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*Original Article*

# Hepatokines fetuin A and fetuin B status in women with/without gestational diabetes mellitus

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**Abstract:** Objectives: To assess circulating fetuin A and fetuin B levels in participants with and without Gestational Diabetes Mellitus (GDM) and to find out their correlations with other different parameters relating to gestational diabetes in Saudi women. Methods: A total of 123 Saudi pregnant women (N: 46 GDM and N: 77 healthy control) were included in this observational study. Fasting blood samples were collected to assess serum lipids, insulin and fetuin A and fetuin B. Serum fetuin A and fetuin B were quantified by commercially available kits. Results: The median value of fetuin A was slight lower in GDM patients [2003 pg/ml (866-3369)] than in the control group [2015 pg/ml (1060-2951)] without significant difference (P=0.95). The median value of fetuin B was also slight lower in GDM patients [3292 ng/ml (782-6740)] than the control group [3514 ng/ml (364-14854)] but without significant difference (P=0.564). There was a significant inverse correlation between fetuin B and total cholesterol in control group. Conclusions: The present study did not find a significant association between fetuins A and B with GDM or insulin resistance, but there was a significant inverse correlation between fetuin B and total cholesterol in the control group, reflecting good glucose control and adequate use of lipids in the nutrition of the fetus. Further research is required in the future to understand fetuin's role in the progression of GDM in Saudi women.

**Keywords:** Fetuin-A, fetuin-B, gestational diabetes mellitus, Saudi Arabia

## Introduction

According to the American Diabetes Association (ADA), gestational diabetes mellitus (GDM) is glucose intolerance detected at any time of pregnancy but later redefined as glucose intolerance during the second or third trimester of pregnancy [1]. A meta-analysis research that included 20 countries in the continent of Asia ranked Saudi Arabia third in the prevalence of GDM (22.9%) while another research ranked Saudi Arabia second among 6 East Mediterranean countries (17.6%) [2, 3]. In Saudi Arabia, primary risk factors for GDM include excessive weight gain and obesity, a family history of diabetes or a history of GDM in previous pregnancies, geriatric pregnancy, and hypertension [4, 5]. The disease usually subsides after delivery, but follow-up studies showed approximately 50% of GDM mothers develop type 2 diabetes mellitus (T2DM) after giving birth, meaning that an underlying T2DM might be the root cause of the widespread GDM. Due to the great possibility of developing T2DM, it is important to screen continually postpartum [6, 7].

Fetuins was first discovered in large quantities in fetal bovine serum by Pederson et al. in 1944, thus, the name fetuin was derived from the word fetus [8]. The glycoprotein was renamed α2-Heremans-Schmid glycoprotein (AHSG) in honor of Heremans, Bürgi, and Schmid, the scientists who discovered the human homologue in the 1960s [9]. In 2000, Olivier et al. discovered a homologue similar to AHSG named fetuin B and AHSG was renamed to fetuin A to differentiate the similar glycoproteins [10].

Fetuins A and B are both members of the cystatin superfamily. They are mainly expressed in the liver (hepatokines), as well as other tissues such as the kidneys, tongue, and placenta [11, 12]. Fetuin A consists of a heavy A chain containing 282 amino acids and a light B chain

of about 12 amino acids. The two chains are linked through half-cystine residues of their amino acid sequences, which are consequently arranged into a loop structure [13]. Fetuin B has 22% sequence similarities with fetuin A in humans and mice [10]. Both the fetuins A and B genes reside next to each other on chromosome region 3q27 [12].

Fetuin A has an innate function in bone mineralization and protection against vascular calcification [14, 15]. It also has a role in insulin resistance (IR), working as an innate inhibitor of the insulin receptor tyrosine kinase action, causing a halt in the downstream signaling cascade. It inhibits insulin receptor substrate-1 (IRS-1) autophosphorylation, resulting in decreased insulin sensitivity in the muscles and liver, as well as decreased glucose uptake into skeletal tissues by inhibiting glucose transporter-4 (GLUT-4) translocation to the plasma membrane [16, 17].

Fetuin A is an independent risk factor for T2DM, acting as a novel mediator between insulin resistance and diet [18, 19]. Furthermore, fetuin A acts as a negative acute phase protein, correlating inversely in the presence of different proinflammatory cytokines such as interleukins (IL)-1 and IL-6. Several inflammatory conditions and diseases, including systemic lupus erythematosus, rheumatoid arthritis, and chronic kidney disease, have been linked to lower fetuin A concentrations [20-23]. Fetuin A was also identified to stimulate the signaling of Toll-like receptor 4 (TLR4) by acting as an adaptor protein for saturated fatty acids, thus activating subclinical inflammation and subsequently causing lipid-induced insulin resistance through this pathway [24].

Fetuin B is expressed at a higher level in females than in males in humans and has been associated with female fertility [12, 25]. Fetuin B has been identified as a novel adipokine/hepatokine that significantly increases hepatic steatosis and mediates impaired insulin action and glucose intolerance [26].

The significant associations of these hepatokines with diabetes complications are well established, but few studies have been undertaken as to whether these associations exist among women at risk for gestational diabetes. The objective of this study is to assess circulating levels of fetuin A and fetuin B in participants with and without GDM and to find out their correlations with other different parameters relating to gestational diabetes in Saudi women.

## Materials and methods

### *Study population*

A total of 123 Saudi pregnant women with the age range of 27-35 were enrolled in this study and divided into two groups, the control group (n=77) and the GDM group (n=46). Written informed consent was obtained from patients prior to inclusion in the study. All participants completed a questionnaire on demographic information, general health status, and past medical history. Approval of the study was obtained from the institutional review board (IRB) of King Khalid University Hospital, College of Medicine, King Saud University, Riyadh, Saudi Arabia (Ref No. 14/4067/IRB). Anthropometry included height (cm), weight (kg), waist and hip circumference (cm), mean systolic and diastolic blood pressure was measured. Body mass index (BMI) was calculated. Fasting blood samples (>10 h) were collected from participants in their second trimester and third trimester (24-28 weeks).

### *Biochemical analysis*

Lipid profile and glucose were measured routinely using a Konelab analyzer (ThermoFisher Scientific, Vantaa, Finland). Serum insulin was determined using the LIAISON XL automated quantitative analyzer (DiaSorin, Saluggia, Italy) as used in previous studies [27, 28]. It uses an advanced chemiluminescence technique with magnetic microparticle separation to achieve the best sensitivity and accuracy of the assay.

The serum Fetuin-A and C-Reactive Protein (CRP) levels were measured using sandwich enzyme-linked immunoassay (ELISA) kits (R&D Systems Quantikine, Minneapolis, Minnesota, USA, Cat. #DFTA00), (R&D Systems Quantikine, Minneapolis, Minnesota, USA, Cat. #DCRP00, #SCRP00, #PDCRP00), respectively. The intra- and inter-coefficients of variation for fetuin-A were 4.3% and 7.3%, respectively and the intra- and inter-coefficient of variation for CRP were 5.5% and 6.5%, respectively. The serum fetuin-B levels were measured also using ELISA kits (Biovendor, Karasek, Czech Republic, Cat.

**Table 1.** Descriptive characteristics according to GDM and control groups

| Parameters | All | Control | GDM | *P*-Value |
|---|---|---|---|---|
| N | 123 | 77 | 46 | |
| Age (years) | 29.2 ± 5.4 | 28.5 ± 5.1 | 30.4 ± 5.8 | 0.06 |
| BMI (kg/m$^2$) | 31.9 ± 6.8 | 31.3 ± 6.8 | 33.1 ± 6.6 | 0.16 |
| Waist-Hip Ratio | 0.91 ± 0.10 | 0.90 ± 0.10 | 0.91 ± 0.10 | 0.63 |
| Systolic BP (mmHg) | 111.7 ± 11.1 | 109.9 ± 10.8 | 113.9 ± 11.3 | 0.15 |
| Diastolic BP (mmHg) | 67.2 ± 9.0 | 64.4 ± 7.7 | 70.5 ± 9.5 | 0.006 |
| Glucose (mmol/l) | 4.9 ± 0.7 | 4.56 ± 0.5 | 5.54 ± 0.6 | <0.001 |
| Glucose-2 hours (mmol/l) | 7.3 ± 2.3 | 5.8 ± 1.1 | 9.7 ± 1.4 | <0.001 |
| T. Cholesterol (mmol/l) | 4.94 ± 1.1 | 4.70 ± 1.0 | 5.35 ± 1.2 | 0.001 |
| HDL-Chol (mmol/l) | 1.33 ± 0.3 | 1.34 ± 0.3 | 1.33 ± 0.4 | 0.85 |
| LDL-Chol (mmol/l) | 3.1 ± 0.9 | 2.9 ± 0.7 | 3.4 ± 1.1 | 0.008 |
| Triglycerides (mmol/l) | 1.35 (1.0-1.8) | 1.29 (0.9-1.7) | 1.47 (1.1-2.0) | 0.025 |
| Insulin (μU/ml) | 11.4 (6.2-20.2) | 11.3 (4.8-20.1) | 12.4 (7.5-20.2) | 0.18 |
| HOMA-IR | 2.3 (1.3-4.2) | 2.2 (1.1-4.2) | 2.8 (1.8-4.6) | 0.03 |
| HOMA-β | 42.1 (21.0-85.6) | 44.9 (16.7-87.5) | 37.9 (24.8-77.5) | 0.53 |
| Fetuin-A (pg/ml) | 2016 (1032.4-3284.2) | 2015 (1060-2951) | 2003 (866-3369) | 0.98 |
| Fetuin-B (ng/ml) | 3300 (462-10156) | 3514 (364-14854) | 3292 (782-6740) | 0.56 |

Note: Data presented as Mean ± SD for normal variables while Median (1$^{st}$-3$^{rd}$) percentile for non-normal variables; *P*-values <0.05 considered significant. BMI: body mass index; BP: blood pressure; Chol: cholesterol; HDL: high-density lipoprotein; HOMA-β: homeostasis model assessment-insulin sensitivity; HOMA-IR: homeostasis model assessment-insulin resistance; LDL: low-density lipoprotein; T: total.

#RD191172200R). The intra- and inter-coefficient of variation for fetuin-A were 3.7% and 5.2%. Fasting blood glucose (FBG), high-density lipoprotein-cholesterol (HDL-C), total cholesterol (TC) and triglycerides (TG) were measured by colorimetric methods using an automated chemistry analyzer (Konelab, Thermo Scientific, Vantaa, Finland). Homeostasis model for insulin resistance (HOMA-IR) was calculated as (insulin x FBG)/22.5 as well as insulin sensitivity (HOMA-β).

*Statistical analysis*

Continuous data were presented as mean ± standard deviation (SD) for normal variables and non-Gaussian variables were presented in median (Quartiles 1 and 3). Categorical data presented as frequencies and percentages (%). An independent sample T-test and Mann-Whitney U tests were used to compare mean and median difference between control and GDM subjects for normal and non-normal variables respectively. Spearman's correlation analysis was performed for fetuin-A and fetuin-B with anthropometrics, biochemical and pro-inflammatory markers. *P*-value <0.05 was considered statistically significant.

## Results

**Table 1** shows the descriptive statistics according to presence of GDM. Glucose levels in GDM group was significantly higher than the control group [5.54 ± 0.6 vs 4.56 ± 0.5 *P<0.001*]. Furthermore, 2-hr glucose level was also significantly higher in GDM than the control group [9.7 ± 1.4 vs 5.8 ± 1.1 *P*<0.001]. There was no significant difference in insulin levels between the two groups (*P*=0.184), but a significant difference between GDM and control group was observed in HOMA-IR as it was significantly higher in GDM than the control group [2.8 (1.8-4.6) vs 2.2 (1.1-4.2) *P*=0.026]. Lipid profile also showed significantly high concentrations in GDM than control group (all *P*<0.05) except HDL-cholesterol which was not significant (*P*=0.848). Furthermore, **Table 1** showed that the median value of fetuin A and fetuin B was lower in GDM patients than the control group without significant difference (*P*>0.05).

A Spearman's correlation analysis was performed to explore the associations between fetuin A, fetuin B and other clinical parameters (**Table 2**). Findings revealed that serum fetuin B level was negatively associated with total cho-

**Table 2.** Correlations between fetuin A and fetuin B with select parameters according to all, GDM and Control groups

| Parameters | All | | Control | | GDM | |
|---|---|---|---|---|---|---|
| N (Females) | Fetuin-A | Fetuin-B | Fetuin-A | Fetuin-B | Fetuin-A | Fetuin-B |
| Age (years) | -0.12 | -0.11 | -0.22 | -0.12 | 0.04 | -0.10 |
| BMI (kg/m$^2$) | -0.01 | -0.05 | -0.10 | -0.10 | 0.15 | 0.06 |
| Waist-Hip Ratio | -0.11 | -0.22 | -0.02 | -0.14 | -0.17 | -0.19 |
| Systolic BP (mmHg) | -0.07 | -0.04 | -0.31 | 0.03 | 0.14 | -0.19 |
| Diastolic BP (mmHg) | -0.21 | -0.16 | -0.39 | -0.20 | -0.10 | -0.28 |
| Glucose (mmol/l) | 0.10 | -0.02 | 0.23 | 0.10 | 0.05 | -0.10 |
| Glucose-2 hours (mmol/l) | 0.06 | -0.12 | 0.08 | -0.16 | 0.15 | 0.05 |
| T. Cholesterol (mmol/l) | -0.10 | -0.26** | 0.01 | -0.25* | -0.10 | -0.26 |
| LDL-Chol (mmol/l) | -0.20 | -0.14 | -0.24 | -0.18 | -0.07 | 0.12 |
| HDL-Chol (mmol/l) | -0.16 | -0.13 | -0.03 | -0.12 | -0.28 | -0.10 |
| Triglycerides (mmol/l) | 0.13 | -0.10 | 0.17 | -0.10 | 0.12 | -0.10 |
| Insulin (μU/ml) | -0.10 | 0.10 | -0.07 | 0.12 | -0.05 | -0.07 |
| HOMA-IR | -0.06 | 0.04 | -0.17 | -0.12 | -0.03 | -0.12 |
| HOMA-β | -0.10 | 0.11 | -0.10 | 0.13 | -0.03 | 0.10 |

Note: Data presented as coefficient (R); *denotes significance at 0.05 level; **denotes significance at 0.01 level. BMI: body mass index; BP: blood pressure; Chol: cholesterol; HDL: high-density lipoprotein; HOMA-β: homeostasis model assessment-insulin sensitivity; HOMA-IR: homeostasis model assessment-insulin resistance; LDL: low-density lipoprotein; T: total.

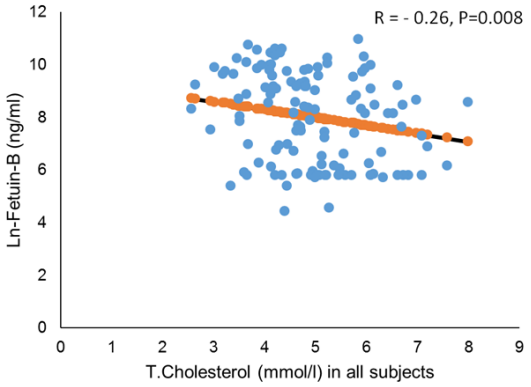


**Figure 1.** Inverse correlation (R) between linear (Ln) fetuin B (ng/ml) and T. (total) cholesterol (mmol/l) in all subjects.

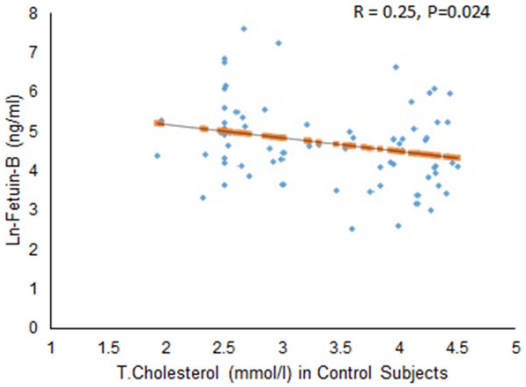


**Figure 2.** Positive correlation between linear (Ln) fetuin B (ng/ml) and T. (total) cholesterol (mmol/l) among control subjects.

lesterol in control group (R=-0.25, *P*=0.024) (**Figure 1**). Fetuin A was not found to be correlated with any of the parameters.

**Figures 1** and **2** show the scatter plots between TC (mmol/l) and fetuin B (ng/ml) in all subjects and control group respectively. There is an inverse and significant correlation [R=-0.26, *P*=0.008] between TC and fetuin B.

## Discussion

In our study the level of HOMA-IR in GDM group was 2.8 (1.8-4.6) and 2.2 (1.1-4.2) in the control group showing a significant difference between the two groups. This was expected since in GDM insulin resistance is elevated and different investigations revealed the HOMA-IR index in GDM to be in contrast to HOMA-IR index of normal pregnancy.

Endo et al. compared between pregnant women with GDM in three groups in three different trimesters matched with three groups of healthy pregnant subjects also in different stages of pregnancy. The results displayed an increasing HOMA-IR in the GDM groups while no substantial change was marked in healthy pregnant

groups through different stages of gestation [29]. This is because in GDM peripheral insulin sensitivity is decreased in targeted cells. Sokup et al. studied the HOMA-IR in second and third trimester and detected values of HOMA-IR as indication of beta-cell dysfunction and may possibly be facilitated as marker of GDM prognosis [30]. GDM also causes a rise in oxidative stress and stress adaptations leading to higher insulin resistance compared to healthy controls as a result of higher levels of energy and oxygen necessary to sustain the gestation state [31].

Lipid profile showed highly significant differences in the GDM in comparison to control group except HDL-cholesterol. Wang et al. searched the discrepancy of lipid profile in 1st, 2nd, and 3rd trimesters of pregnancy between normal gestation and GDM and demonstrated that increased levels of TG and low concentration of HDL-C throughout pregnancy in the GDM group compared to normal glucose tolerant pregnant women [32]. Shen et al. also explored hyperlipidemia in pregnancy and detected high levels of TG, LDL-C and TC throughout gestation and found it to be associated with developing GDM. This physiological phenomenon is essential to maintain energy reservoir for fetus and to ensure proper development. No statically significant difference was found in HDL-cholesterol concentration between healthy pregnant group and GDM group in agreement with our findings [33]. A meta-analysis investigation showed HDL-C to be lower in GDM compared to controls while TG is elevated [34].

The present concentrations of fetuin A [GDM 2003 pg/ml (866-3369)] vs the control group [2015 pg/ml (1060-2951)] in our studies are lower compared to previous studies that explored fetuin A in the state of GDM. For ristance Iyidir et al. reported that the mean concentration of fetuin A in Turkish women between 24th and 28th gestational weeks to be 35 ng/ml in GDM group and 32 ng/ml in control [35000 pg/ml GDM] vs [32000 pg/ml control] [35]. The difference in concentration between Iyidir and our study could be due to discrepancy between ethnicities.

Kansu-Celik et al. presented lower levels of fetuin A in his inquiry of the protein in pregnant women at 11 and 14 gestational weeks and this was associated with greater incidence of developing GDM later in pregnancy [36].

Precious studies have examined fetuin A levels and its association with testosterone in the development of atherosclerosis in diabetic Saudi men and found that fetuin A in mean range of 275 pg/dl in T2DM subjects and 71 pg/ml in controls [2.75 pg/ml T2DM] vs [0.71 pg/ml] controls [37]. These results are lower than our findings and this might be due to different physiological condition (pregnancy) and difference in sex. This was explored as the treatment of sex hormones in mice showed different levels of fetuin A and marked its direct expression via sex-hormones receptors [38]. The state of pregnancy and fetal growth is also influenced by fetuin A levels as its expression is elevated in the fetus and has an essential role in post-partum fetal bone growth [39].

Fetuin B concentrations in our cohort is [GDM 3292 ng/ml (782-6740)] vs the non-GDM group [3514 ng/ml (364-14854)]. These concentrations are correspondent to concentration of fetuin B searched in obese Chinese adults in a tertile with mean concentration of 3.85 μg/ml [3850 ng/ml] according to risk of developing atherosclerosis [40]. Concentration of fetuin B in our study was lower compared to previous studies observing its levels in GDM in the same fashion as fetuin A, although some studies have also disclosed its exact concentration [41, 42].

Fetuin A and fetuin B showed surprisingly no significant difference between the two groups of pregnant women in our research. Farhan et al. conducted a case control study in Austria and recruited 10 women with GDM and 10 healthy pregnant women at 28 weeks of pregnancy and marked no change in the concentration of fetuin A following an acute glucose intake [43]. This is opposing to a study that showed high level of glucose to activates fetuin A gene promoter and subsequent higher expression of the protein in cultured hepatic cell lines (HepG2) [44]. Another study in Czech Republic by ŠIMJÁK et al. recently also showed no significant difference in the levels of fetuin A and fetuin B in second and third trimester between two groups [42].

Other studies are inconsistent with these findings, Kralisch et al. in Germany investigated the levels of fetuin B in 74 healthy pregnant women and 74 age matched GDM women with mean gestational age of 201 days (second trimester) showed increased levels of fetuin B in GDM

subjects and HOMA-IR as an independent positive indicator of fetuin B. Fetuin A was not of an important variance in investigated groups [41]. Kalabay et al. showed a higher level of fetuin A in both healthy pregnant women and GDM subjects in comparison to non-pregnant subjects while stating it to be relatively in higher concentration in GDM group [45]. Iyidir et al. in Turkey revealed the high levels of fetuin A in GDM group compared to control group that were in their second and third trimester and found a positive correlation between fetuin A and glycated hemoglobin levels (HbA1c) [35].

In our study the insignificance in the concentration of fetuin A and B between GDM group and healthy pregnant group could be due to the indifference in maternal age, BMI (both investigated groups were obese), fasting serum insulin and HOMA-β. thus exhibiting somewhat a similar glucose control in the face of greater insulin resistance especially for GDM subjects [29].

Another reason could be involved and can be behind the insignificance of fetuin A and B in our study such as a decreased phosphorylation of those proteins in GDM [46].

The absence of significance could also be due to the type of treatment used during pregnancy in GDM group. Treatments such as metformin increases insulin sensitivity and the peripheral uptake of glucose due to it is action on insulin receptors tyrosine kinase. This drug also increases activity of GLUT4 transporters [47, 48].

Fetuin A and fetuin B in our cohort did not correlate with any of the insulin resistance parameters in both GDM and healthy pregnant group. This finding is consistent with a previous study that showed no correlation between fetuin A and parameters of insulin resistance while other studies are in disagreement with this and showed correlation of fetuin A and fetuin B with insulin resistance in gestational diabetes and T2DM [18, 41, 43, 49, 50].

Fetuin B demonstrated an inverse association with total cholesterol but in control group and this could be due to good insulin sensitivity (as mentioned above the HOMA-IR was higher in GDM group) this helps in maintaining normal maternal plasma glucose while lipids are used for fetus development [51]. Gene polymorphism of these proteins in the case of Saudi GDM subjects has not been explored. Genetic based studies in Saudi subjects are also suggested to understand these proteins at a molecular level.

The authors acknowledge some limitations including the lack of significance in the concentration of our interest hepatokinase that might be due small sample number that contributed to failure of some comparisons to reach statistical significance. The regulation of fetuin A and fetuin B could be in an age-dependent matter in GDM and this should be considered in future investigation. Gene polymorphism of these proteins in the case of Saudi GDM subjects has not been explored, it is important to also mention the cross-sectional nature making the interpretation of the results limited.

## Conclusion

Our study was of cross-sectional design and no association between fetuin A, fetuin B and GDM or insulin resistance was established but there was a significant inverse correlation between fetuin B and total cholesterol in non-diabetic pregnant Saudi women reflecting a normal insulin sensitivity of this group. Preexisting overweight disguised by pregnancy might have affected the release of fetuins and further studies are needed to show the exact mechanism this have been achieved. Further studies with larger sample including non-pregnant subject are needed to confirm the relationship of fetuin A, fetuin B and insulin resistance with GDM in Saudi women while elements such as changing body weight and liver diseases that affects fetuins production and release are advised to be investigated in the case of GDM.

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## Disclosure of conflict of interest

None.

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