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Exploring Irisin and Nesfatin-1 in second trimester amniotic fluid: a comparative study of obese and normal weight pregnant women

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Abstract

Objective: Obesity is characterized by dysregulated adipokine production patterns, leading to low-grade inflammation. Irisin has been identified as a potential target for the treatment of diabetes and obesity. Nesfatin-1, another adipokine, plays a significant role in various metabolic processes, including glucose homeostasis. This study investigated the levels of the new generation adipokines, irisin and nesfatin-1, in the amniotic fluid during the second trimester of normal and obese pregnancies.

Methods: Amniotic fluid samples were collected following established protocols. The first 2 mL portion of fluid obtained during amniocentesis was retained aAfter centrifugation at 1500 g for 10 minutes, the cell-free amniotic fluid was transferred to Eppendorf tubes and quantitative measurements of irisin and nesfatin-1 levels were performed using the Enzyme-Linked Immuno Sorbent Assay (ELISA) method.

Results: Our results demonstrate, for the first time in the literature, the presence of irisin and nesfatin-1 in amniotic fluid. Additionally, we found that the levels of these adipokines were significantly lower in obese pregnant women compared to the control group (both $p<0.05$).

Conclusion: By establishing the presence of these hormones in amniotic fluid, our study provides a new perspective on the examination of molecules associated with obesity. Further investigation and confirmation of our results, involving a larger cohort of patients and additional parameters, are necessary to enhance our understanding of the impact of obesity on pregnancy.

Keywords: Adipokine, amniotic fluid, irisin, maternal obesity, nesfatin-1

Introduction

Obesity during pregnancy poses significant risks not only to the mother but also to the developing fetus. The environment created by increased pro-inflammatory cytokines and adipokines in obese pregnant women may contribute to fetal programming, potentially leading to various pregnancy complications and influencing the future health status of the fetus.[1] These inflammatory markers can disrupt normal physiological processes and alter the intrauterine environment, creating conditions that may predispose the fetus to metabolic disorders later in life.[2]

Amniotic fluid, which serves as a dynamic medium between the fetus and the placenta, plays a crucial role in fetal development. In the second trimester, amniotic fluid is primarily produced by the fetal urinary system and lungs and its composition closely resembles that of fetal plasma.[2] By examining the composition of amniotic fluid, researchers can gain valuable insights into the environmental conditions surrounding the developing fetus.[3] This analysis can reveal the presence of various biomolecules, including adipokines, which are secreted by

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adipose tissue and have significant paracrine and endocrine functions.

Adipose tissue is recognized as an active endocrine organ that secretes various functional molecules called adipokines, which possess significant paracrine/neuroendocrine properties.[4] These adipokines play roles in lipid and glucose metabolism, inflammation, vascular hemostasis, endothelial function, and immune system activation.[5,6] Obesity is characterized by dysregulated adipokine production patterns, leading to low-grade inflammation associated with insulin resistance and increased cardiovascular risk.[7] Many studies have focused on adipokines such as leptin, adiponectin, resistin, visfatin, chemerin, ghrelin, and omentin-1. Newer generation adipokines like irisin and nesfatin-1 have also been identified.[8] Investigating the levels of these adipokines in amniotic fluid can shed light on the impact of maternal obesity on fetal development.

Irisin, a newly discovered hormone released from skeletal muscle and adipose tissue, is a target for diabetes and obesity treatment.[9] As an indicator molecule in the regulation of glucose homeostasis, irisin is a proteolytic product of domain 5 of fibronectin type 3, a transmembrane protein.[10] Exercise and cold stimulate irisin, which increases the expression of uncoupling proteins 1 in white adipose tissue cells, enhancing mitochondrial activity.[11] Irisin shows promise in treating metabolic diseases by increasing energy expenditure and causing weight loss.[12] While studies have focused on plasma irisin levels' effects on metabolic syndrome, obesity, type 2 diabetes mellitus, and cardiovascular diseases, there are also studies on serum and cord blood levels in gestational diabetes.[13] Irisin is suggested to have anti-inflammatory effects and can reduce the gene expression of inflammatory cytokines such as IL-6, TNF-α, and macrophage inflammatory protein 1 α and 1 β.[14]

Nesfatin-1 is a satiety molecule found in the hypothalamus and associated with obesity, also produced in peripheral tissues such as adipose cells, gastric mucosa, and pancreatic B cells.[15] It plays a crucial role in many metabolic processes such as food intake and glucose homeostasis, and is associated with adverse pregnancy outcomes through maternal serum levels. Though its mechanism of action on glucose metabolism regulation is not fully understood, it is primarily thought to act insulin-dependently.[16] During obesity, white adipose tissue (WAT) decreases the concentration of the anti-inflammatory adipokine nesfatin-1, which weakens immunity and contributes to the progression of obesity-associated metabolic disorders.[17,18] Irisin stimulates the browning of WAT and consequently in-creases energy expenditure, additionally regulating glucose metabolism.[19]

To the best of our knowledge, no study has demonstrated the presence of irisin and nesfatin-1 in amniotic fluid. This study aimed to determine the levels of amniotic fluid irisin and nesfatin-1 in normal and obese pregnant women and discuss the relationship between these adipokines and cytokine levels in amniotic fluid with maternal body mass index (BMI) and obesity.

Methods

This study included all pregnant women who presented to the Department of Obstetrics and Gynecology, Division of Prenatal Diagnosis and Treatment Unit, Inonu University, between April 1, 2021, and April 1, 2022, for second-trimester fetal anomaly diagnosis and underwent prenatal diagnostic amniocentesis. Among these women, those with obesity (BMI >30 kg/m²) constituted the study group (30 obese pregnant women), while normal-weight pregnant women matched for maternal age and gestational week formed the control group (30 pregnant women with a normal BMI of 18.5-24.9). Gestational weeks of the participants were confirmed based on first-trimester ultrasound measurements. The study included singletonpregnant women aged 18-45 years who underwent elective amniocentesis for karyotype analysis and had normal obstetric and medical histories. Exclusion criteria included multiple pregnancies, pregestational diabetes mellitus (DM), gestational DM (GDM), chronic hypertension, dyslipidemia, chronic kidney failure, malignancy, asthma, pulmonary or cardiac diseases, abnormal karyo-

types, fetal malformations, alcohol and tobacco use, maternal blood contamination, clinical evidence of infection, antibiotic use in the previous two weeks, and early membrane rupture.

During the second trimester, transabdominal amniocentesis for prenatal diagnosis was performed by a single clinician certified by the Fetal Medicine Foundation in invasive procedures (RM). Amniotic fluid samples were collected following established protocols. The first 2 mL portion of fluid obtained during amniocentesis was discarded to avoid maternal contamination. However, for this study, the initial 2 mL of amniotic fluid was retained and not discarded. After centrifugation at 1500 g for 10 minutes, the cell-free amniotic fluid was transferred to Eppendorf tubes and stored at -80°C until analysis. Upon reaching the desired sample size, the amniotic fluids were thawed, and quantitative measurements of irisin and nesfatin-1 levels were performed using the Enzyme-Linked ImmunoSorbent Assay (ELISA) method. Blood samples were collected from participants into gel separator tubes (serum) in the morning after overnight fasting. The serum tubes were kept at room temperature for 30 minutes to coagulate and then centrifuged at 1200 g for 10 minutes. After centrifugation, serum samples were transferred to sterile 1.5 mL Eppendorf tubes and stored at -80°C until biochemical analysis using commercial ELISA kits. Stored serum samples were prepared for analysis under appropriate conditions. Irisin and nesfatin-1 levels were determined using ELISA kits from Cloud-Clone Corp (Cat. No: SEN576Hu for irisin and CEA242Hu for nesfatin-1, China) according to the manufacturer's recommendations.

The sample size calculation was based on a power analysis, which considered an effect size of 1.0 ng/dL (1.7 standard deviations) for the amniotic fluid irisin level in pregnant women with class III obesity as statistically significant. To detect this difference with 80% power and a 5% (two-tailed) significance level, at least 20 samples were required in each group. The parameters analyzed included age (years), gravidity, parity, pre-pregnancy body mass index (BMI, kg/m^2), BMI at the time of amniocentesis (kg/m^2), gestational age at the time of amniocentesis (weeks), indication for amniocentesis, fetal gender, and estimated fetal weight determined during amniocentesis. Data analysis was conducted using SPSS (Statistical Program for Social Sciences) version 25. The normality of the data was assessed using the Kolmogorov-Smirnov test. A significance level (p) of 0.05 was used for comparison tests. Since the variables did not follow a normal distribution ($p > 0.05$), non-parametric test methods were employed.Comparisons between independent binary groups were performed using the Mann-Whitney U test due to the lack of normality. Chi-square analysis was used for comparing categorical data by creating cross-tables. Descriptive statistics, including mean, standard deviation, count, and percentage, were used to present the data.

Results

Statistically significant differences were found between the patient and control groups regarding pre-pregnancy weight, BMI, and the presence of additional diseases (both $p < 0.05$). However, no statistically significant differences were observed between the groups in terms of age, medication usage, smoking status, and kinship (both $p > 0.05$) (Table 1). There were no statistically significant differences between the patient and control groups regarding the history of anomalous fetuses, cesarean section operations, indications for amniocentesis, gestational age, gravida, parity, abortion, and number of living children (both $p > 0.05$) (Table 2). Statistically significant differences were found between the patient and control groups in terms of irisin and nesfatin-1 levels (both $p < 0.05$). Box-plots of these variables are presented in Figures 1 and 2. The average irisin level in obese pregnant women was 296.14 ± 19.98, compared to 395.76 ± 36.36 in the control group. The average nesfatin-1 level in obese pregnant women was 1161.00 ± 108.2, while in the control group, it was 1659.39 ± 107.44 (Table 3).

Table 1. Comparison of Demographical Data of Patients with Obese pregnant and Controls

Variable	Groups		Experiment	Control	Total	χ^2	p
Comorbidity	**(-)**	**n**	22	28	50	0.268	0.038*
		%	73.30%	93.30%	83.30%		
	(+)	**n**	8	2	10		
		%	26.70%	6.70%	16.70%		
Drug Use	**(-)**	**n**	22	25	48	0.167	0.197
		%	73.30%	86.70%	80.00%		
	(+)	**n**	8	4	12		
		%	26.70%	13.30%	20.00%		
Smoking	**(-)**	**n**	28	24	52	-0.196	0.129
		%	93.30%	80.00%	86.70%		
	(+)	**n**	2	6	8		
		%	6.70%	20.00%	13.30%		
Kinship	**(-)**	**n**	25	25	50	0.001	1.000
		%	83.30%	83.30%	83.30%		
	(+)	**n**	5	5	10		
		%	16.70%	16.70%	16.70%		
TOTAL			**30 (%100)**	**30 (%100)**			
Variables			**Experiment**	**Control**	**Mann Whitney U**		**p**
BMI M (Min - Max)		**Mean ± sd**	22.88 ± 1.74	33.31 ± 2.93	10		0.001*
		32.85(30.08-40.5)	22.9(19.47-24.97)				
Prepregnancy weight M (Min - Max)		**Mean ± sd**	80.92 ± 10.69	58.13 ± 5.24	16		0.001*
		79.5(58-110)	58(47-69)				
Age M (Min - Max)		**Mean ± sd**	34.77 ± 5.47	32.73 ± 6.2	376		0.266
		35.5(24-45)	33(20-43)				

n; frequency, %; percent, M; Median; *p<0.05:There is a statistically significant difference between the groups. Bold values are statistically significant values (p<0.05).

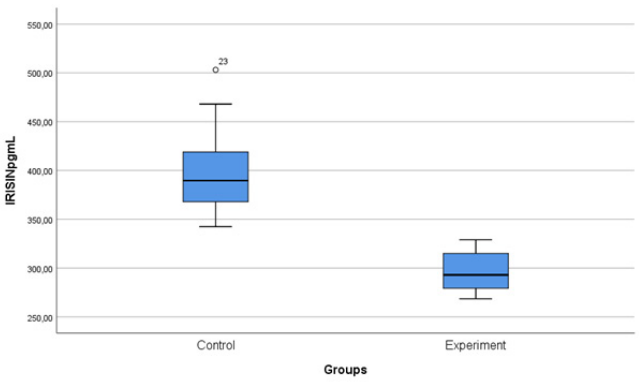

Fig 1. Box-plot of the distribution of irisin in all study groups

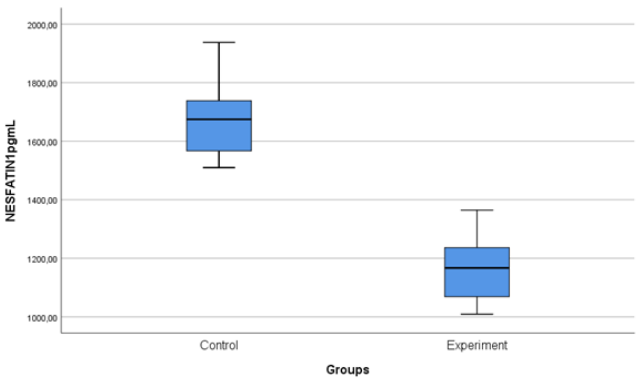

Fig 2. Box-plot of the distribution of nesfatin-1 in in all study groups

Table 2. Comparison of Pregnancy Variables of Patients with Obese pregnant and controls

Variable	Groups		Experiment	Control	Total	χ^2	p
History of fetus with abnormality	(-)	n	26	28	54	0.111	0.389
		%	86.70%	93.30%	90.00%		
	(+)	n	4	2	6		
		%	13.30%	6.70%	10.00%		
operation_cs1	(-)	n	16	21	37	0.171	0.184
		%	53.30%	70.00%	61.70%		
	(+)	n	14	9	23		
		%	46.70%	30.00%	38.30%		
AS indication	History of fetus with abnormality	n	4	6	10	0.133	0.587
		%	13.30%	20.00%	16.70%		
	Advanced maternal age	n	4	2	6		
		%	13.30%	6.70%	10.00%		
	Risk in screening test	n	22	22	44		
		%	73.30%	73.30%	73.30%		
Variables			Experiment	Control	Mann Whitney U		p
Gestational age procedure M (Min - Max)		Mean ± sd	17.9 ± 1.97	18.43 ± 2.7	405.500		0.504
		17.5(15-23)	17.5(13-23)				
G M (Min - Max)		Mean ± sd	3.13 ± 1.46	2.8 ± 1.45	387.500		0.346
		3(1-6)	2.5(1-6)				
P M (Min - Max)		Mean ± sd	1.67 ± 1.32	1.23 ± 1.1	368.000		0.210
		1.5(0-5)	1(0-4)				
A M (Min - Max)		Mean ± sd	0.43 ± 0.63	0.6 ± 1.04	445.500		0.937
		0(0-2)	0(0-4)				
Y M (Min - Max)		Mean ± sd	1.47 ± 1.28	1.1 ± 0.99	383.000		0.303
		1(0-5)	1(0-3)				

M; Median; *p<0.05:There is a statistically significant difference between the groups; G; gravida, P; parity, A; abortion, and Y; alive

Table 3. Comparison of Parameters Across Groups

Variables	Experiment		Control		p
	Mean ± sd	M (Min - Max)	Mean ± sd	M (Min - Max)	
Irisin(pg/mL)	296.14 ± 19.98	293(268.45-329.02)	395.76 ± 36.36	389.6(342.41-503.1)	0.001*
Nesfatin1(pg/mL)	1161.00 ± 108.2	1166.9(1009.05-1364.46)	1659.39 ± 107.44	1674.62(1509.72-1937.29)	0.001*

M; Median; *p<0.05:There is a statistically significant difference between the groups.

The most important characteristic of a good estimator is its efficiency, often measured by standard deviation. Smaller standard deviations indicate more efficient statistical predictions.[20] The low standard deviations of irisin and nesfatin-1 levels in the obese subjects suggest that these variables are reliable predictors in these groups.

Discussion

This study investigated the presence and levels of irisin and nesfatin-1 in the second-trimester amniotic fluid of normal and obese pregnant women. Our results revealed significantly lower levels of irisin and nesfatin-1 in obese pregnant women compared to normal pregnant women. To our knowledge, this is the first study to demonstrate the presence of iri-

sin and nesfatin-1 in the second-trimester amniotic fluid and to highlight the differences associated with obesity. The lower standard deviation values in the obese group suggest that irisin and nesfatin-1 are effective and reliable biomarkers in this population. These findings suggest that irisin and nesfatin-1 levels in the amniotic fluid may play a role in the pathophysiology of obesity during pregnancy. Further research is warranted to elucidate the mechanisms underlying these differences and their potential implications for maternal and fetal health. Obesity is a global disease characterized by excessive adipose tissue accumulation and associated metabolic consequences.[21] Adipokines and myokines, secreted by adipose tissue and skeletal muscle respectively, play crucial roles in energy homeostasis and insulin sensitivity.[22,23] Irisin and nesfatin-1, the focus of our study, are among these cytokines.

The relationship between irisin levels and obesity is complex. While several studies report lower irisin expression in obese individuals and those with type 2 diabetes (T2D), other studies show higher irisin levels in obese individuals compared to those with normal BMI.[24,25] This discrepancy may arise from factors such as irisin receptor dysfunction in WAT.[26,27] It is suggested that measuring irisin levels in different obesity phenotypes—normal weight obesity (NWO), metabolically obese normal weight (MONW), metabolically healthy obesity (MHO), and metabolically unhealthy obesity (MUO) could provide clearer insights.[28-30] In our study, we used BMI to classify obesity. Given the unclassified phenotypes, our assessment may be limited, but our findings align with several reports indicating lower irisin levels in obese individuals, emphasizing the need for further research to clarify these associations and their implications for obesity management.

Yosaee et al., in agreement with our findings, observed that irisin levels were lower in obese patients compared to controls.[31] These results were further confirmed in a study by Castillo et al., which compared metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO) children with their normal-weight pers.[32] However, Abulmeaty et al. reported higher irisin levels in MUO individuals compared to normal-weight individuals.[33] These inconsistent results underscore the need for additional studies to explore the relationship between irisin and various obesity phenotypes to clarify these discrepancies. Currently, there is no established reference range for plasma and serum irisin levels, with circulating irisin levels in humans varying widely from 0.01 ng/mL to 2000 ng/mL.[34] A study involving 81 boys and 72 girls found that elevated circulating irisin levels were associated with impaired glucose tolerance, with this association being more pronounced in girls.[35] The authors suggested that this sexual dimorphism could be attributed to hormonal differences between the sexes, which confirms the sex-related variation in irisin production. Our study adds to this body of literature by demonstrating that irisin levels are significantly lower in the second-trimester amniotic fluid of obese pregnant women compared to normal-weight controls. This finding aligns with several studies indicating lower irisin levels in obesity but contrasts with others, emphasizing the complexity of irisin regulation in different metabolic states. The absence of a standardized reference range for irisin further complicates the interpretation of these results. Our study highlights the potential role of irisin as a biomarker for obesity-related complications during pregnancy and underscores the importance of further research to better understand the mechanisms driving these observations.

Due to its regulatory role in energy and glucose metabolism, nesfatin-1 has emerged as a promising therapeutic target for the treatment of metabolic disorders, including obesity.[36] However, nesfatin-1 levels may vary across different obesity phenotypes. Therefore, it is necessary to consider these variations in new clinical trials to better understand the therapeutic potential of nesfatin-1 in metabolism. Studies have reported higher levels of nesfatin-1 in individuals with metabolically healthy obesity (MHO) compared to those with metabolically unhealthy obesity (MUO). Consistent with our findings, one study found that serum nesfatin-1 concentrations were

significantly lower in obese individuals compared to non-obese individuals.[37] Another study supporting our results reported that nesfatin-1 levels were negatively associated with obesity, BMI, body fat percentage, body fat weight, and blood glucose levels.[38] Additionally, a study similar to our research found lower nesfatin-1 levels in individuals with metabolic syndrome (MetS) and obesity, noting associations between nesfatin-1 levels and BMI, waist circumference, and body weight.[39] These findings underscore the potential of nesfatin-1 as a biomarker for metabolic health in various obesity phenotypes and highlight the need for further research to elucidate its role and therapeutic applications.

Although the exact role of nesfatin-1 in different obesity phenotypes remains unclear, it has been observed that nesfatin-1 levels may vary in response to changes in body weight, adipose tissue distribution and metabolic function. The results of studies investigating serum nesfatin-1 levels in patients with gestational diabetes mellitus (GDM) are inconsistent. In addition, cord blood nesfatin-1 levels were measured and it was reported that these levels were similar in the GDM and control groups, with a positive correlation between maternal serum nesfatin-1 levels and corresponding cord blood levels. As our study measured nesfatin-1 levels from amniotic fluid samples, we cannot directly compare these findings; however, 40 GDM patients at 24-28 weeks' gestation were shown to have lower maternal serum nesfatin-1 concentrations compared to healthy controls, which is consistent with our amniotic fluid measurements.[40]

It is important to consider certain limitations of this study. As this is the first report of irisin and nesfatin-1 in amniotic fluid, comparisons with existing literature are limited. Additionally, the use of BMI for defining obesity does not account for different metabolic responses among obesity phenotypes, which were not discussed in most related studies. Future research should include detailed assessments of obesity phenotypes. Another limitation is the absence of a normal reference range for irisin, complicating the evaluation of our findings. Nonetheless, this study has achieved its primary objective by measuring irisin and nesfatin-1 levels in amniotic fluid. Further comprehensive research is needed to propose these markers for clinical use in obesity.

Conclusion

This study highlights the presence and differential levels of irisin and nesfatin-1 in the amniotic fluid of obese and normal pregnant women, suggesting their potential roles in the pathophysiology of obesity during pregnancy. Further studies are essential to understand these mechnisms and their implications for maternal and fetal health.

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