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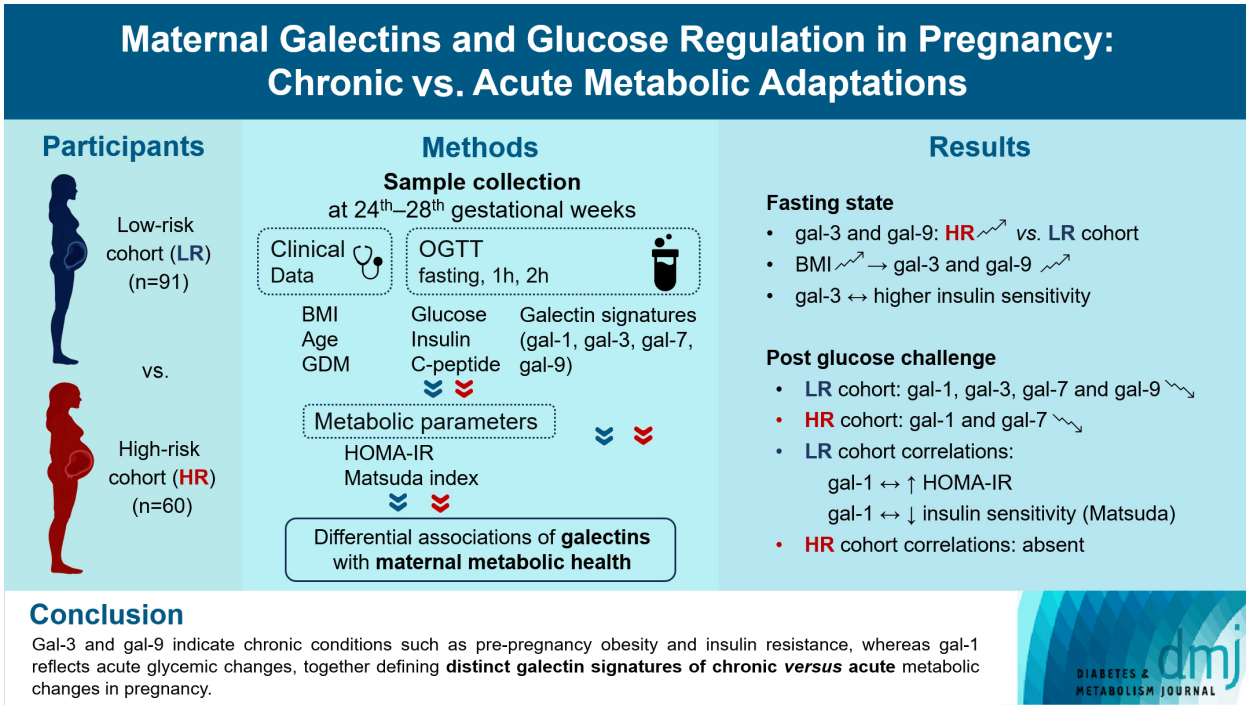

Highlights

- **Maternal gal-3 and gal-9 at 2nd trimester correlate positively with pre-pregnancy BMI.**
- **Gal-3 shows a positive association with insulin sensitivity.**
- **Serum galectins after glucose load show dynamic responses in normal-weight pregnancies.**
- **Gal-1 correlates positively with HOMA-IR during OGTT in normal-weight pregnancies.**

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Maternal Galectins and Glucose Regulation in Pregnancy: Chronic vs. Acute Metabolic Adaptations

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Background: Galectins (gal) are glycan-binding proteins that regulate maternal adaptations during pregnancy, but their role in pregnancy-associated metabolic homeostasis is unclear. This study characterizes the maternal galectin profile in response to an oral glucose tolerance test (OGTT) in pregnant women with varying body weight.
Methods: In a two-center prospective study, pregnant women were recruited into two cohorts: low-risk (LR) with normal weight and high-risk (HR) with overweight or obesity. Circulating levels of gal-1, -3, -7, and -9 were measured at fasting, 1 hour, and 2 hours during the OGTT between 24 and 28 weeks of gestation. Correlations with clinical and metabolic parameters were assessed (HMO study: ClinicalTrials.gov Identifier NCT05496712; FitFor2 trial: trial registration number NTR1139).
Results: Fasting gal-3 and gal-9 were elevated in the HR cohort compared to the LR cohort. Body mass index was positively associated with gal-3 and gal-9, while gal-3 was also linked to insulin sensitivity. After glucose challenge, gal-1, -3, -7, and -9 decreased in the LR cohort; in the HR cohort, only gal-1 and gal-7 decreased after 2 hours, while gal-3 and gal-9 remained unchanged. Gal-1 correlated positively with homeostasis model assessment for insulin resistance (HOMA-IR) and inversely with insulin sensitivity across the OGTT in the LR cohort, but some of these correlations were not observed in the HR cohort.
Conclusion: Galectins exhibited distinct patterns of association with glucose homeostasis during the second trimester of pregnancy. Gal-3 and gal-9 are associated with chronic conditions such as pre-pregnancy obesity and insulin resistance, whereas gal-1 appears to be particularly sensitive to the acute glucose challenge.

Keywords: Galectin 1; Galectin 3; Galectin 9; Glucose tolerance test; Insulin resistance; Obesity, morbid; Pregnancy

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INTRODUCTION

Pregnancy is a dynamic process that involves structural and functional changes in the maternal body, often described as a biological 'stress test' for multiple organ systems. These adaptations include physiological changes in glucose and lipid metabolism, which are critical for ensuring glucose transport from the maternal to the fetal circulation. During the first trimester, placental-derived hormones and growth factors lead to enhanced proliferation of pancreatic β-cells and thus, increased insulin secretion and insulin sensitivity. However, as pregnancy progresses, insulin sensitivity gradually decreases, especially in late gestation [1]. Growing evidence suggests that pregnancy complications arise when these maternal adaptations fail, particularly within the metabolic and cardiovascular systems [2].

The rising prevalence of obesity worldwide, especially in the younger population, has exacerbated the public health burden. As a result, women of reproductive age are more likely to have metabolic characteristics that increase the risk of pregnancy complications [3]. Maternal metabolic disorders during pregnancy negatively impact pregnancy outcomes but also increase susceptibility to long-term health problems in both mother and child [4,5]. Mothers who experience obesity before or during pregnancy, as well as their children, face an increased risk of developing metabolic syndrome, high blood pressure, early-onset type 2 diabetes mellitus, and cardiovascular disease later in life [6,7]. In women suffering from overweight or obesity, pre-conceptional insulin resistance is superimposed on pregnancy-related insulin resistance leading to an increased risk of gestational diabetes mellitus (GDM) [8,9]. Therefore, a deeper understanding of the mechanisms underlying these metabolic disorders is crucial for improving the metabolic trajectory towards better health of both mother and child.

Galectins belong to a family of β-galactoside-binding proteins that emerge as key regulators of several processes during pregnancy, including maternal vascular and immune adaptations and placentation [10]. Based on their structure, galectins are classified as prototype (e.g., galectin [gal]-1, gal-7), containing one carbohydrate recognition domain (CRD), chimeric (gal-3), or tandem repeat galectins with two CRDs (e.g., gal-9 and gal-12) [10]. During healthy pregnancies, maternal serum levels of gal-1, gal-3, gal-7, and gal-9 progressively increase [11-13]. However, dysregulation of galectin expression has been identified in several pregnancy complications such as preeclampsia and GDM [11,14,15]. Indeed, we have previously shown that in pregnancies complicated with GDM, circulating gal-1 levels fail to rise during the second and third trimesters, and gal-3 levels are reduced at the end of gestation compared to healthy pregnancies [12,15]. However, other reports demonstrated that gal-3 were increased in women with GDM during the first and second trimesters compared to healthy pregnant women [16-18]. Additionally, increased gal-1 and gal-3 levels have been associated with obesity-related metabolic dysfunction in the non-pregnant state [19-23].

Despite these observations, the role of these galectins in overweight, obesity and metabolic disorders during pregnancy remains poorly understood. While galectin levels and glucose metabolism appear to be linked, it is unclear whether hyperglycemia during pregnancy alters maternal galectin levels or if dysregulated galectins contribute to an abnormal glucose response. To address this gap, we assessed the maternal serum galectin profile (including the four predominant galectins gal-1, gal-3, gal-7, and gal-9) in two cohorts of pregnant women: one at low-risk (LR) for metabolic complications, consisting primarily of women with normal weight, and a high-risk (HR) group, including women with overweight and obesity. Maternal galectin levels were measured in both the fasting state and after the hyperglycemic challenge (standardized oral glucose tolerance test [OGTT]) at 24 to 28 weeks of gestation, to better understand how metabolic stress influences galectin profile in pregnancy.

METHODS

Data collection and study designs

This study was a secondary analysis of samples available from two longitudinal pregnancy cohorts conducted in Graz, Austria (HMO study; ClinicalTrials.gov Identifier: NCT05496712) (n=91) [24] and Amsterdam, The Netherlands (FitFor2 trial; trial registration number: NTR1139) (n=60) (Fig. 1) [25]. Participants of the Graz cohort, a prospective, observational, longitudinal study of pregnant women with predominantly normal weight, were recruited at the Department of Obstetrics and Gynecology of the Medical University of Graz between 2014 and 2021. Pregnant women came to the clinics for routine prenatal care at multiple time points during pregnancy and for delivery. The study was approved by the ethical committee of the Medical University of Graz, under the number EK# 26-380 ex13/14. Due to the clinical characteristics of the cohort, we considered it as LR cohort. Participants from the Amsterdam cohort had been recruited in three large hospitals and ten mid-

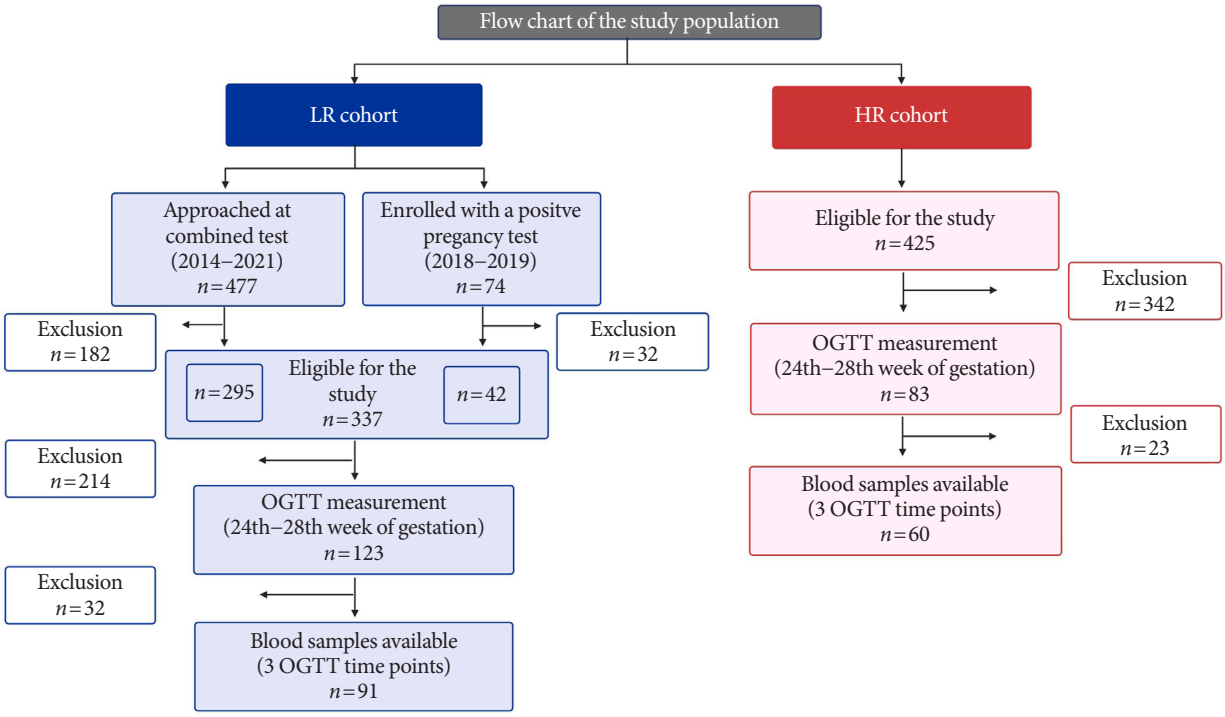

Fig. 1. Flow chart summarizing participants in the low-risk (LR) cohort and high-risk (HR) cohort. The reasons for exclusion of both cohorts are lost for follow-up/missed visits or because of personal/medical reasons. OGTT, oral glucose tolerance test.

wifery practices in Amsterdam, the Netherlands, between 2007 and 2011. The study was approved by the Medical Ethics Committee of VU University Medical Center in Amsterdam (2007/133). This cohort was originally designed as a randomized controlled trial with a lifestyle intervention in women with overweight and obesity at increased risk of GDM. Therefore, we herein refer to it as HR cohort. Inclusion criteria for this study were pre-pregnancy overweight (body mass index [BMI] ≥25 kg/m^2) in combination with at least one other risk factor (history of macrosomia, history of GDM, or type 2 diabetes mellitus in family history) or obesity (BMI ≥30 kg/m^2). In both cohorts, study participants had given informed consent and exclusion criteria included multiple pregnancies, pre-existing diabetes, the use of medication affecting insulin secretion or sensitivity and hypertension.

OGTT and GDM diagnostic criteria

Both cohort studies had a total of three visits during pregnancy at the outpatient clinics. Study participants underwent an OGTT in mid-pregnancy, between the 24th and 28th gestational week after overnight fasting (at least 8 hours prior to the test). In the LR cohort, women received a standardized 75 g glucose load (2-hour OGTT), while in the HR cohort women received 100 g glucose (3-hour OGTT). From both cohorts, venous blood samples drawn in the fasting state, 1 hour, and 2 hours after glucose load were used in this study. GDM was diagnosed according to the International Association of Diabetes and Pregnancy Study Groups/World Health Organization 2013 criteria for 75 g OGTT if fasting plasma glucose was ≥5.1 mmol/L, 1 hour post-load ≥10.0 mmol/L, and after 2 hours ≥8.5 mmol/L [26]. For the 100 g OGTT, GDM was defined as ≥2 plasma glucose concentrations >5.3 mmol/L (fasting), >10.0 mmol/L (1 hour), >8.6 mmol/L (2 hours), or >7.8 mmol/L (3 hours) according to American Diabetes Association criteria [27].

Measurement of glucometabolic parameters and calculation of indices

Concentrations of glucose, insulin and C-peptide were measured in fasting, 1-hour, and 2-hour blood samples. Plasma glucose was measured using a Gluco-quant glucose/HK kit (Glucoquant/Hitachi Modular P analyzer, Roche Diagnostics, Basel, Switzerland). Serum concentrations of insulin and C-peptide were measured by a chemiluminescent immunoassay

(Luminescence Advia Centaur, Siemens Medical Solutions Diagnostics, Erlangen, Germany). Concentrations are given as pmol/L for insulin, mmol/L for glucose, nmol/L for C-peptide. Based on the measured metabolic parameters, the respective indices were calculated. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated from the fasting glucose and insulin values, according to the formula [fasting glucose concentration (mmol/L)×fasting insulin concentration (pmol/L)/6.945]/22.5 [28]. Whole body insulin sensitivity was estimated by calculating the Matsuda index according to the formula=10,000/√[(fasting glucose×fasting insulin)×(mean glucose during OGTT×mean insulin during OGTT)] [29].

Determination of circulating galectins levels

Galectins were measured in serum samples collected during the OGTT (fasting, 1 hour, and 2 hours) by enzyme-linked immunosorbent assay (ELISA) as previously described [30]. Briefly, 96-well half-area plates (CLS3690, Corning Inc., Corning, NY, USA) were coated overnight with either polyclonal anti-human gal-1, gal-3, gal-7, or gal-9 antibodies (500-P210, PeproTech, Cranbury, NJ, USA; AF 1154, 842118 and AF 2045, R&D Systems, Minneapolis, MN, USA, respectively) and washed with washing buffer. Plates were blocked with 1%–2% bovine serum albumin in phosphate-buffered saline. Individual wells were incubated with serial dilutions of galectin standards or serum samples for 1 to 2 hours at room temperature. Wells were washed and incubated with biotinylated polyclonal anti-human galectin antibodies (500-P210BT, PeproTech; BAF 1154, 842119, and BAF 2045, R&D Systems, respectively). Plates were washed three to six times and incubated with horseradish peroxidase-conjugated streptavidin (189733, Calbiochem, San Diego, CA, USA). After 3–8 additional washes, a colorimetric reaction was developed with the 3,3,5,5′-tetramethyl benzidine (TMB). The reaction was stopped by adding one volume of 4 N H_2SO_4 and absorbance at 450 nm was recorded. In each ELISA, the reported value is the mean of triplicate assays.

Statistical analysis

GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA, USA), R software version 4.2.3 (R Foundation for Statistical Computing, Vienna, Austria), or IBM SPSS statistics version 29.0 (IBM Co., Armonk, NY, USA) were used for all statistical analyses. Data was tested for normality and appropriate parametric or non-parametric tests were selected. Multivariable linear regression models were used for assessing the association of cohort (LR vs. HR) and metabolic parameters (BMI, insulin sensitivity, and GDM status) with fasting galectin levels, adjusted for maternal age, ethnicity, and smoking. Possible interaction of metabolic parameters with cohort was tested by adding the interaction term to the model. When significant, analyses were performed for each cohort separately. Since the glucose load used in the OGTT was different between cohorts, analyses with non-fasting galectin levels were performed for each cohort separately. Changes in galectin levels between fasting, 1-hour, and 2-hour time points were tested using the Friedman *post hoc* Dunn test. Partial correlations between metabolic parameters and galectin levels at different time points of the OGTT were evaluated by the Spearman rank test, with adjustment for maternal age, ethnicity and smoking. *P* values less than 0.05 were considered statistically significant.

RESULTS

General and biochemical characteristics of the study participants

A total of 151 pregnant women from two independent cohorts were included in this study, 91 women with predominantly normal weight (LR cohort) and 60 women with overweight and obesity (HR cohort). Table 1 provides clinical characteristics from participants of the respective cohort included in this study. In the LR cohort, 74.7% of women had a pre-pregnant BMI <25 kg/m^2 (68/91), 17.6% had overweight (16/91), and 7.7% suffered from obesity (7/91). Overall, the GDM incidence was 5.5% (5/91). In contrast, the HR cohort consisted of 20% (12/60) of women with overweight, 80% (48/60) with obesity. In the HR cohort 20% (12/60) of women developed GDM. As expected from the study design, the median BMI was significantly higher in the HR cohort compared to the LR cohort (32.1 kg/m^2 vs. 22.7 kg/m^2, respectively; $P<0.0001$) along with an increased GDM prevalence in the HR cohort.

Biochemical characteristics also differed markedly between the two study populations, with significant differences observed in fasting glucose, insulin, C-peptide and the Matsuda and HOMA-IR indexes (Table 2), further underscoring the metabolic disparities between the LR and HR cohorts.

Galectin signature during fasting reflects maternal metabolic status

We first assessed how maternal metabolic status influences the galectin profile during the second trimester of pregnancy. As

Table 1. Clinical characteristics in the low-risk and high-risk cohort

Cohorts feature	LR (n=91)	HR (n=60)
Age, yr	35.1±4.3	30.4±4.7
Pre-pregnant BMI, kg/m^2, median (IQR)	22.7 (20.4–25.4)	32.1 (30.2–35.0)[a]
Weight		
Normal weight (BMI <25 kg/m^2)	68 (74.7)	0[c]
Overweight (BMI 25–29.9 kg/m^2)	16 (17.6)	12 (20)
Obesity (BMI ≥30 kg/m^2)	7 (7.7)	48 (80)[c]
Ethnicity		
Caucasian	90 (98.9)	28 (46.7)
Non-Caucasian	1 (1.1)	29 (48.3)
Unknown	0	3 (5)
GDM	5 (5.5)	12 (20)[b]
Gestational week at sampling, median (IQR)	25 (24–25)	25 (24–25)
Newborn		
Birthweight, g, median (range)	3,357 (2,100–4,594)	3,360 (2,530–4,800)
Sex		
Male	30 (33.0)	28 (46.7)
Female	54 (59.3)	32 (53.3)
Unknown	7 (7.7)	0

Values are presented as mean±standard deviation or number (%) unless otherwise indicated.
LR, low-risk; HR, high-risk; BMI, body mass index; IQR, interquartile range; GDM, gestational diabetes.
[a]P<0.0001 analyzed by Mann-Whitney test, and [b]P<0.05, [c]P<0.0001 analyzed by chi-square test compared to LR cohort.

Table 2. Biochemical parameters of the low-risk and high-risk cohorts

Cohorts feature	LR (n=91)	HR (n=60)
Glucose, mmol/L		
Fasting	4.3 (4.1–4.4)	4.8 (4.4–5.1)[a]
1 hour	6.7 (5.6–7.7)	7.4 (6.2–8.7)
2 hours	5.7 (4.8–6.5)	6.4 (5.7–7.2)
Insulin, pmol/L		
Fasting	53.4 (37.5–70.8)	100.8 (70.1–131.0)[a]
1 hour	426.1 (316.5–676.7)	740.3 (483.3–954.9)
2 hours	349.1 (245.7–444.2)	554.4 (360.8–986.9)
C-peptide, nmol/L		
Fasting	0.36 (0.28–0.44)	0.55 (0.42–0.68)[a]
1 hour	2.04 (1.67–2.53)	2.26 (1.55–2.85)
2 hours	1.80 (1.50–2.30)	2.39 (1.59–3.16)
Matsuda	6.59 (4.72–8.55)	3.14 (2.18–4.40)[a]
HOMA-IR	1.42 (1.04–2.05)	3.04 (2.08–4.08)[a]

Values are presented as median (interquartile range).
LR, low-risk; HR, high-risk; HOMA-IR, homeostasis model assessment for insulin resistance.
[a]P<0.0001 vs. LR (fasting) analyzed by Mann-Whitney test.

shown in Fig. 2A, circulating levels of gal-3 and gal-9 were significantly higher in the HR cohort compared to the LR cohort. Similarly, when pregnant women were categorized based on pre-pregnancy BMI, increased levels of gal-3 and gal-9 were observed among pregnant women with obesity (Supplementary Fig. 1). After adjusting for potential confounders, no significant differences were observed between cohorts regarding maternal circulating concentrations of gal-1 and gal-7 in the fasting state. However, pregnant women in the HR cohort exhibited significantly higher levels of gal-3 and gal-9 in the fasting state compared to the LR cohort (Table 3, model 1). These differences were only minimally attenuated when adjusting for metabolic characteristics such as pre-pregnant BMI, Matsuda index, and GDM (Table 3, model 2). Additionally, pre-pregnant BMI was positively associated with the levels of gal-1, gal-3, and gal-9, with this association observed for gal-1 only in the HR cohort. Insulin sensitivity was positively associated with gal-3 levels.

Further analysis of the relative abundance of each galectin revealed that gal-1 was the most abundant in maternal circulation, followed by gal-3, gal-7, and gal-9 (Supplementary Fig. 2).

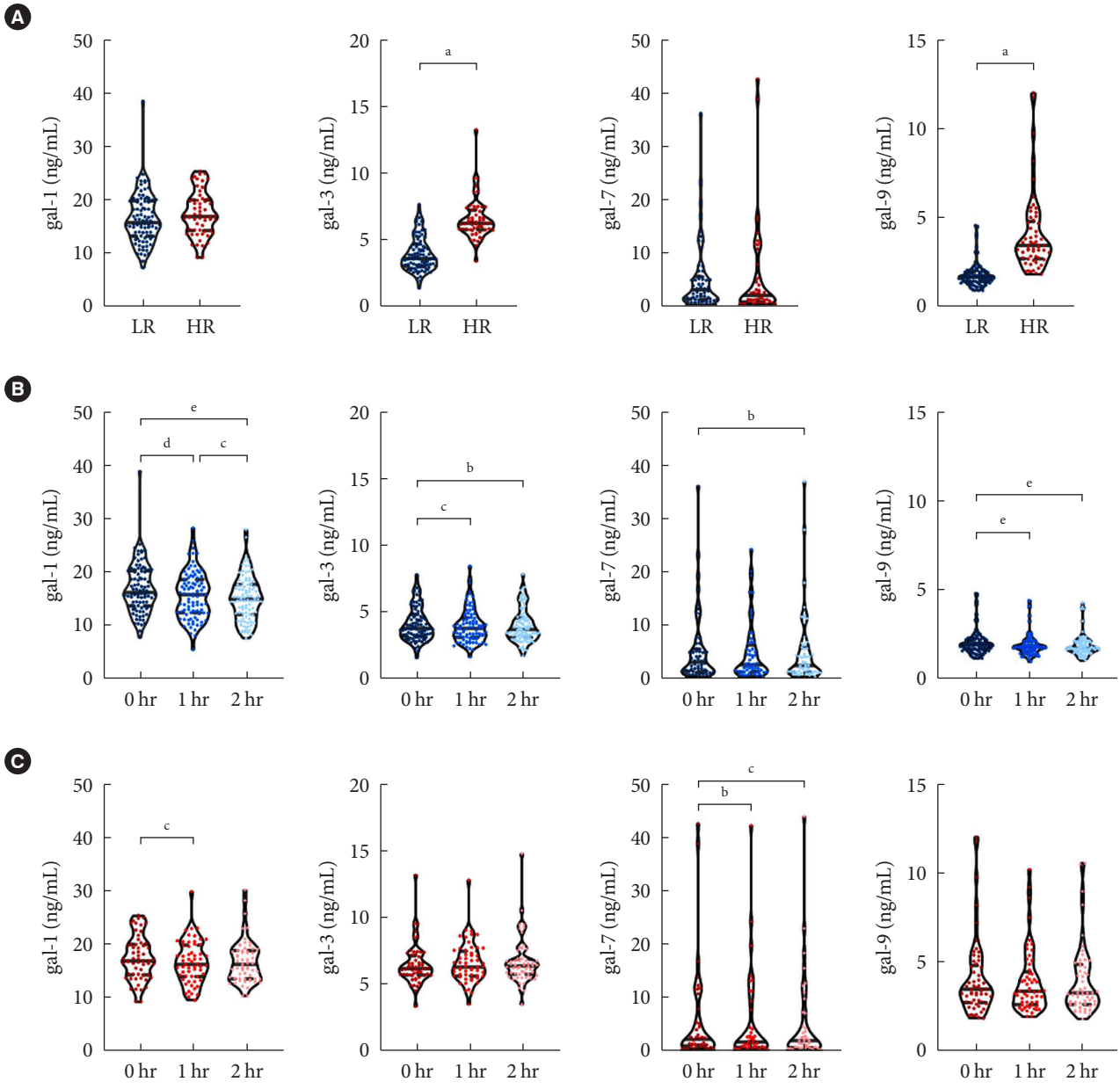

Fig. 2. Unadjusted comparison between the serum galectins levels (fasting state) in low-risk (LR) cohort and high-risk (HR) cohort. Data are presented as violin plots with individual values, median and interquartile range (A). Violin plots showing the serum levels (ng/mL) of galectin (gal)-1, gal-3, gal-9, and gal-7 in LR cohort (B) or HR cohort (C) analyzed by enzyme-linked immunosorbent assay (ELISA) at three time points of the oral glucose tolerance test (0, 1, and 2 hours) at 24–28 weeks of gestation. [a]$P<0.0001$ (Mann-Whitney test); [b]$P<0.05$, [c]$P<0.01$, [d]$P<0.001$, and [e]$P<0.0001$ (Friedman and Dunn's post-test).

Interestingly, we observed a lower relative concentration of gal-1 (57.1% vs. 65.2%) and a higher percentage of gal-3 (21.4% vs. 15.9%) in the HR cohort compared to the LR cohort. This suggests that both absolute concentrations and relative distributions of galectins are sensitive to maternal metabolic status.

Dynamics of circulating galectins during the OGTT and correlations with glucometabolic parameters

Next, we measured maternal serum galectin concentrations during the OGTT at fasting (0 hour), 1 hour, and 2 hours after glucose load in each cohort separately. In the LR cohort, gal-1 significantly decreased after glucose load (1 hour and 2 hours),

Table 3. Multivariable linear regression analyses of associations between maternal metabolic characteristics and galectins

	Model 1		Model 2	
	Beta (95% CI)	*P* value	Beta (95% CI)	*P* value
gal-1				
Cohort (LR vs. HR)	−1.41 (−3.58 to 0.76)	0.202	0.68 (−2.02 to 3.39)	0.618
Pre-pregnant BMI	-	-	0.18 (0.01 to 0.35)	0.039[a]
LR	-	-	−0.04 (−0.29 to 0.21)	0.759
HR	-	-	0.39 (0.17 to 0.62)	0.001[a]
Matsuda index	-	-	−0.24 (−0.53 to 0.05)	0.097
GDM (no vs. yes)	-	-	−1.21 (−3.89 to 1.48)	0.375
gal-3				
Cohort (LR vs. HR)	−3.12 (−3.75 to −2.48)	<0.001[a]	−2.60 (−3.40 to −1.81)	<0.001[a]
Pre-pregnant BMI	-	-	0.07 (0.02 to 0.12)	0.009[a]
Matsuda index	-	-	0.12 (0.04 to 0.20)	0.006[a]
GDM (no vs. yes)	-	-	0.60 (−0.19 to 1.38)	0.135
gal-7 (ln)				
Cohort (LR vs. HR)	0.31 (−0.41 to 1.02)	0.392	0.59 (−0.39 to 1.56)	0.238
Pre-pregnant BMI	-	-	0.03 (−0.04 to 0.09)	0.388
Matsuda index	-	-	−0.01 (−0.11 to 0.08)	0.766
GDM (no vs. yes)	-	-	−0.12 (−1.04 to 0.80)	0.799
gal-9 (ln)				
Cohort (LR vs. HR)	−0.74 (−0.90 to −0.57)	<0.001[a]	−0.55 (−0.75 to −0.34)	<0.001[a]
Pre-pregnant BMI	-	-	0.02 (0.01 to 0.04)	0.002[a]
Matsuda index	-	-	0.01 (−0.01 to 0.04)	0.216
GDM (no vs. yes)	-	-	0.07 (−0.13 to 0.28)	0.486

Models were adjusted for maternal age, ethnicity, and smoking.
CI, confidence interval; gal, galectin; LR, low-risk; HR, high-risk; BMI, body mass index; GDM, gestational diabetes; ln, natural logarithm.
[a]Statistical significance.

as shown in Fig. 2B and Supplementary Table 1, with the most marked difference found between the fasting and 2-hour values ($P<0.0001$). Gal-3 concentrations also significantly decreased from fasting to 1-hour and to 2-hour post-glucose load ($P<0.01$ and $P<0.05$, respectively). In addition, gal-9 significantly decreased over time between fasting and 1-hour and 2-hour measurements ($P<0.0001$). Gal-7 significantly decreased from fasting state to 2-hour measurement after glucose load ($P<0.05$). The HR cohort showed different dynamics with less pronounced changes in galectins in response to glucose load (Fig. 2C and Supplementary Table 1). Like the LR cohort, circulating gal-1 levels also significantly decreased in this cohort between fasting and 1 hour post-glucose load ($P<0.01$), and maternal gal-7 levels decreased from fasting to 1 hour ($P<0.05$) and from fasting to 2 hours ($P<0.01$). In contrast to the LR cohort, gal-3 and gal-9 concentrations did not fluctuate during the OGTT.

We then assessed correlations between the most abundant maternal circulating galectins (gal-1, gal-3, and gal-9) at the different time points of the OGTT. In the LR cohort, a negative correlation was found between gal-1 and gal-3 levels and a positive correlation between gal-3 and gal-9 levels at all OGTT time points (Supplementary Fig. 3A). In the HR cohort, however, only the positive correlation between maternal circulating levels of gal-3 and gal-9 persisted (Supplementary Fig. 3B).

To evaluate the relationship of galectins with maternal metabolic adaptations during the response to glucose load, Spearman correlation was performed. As shown in Fig. 3A, in the LR cohort, maternal serum gal-1 (0, 1, 2 hours) levels positively correlated with insulin (fasting), and gal-1 (1 hour) also positively

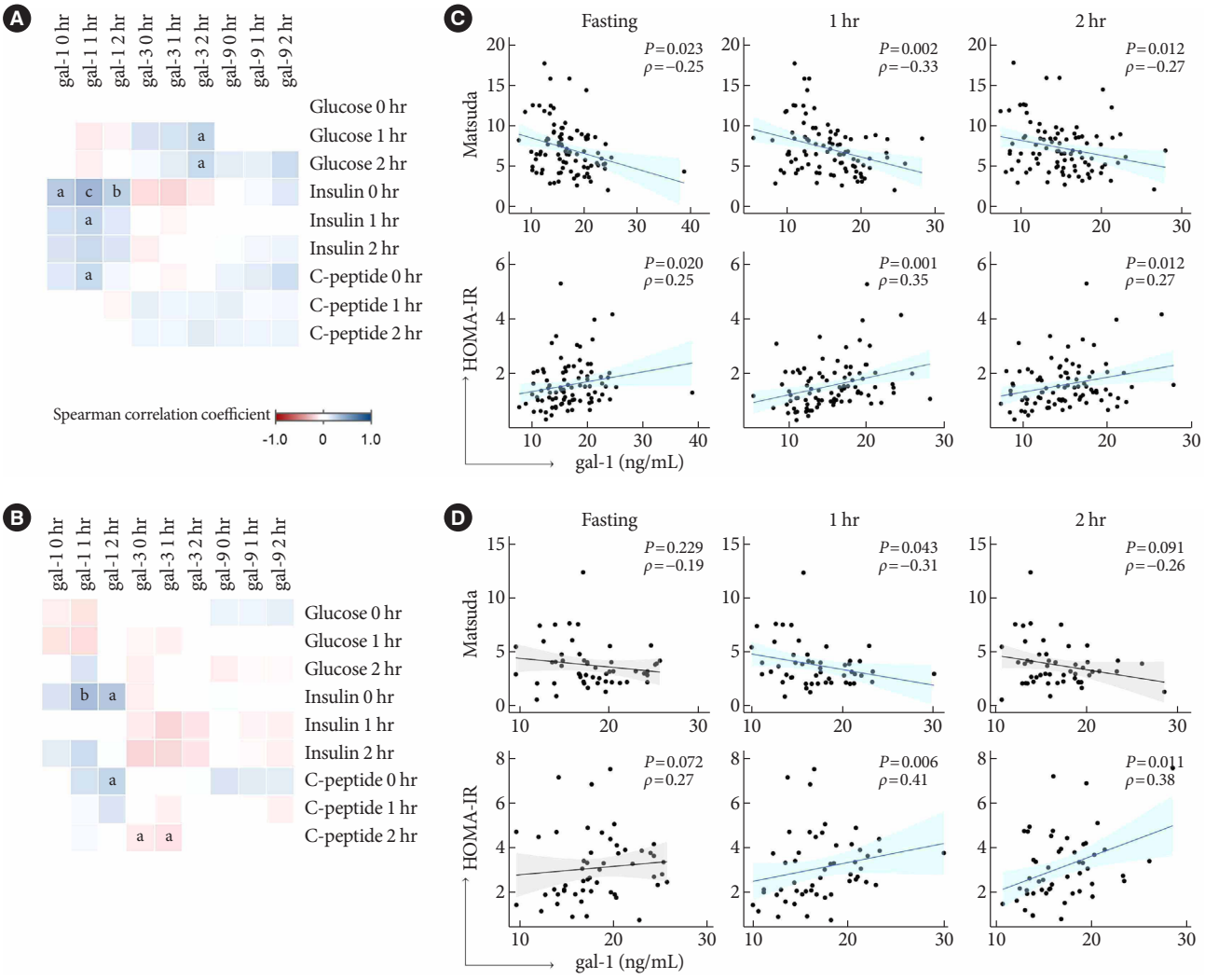

Fig. 3. Correlation between serum levels of galectins and oral glucose tolerance test measurements in the (A) low-risk (LR) and (B) high-risk (HR) cohort. Partial correlation coefficient values are indicated by the color (blue positive and red negative). Partial correlations between gal-1 and Matsuda (whole body insulin sensitivity index) or homeostasis model assessment for insulin resistance (HOMA-IR) in (C) LR cohort and in (D) HR cohort. The partial Spearman correlation coefficient (ρ) is shown. $P<0.05$ was considered statistically significant (light blue) and $P>0.05$ not significant (grey). The significance levels are indicated within squares: $^{a}P<0.05$, $^{b}P<0.01$, and $^{c}P<0.001$.

correlated with insulin (1 hour) and C-peptide (fasting). Furthermore, gal-3 (2 hours) positively correlated with glucose (1 and 2 hours). In the HR cohort, serum levels of gal-1 (1 and 2 hours) were significantly correlated with fasting insulin, and gal-1 (2 hours) with C-peptide (fasting). Gal-3 (fasting and 1 hour) was negatively correlated with C-peptide (2 hours) (Fig. 3B).

Correlations between gal-1 and insulin sensitivity/resistance during the OGTT

Considering that both gal-1 and gal-3 positively correlated with insulin and C-peptide during the OGTT, we further examined the relationship between these galectins and indices for insulin sensitivity (Matsuda index) and insulin resistance (HOMA-IR index). Within the LR cohort, we observed significant negative correlations between gal-1 and the Matsuda index at all three time points (Fig. 3C upper panel). We also found significant positive correlations between serum gal-1 levels and the HOMA-IR index, which serves as a measure of insulin resistance at all three time points (Fig. 3C lower panel). These results indicate that higher gal-1 levels were correlated

with reduced insulin sensitivity and increased insulin resistance. However, in the HR cohort, the correlation between gal-1 and the Matsuda index was only observed at 1 hour, and between gal-1 and the HOMA-IR index at both 1 and 2 hours (Fig. 3D). Correlation analyses between Matsuda or HOMA-IR and gal-3, gal-7, and gal-9 during the OGTT revealed no relationship in both LR and HR cohorts (data not shown).

DISCUSSION

This study provides a detailed characterization of circulating galectin profiles (specifically gal-1, gal-3, gal-7, and gal-9) during the second trimester of pregnancy in two distinct cohorts: a LR group of women with normal weight and a HR group of women with overweight or obesity, and thus, predisposed to GDM. Our findings show elevated fasting levels of gal-3 and gal-9 in the HR cohort, and positive associations between pre-pregnant BMI and both gal-3 and gal-9, and between gal-3 and insulin sensitivity. In response to an OGTT, maternal galectin levels decreased in the LR cohort across all four galectins, suggesting glucose- and insulin-mediated downregulation in metabolically healthy pregnancies. In contrast, the HR cohort exhibited a selective response, with only gal-1 and gal-7 decreasing, while gal-3 and gal-9 remained stable. Further, gal-1 showed a consistent positive correlation with insulin resistance (as determined by HOMA-IR) in the LR cohort across all OGTT time points, a relationship that weakened in the HR cohort. These findings underscore the multifaceted roles of galectins in maternal metabolic adaptation and their potential use as biomarkers of metabolic health during pregnancy.

Our findings revealed significant differences in fasting galectin levels between the LR and HR cohorts, with gal-3 and gal-9 concentrations being higher in the HR cohort. Although the cohorts differ in pre-pregnant BMI, insulin sensitivity, and incidence of GDM, the differences in gal-3 and gal-9 remained independent of these parameters in the multivariable models. Other cohort-related factors, such as sample processing, should also be considered.

Our results align with previous studies in non-pregnant individuals, where gal-3 was positively correlated with BMI [19,20]. The positive association between BMI and gal-9 introduces a new aspect of this galectin's role in metabolic regulation. Although data regarding gal-9 in metabolic disorders is limited, a previous study indicated elevated plasma levels of gal-9 in patients with obesity-related type 2 diabetes mellitus, suggesting its potential involvement in pathophysiological mechanisms [31]. Interestingly, gal-1 was only associated with pre-pregnant BMI in the HR cohort. While earlier studies have shown a positive correlation between gal-1 levels and obesity in non-pregnant populations [32], our results may suggest that the metabolic changes of pregnancy alter the normal relationship between gal-1 and body weight. Alternatively, the absence of an association in the LR cohort could be due to the narrower BMI range within this group, which may limit the ability to detect a significant association. Maternal obesity is a well-established risk factor for the development of GDM. Although our study did not primarily aim to identify GDM-specific galectin signatures due to the limited number of women with GDM in our cohorts, our findings are consistent with previous studies from our laboratory showing that in women with GDM both gal-1 and gal-3 levels did not increase during the second and third trimesters [12,15].

The role of gal-3 in insulin resistance remains complex and context-dependent, as evidenced by conflicting findings from various investigations. Several studies have demonstrated a positive association between gal-3 levels and insulin resistance, measured by the HOMA-IR, in both non-pregnant populations [21] and pregnant women [16]. Consistent with this, studies in mouse models fed a high-fat diet have demonstrated that gal-3 deficiency is associated with reduced insulin levels [21], whereas its overexpression promotes β-cell apoptosis [33]. Additionally, *in vitro* experiments have demonstrated that gal-3 treatment inhibits the insulin signaling pathway in hepatocytes, muscle cells, and adipocytes [21]. These findings suggest that elevated gal-3 may contribute to insulin resistance and impaired glucose metabolism in certain settings.

In contrast, other studies indicate an opposing relationship between gal-3 and metabolic regulation. For instance, experiments with gal-3 deficient mice have revealed hyperglycemia and impaired glucose tolerance [34,35], suggesting that gal-3 may play a protective role in glucose homeostasis. Furthermore, under normal feeding conditions, gal-3 absence impairs adipocyte differentiation, leading to adipose tissue dysfunction, inflammation, insulin resistance, and compromised glucose regulation [36]. In non-pregnant individuals, low gal-3 levels have also been correlated with insulin resistance [37], reinforcing the notion that gal-3 deficiency, rather than excess, may exacerbate metabolic dysregulation. These discrepancies highlight the need for longitudinal studies in metabolically well-characterized cohorts to clarify the role of gal-3 in insulin

resistance and sensitivity, particularly during pregnancy.

The inconsistent associations between gal-3, BMI, and insulin sensitivity may reflect limitations in using BMI as a reliable indicator of metabolic health. It is increasingly recognized that body mass alone does not consistently predict metabolic outcomes [38]. This is evidenced by the existence of metabolically healthy individuals with obesity and insulin-resistant individuals with normal weight [39], which add on to the complexity of gal-3 metabolic effects across different populations. Such heterogeneity underscores the importance of considering additional factors, such as percent body fat, adipose tissue function and inflammatory status, when evaluating gal-3 and its physiological impact.

Our study also identified a shift in the maternal circulatory galectin profile, with decreased relative gal-1 and elevated gal-3 levels in the HR cohort. This result was in accord with earlier work from our group, which demonstrated that gal-1-deficient pregnant dams have a dysregulated placental galectin profile, with elevated gal-3 and gal-9 levels [40]. Taken together, these data suggest that a balanced galectin composition may be critical for maintaining metabolic adaptations and supporting a healthy pregnancy. Disruptions in this balance could contribute to adverse gestational outcomes, warranting further investigation into the interplay of galectins in maternal metabolism.

This study is the first to investigate dynamic changes in serum galectin concentrations in response to a glucose load during an OGTT in the second trimester of pregnancy. In the LR cohort, we observed a consistent reduction in all evaluated galectins (gal-1, gal-3, gal-7, and gal-9) following glucose administration. In contrast, the HR cohort displayed a more selective response, with only gal-1 and gal-7 decreasing at 1 hour post-glucose load, while gal-3 and gal-9 showed no significant changes. These differential patterns suggest that galectin responses are modulated by maternal metabolic status, reflecting distinct regulatory mechanisms in glucose metabolism across risk groups.

The observed reduction in circulating galectin levels following the glucose peak during OGTT may be the consequence of some physiological mechanisms. These include reduced galectin expression or secretion, increased cellular uptake, or cell-surface sequestration. Given the pleiotropic roles of galectins at both extracellular and intracellular sites, such changes are most probably the outcome of a dynamic equilibrium in galectin distribution between compartments. For example, galectins are rapidly endocytosed upon binding to cell-surface glycoproteins, a process critical for modulating receptor exposure and signal transduction [41]. Notably, gal-9 binds to the N-glycosylation site of glucose transporter 2 (GLUT2) in hepatocytes and pancreatic β-cells, stabilizing its expression and influencing insulin secretion [42,43]. Similarly, gal-3 interacts with the insulin receptor, potentially impairing insulin signaling and contributing to insulin resistance [21]. Thus, the glucose-induced modulation of galectin levels observed in this study may represent a physiological mechanism for fine-tuning glucose homeostasis through receptor activity and glycosylation patterns.

One of the interesting observations in the HR cohort, where approximately 70% of women were insulin resistant, was the lack of decrease in gal-3 and gal-9 levels post-glucose load. This could indicate that these galectins are elevated in the fasting state due to underlying metabolic disturbances, thereby masking any subsequent decline. Alternatively, obesity-induced inflammation or hyperglycemia may alter cell-surface glycoproteins glycosylation, thereby impairing galectin binding affinity. Supporting this hypothesis, Lee et al. [44] demonstrated that GDM reduces sialylation of glycosylated glycodelin-A, diminishing its binding to lymphocytes and its immunomodulatory activity. Recent evidence also suggests that nutritional changes can modify O-GlcNAcylation of gal-3, affecting its secretion and role in clathrin-independent endocytosis, with increased gal-3 release linked to reduced endocytic activity [45]. Furthermore, animal studies have shown that high-fat diets downregulate glycosyltransferase enzymes, such as *Mgat4a*, which are essential for gal-9 binding to GLUT2. This reduction impairs GLUT2 expression and glucose uptake, highlighting a potential link between metabolic status and galectin regulation [46].

Our findings also align with prior observations from *ex vivo* placental perfusion models, where high glucose concentrations suppressed gal-1 release [15]. This suggests that acute hyperglycemia may similarly inhibit galectin production *in vivo*, contributing to the decreased levels observed during OGTT in the LR cohort. However, the blunted galectin response in the HR cohort indicates disruption of such regulatory mechanism under conditions of chronic metabolic stress, such as insulin resistance and obesity. We propose that variation in carbohydrate availability in metabolically compromised individuals may affect glycosylation pattern of cell-surface glycoproteins influencing cellular responses to glucose. Nevertheless, differences in glucose loads administered during OGTT across co-

horts may have also contributed to the differential galectin responses observed, warranting further investigation.

Although gal-1 levels did not differ significantly between cohorts, gal-1 in the LR cohort correlated positively with insulin, C-peptide, and HOMA-IR, and negatively with the Matsuda index across the OGTT, suggesting a link between gal-1 and insulin sensitivity as well as glucose homeostasis. These associations weakened in the HR cohort, suggesting metabolic dysregulation compromises the regulatory role of gal-1. In the LR cohort, gal-1 and gal-3 showed an inverse correlation, hinting at complementary functions in metabolically healthy pregnancies, a balance lost in the HR group. Notably, gal-3 and gal-9 were positively correlated in both cohorts, suggesting interrelated roles in metabolism and immune regulation [47]. The novel involvement of gal-9 in pregnancy metabolism merits further study.

Understanding circulating galectin profiles (gal-1, gal-3, gal-7, and gal-9) during pregnancy may help identify biomarkers of maternal metabolic health, particularly in the context of obesity-related metabolic dysregulation. As mentioned above, the elevated fasting gal-3 and gal-9 levels in the HR cohort suggest that these two galectins can be employed as early markers of metabolic disturbances, even when any metabolic complication like GDM is not yet clinically diagnosed.

The differential galectins dynamics throughout the OGTT in both cohorts illustrate the mechanisms by which obesity and insulin resistance disrupt normal pregnancy metabolism. Through the identification of galectin signatures associated with metabolic dysregulation (upregulation of gal-3 and gal-9, impaired gal-1 dynamics), this work offers bases for the use of these proteins as predictors. Early detection of pregnancy-related metabolic risk may enable timely interventions such as lifestyle modification or closer monitoring to prevent or mitigate GDM and associated complications, including macrosomia, preeclampsia, and the future risk of type 2 diabetes mellitus in both mother and child.

This study characterizes the maternal circulating galectin profiles during the 2nd trimester; however, longitudinal research investigating galectin dynamics across all trimesters in the same individuals is needed to completely understand their roles in pregnancy-related metabolic regulation. Having a larger cohort would also enable the inclusion of more patients with pregnancy complications, enhancing the possibility to demonstrate if galectins can serve as predictor for GDM or other disorders. Additionally, our study demonstrates the limitation of BMI as a sole indicator of metabolic health; future investigations should incorporate more comprehensive assessments (e.g., adipose tissue function, inflammation) that could refine interpretations of galectin levels and their metabolic significance.

A major strength of our study includes its comprehensive galectin profiling in two well-characterized cohorts and its pioneering analysis of galectin responses to an OGTT. By measuring gal-1, gal-3, gal-7, and gal-9, particularly the understudied gal-7 and gal-9, we provide novel insights into their metabolic roles, laying groundwork for future research and potential therapeutic applications, especially given ongoing trials of galectin inhibitors in metabolic diseases [48]. However, the principal limitation of this study is the differing glucose loads (75 g in LR vs. 100 g in HR) due to recruitment centers policies. This heterogeneity in glucose exposure makes it difficult to determine whether differences in galectin profiles during the OGTT are associated with the metabolic status of the pregnant women or a consequence of different glucose stimuli. Currently, there is no previous data suggesting that glucose levels modify galectins stability. While we cannot exclude the possibility that the different glucose loads may affect galectins dynamics, it appears unlikely that this factor alone fully account for the observed differences. Fetal sex distribution (33% male in LR vs. 47% in HR) did not significantly affect galectin levels, minimizing its confounding impact.

This study elucidates the distinct dynamics of gal-1, gal-3, gal-7, and gal-9 in pregnant women at varying risks for GDM. Elevated gal-3 and gal-9 in the HR cohort, alongside their associations with BMI and insulin sensitivity, may reflect their links to chronic metabolic states, while the response of gal-1 to acute glucose challenges and correlation with insulin resistance highlight its involvement in acute regulatory mechanisms. These findings enhance our understanding of galectin-mediated metabolic regulation in pregnancy, offering potential biomarkers and therapeutic targets for GDM and related disorders. Future longitudinal studies are needed to validate these roles and explore their clinical implications for maternal and fetal health.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at https://doi.org/10.4093/dmj.2025.0401.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: H.F., E.J.K., S.M.B.
Acquisition, analysis, or interpretation of data: M.G.G., E.H., E.A.H., P.R., M.T.W.F., B.C., B.O.P., M.N.M.P.
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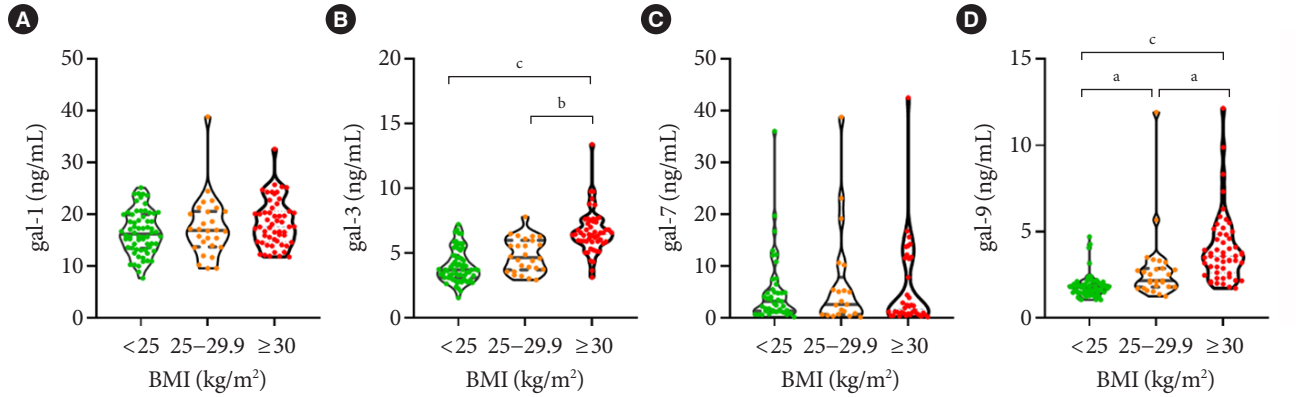
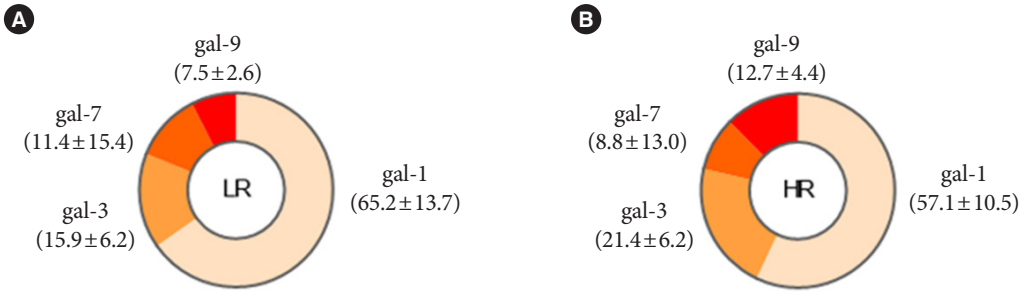
Supplementary Table 1. Galectin signature during the oral glucose tolerance test in pregnancy

Galectin signature	LR	HR
gal-1, ng/mL		
Fasting	16.07 (13.55–20.17)	17.50 (14.63–20.45)
1 hour	15.69 (12.26–18.54)	16.56 (14.29–19.94)
2 hours	14.79 (11.95–17.58)	16.80 (13.90–19.40)
gal-3, ng/mL		
Fasting	3.76 (3.18–4.82)	6.39 (5.90–7.36)[a]
1 hour	3.75 (3.08–4.61)	6.52 (5.84–7.70)
2 hours	3.69 (3.09–4.59)	6.59 (5.97–7.10)
gal-9, ng/mL		
Fasting	1.79 (1.55–2.08)	3.57 (2.84–4.92)[a]
1 hour	1.68 (1.42–1.98)	3.47 (2.71–4.56)
2 hours	1.60 (1.42–1.98)	3.37 (2.73–4.97)
gal-7, ng/mL		
Fasting	3.18 (1.18–6.99)	1.69 (0.63–5.92)
1 hour	2.34 (1.08–6.22)	1.40 (0.54–5.32)
2 hours	2.36 (1.02–5.89)	1.84 (0.53–7.04)

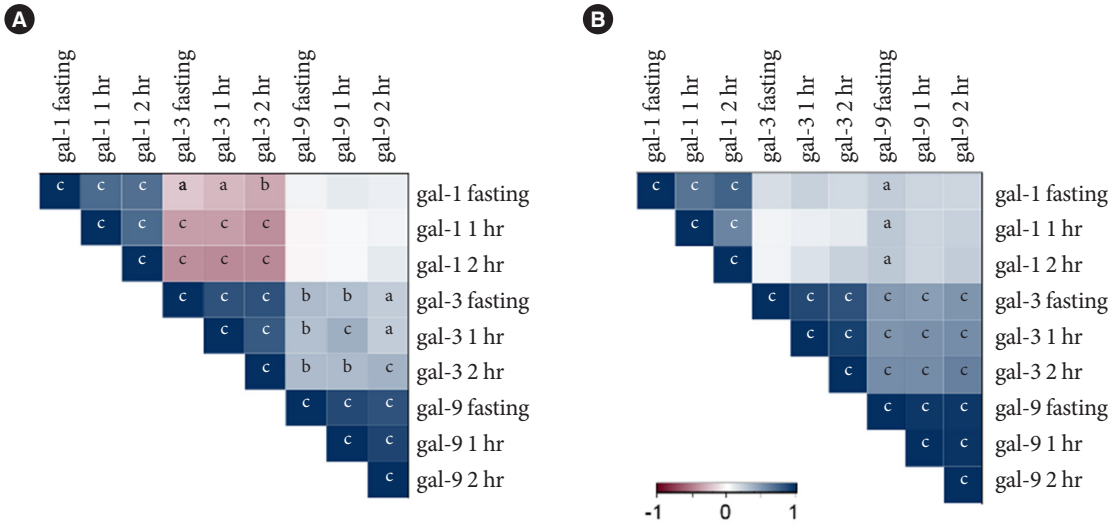
Values are presented as median (interquartile range).
LR, low-risk; HR, high-risk; gal, galectin.
[a]$P<0.0001$ vs. LR (fasting) analyzed by Mann-Whitney test.



Supplementary Fig. 1. Levels of galectins (gal) in pregnant women with pre-pregnant body mass index (BMI) <25 kg/m^2, between 25 and 30 kg/m^2, and ≥30 kg/m^2. (A) gal-1, (B) gal-3, (C) gal-7, and (D) gal-9. Violin plots with individual values, median and interquartile range are shown. [a]$P<0.01$, [b]$P<0.001$, and [c]$P<0.0001$ as analyzed by Kruskal-Wallis and Dunn's post-test.



Supplementary Fig. 2. Percentage of galectins (gal) in maternal circulation in (A) low-risk (LR) and (B) high-risk (HR) cohort. Data are presented as mean±standard deviation.



Supplementary Fig. 3. Correlation between serum levels of galectins (gal) in the (A) low-risk and (B) high-risk cohort during the oral glucose tolerance test. Correlation coefficient values are indicated by the color (blue positive and red negative) and the significance levels are indicated within squares: [a]$P<0.05$, [b]$P<0.01$, and [c]$P<0.001$.