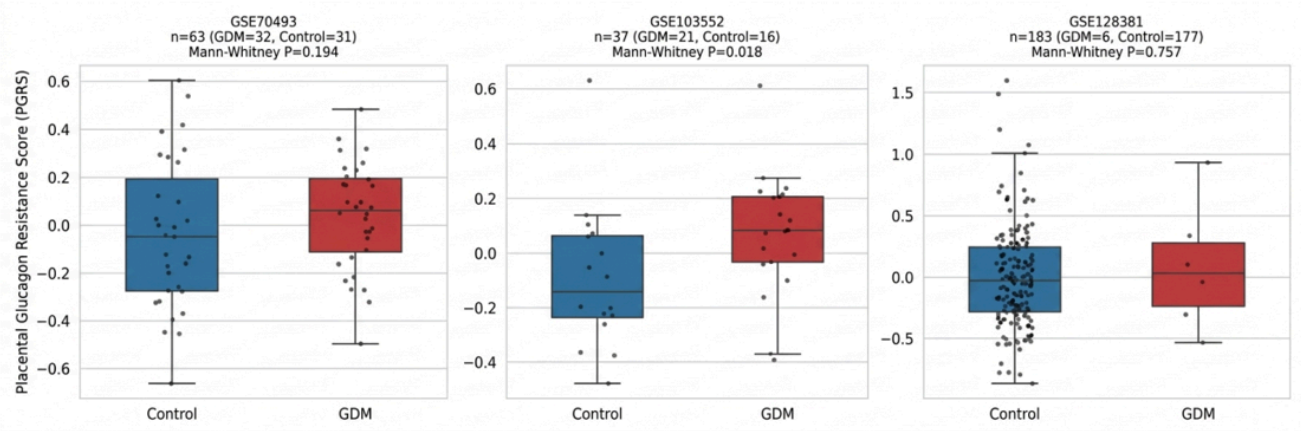


Figure 1

PGRS by group, 3 panels

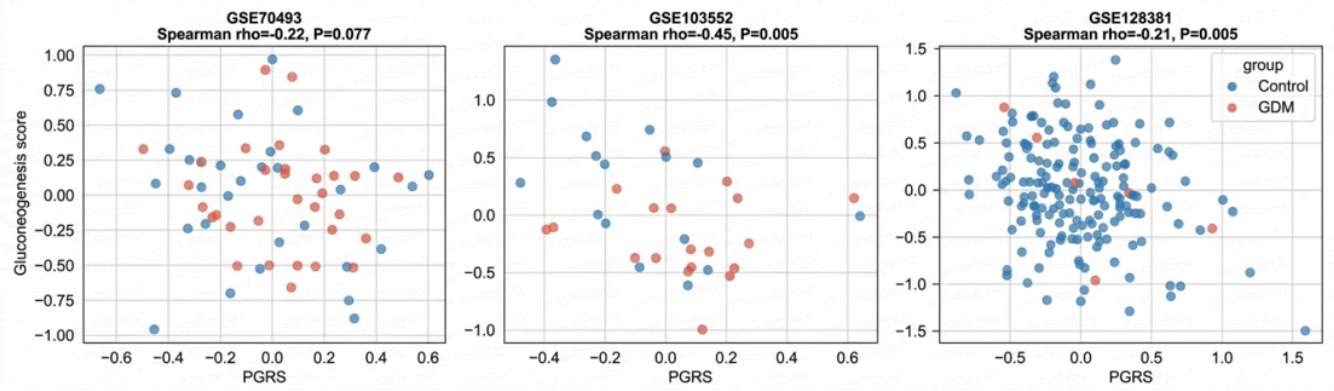


Figure 2

PGRS × gluconeogenesis score

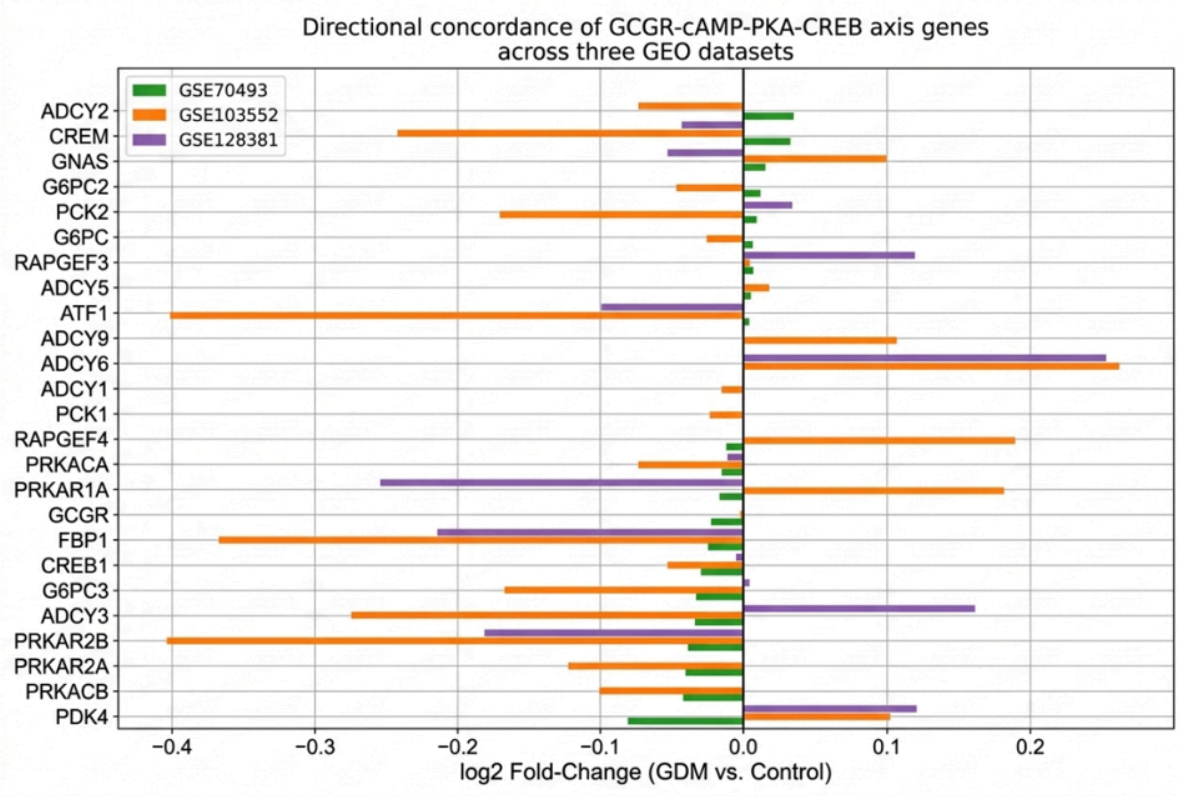


Figure 3

Directional agreement (log2FC) between datasets

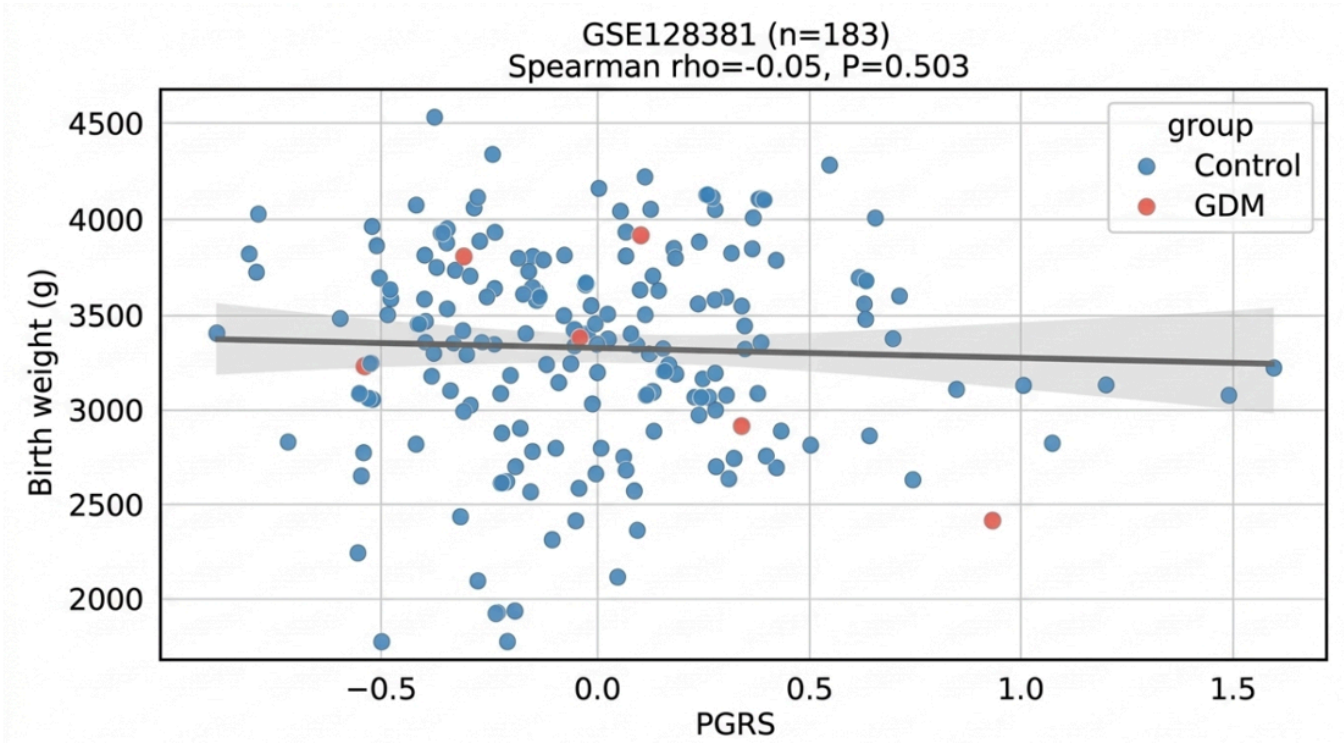


Figure 4

PGRS × birth weight (GSE128381)