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Assessing lipoxin-mediated inflammatory responses in the second trimester of pregnancy among women with obesity: A comprehensive analysis

Obezite ile komplike gebeliklerde ikinci trimester lipoksin aracılı enflamatuvar yanıtların değerlendirilmesi

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Abstract

Objective: This study aimed to explore the relationship between maternal plasma lipoxin A4 (LXA4) levels during the second trimester of pregnancy and certain proinflammatory molecules, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), as well as the antiangiogenic factor vascular endothelial growth factor receptor 1 (VEGFR-1), in conjunction with obesity among pregnant women.

Materials and Methods: A total of 30 pregnant women with obesity were compared with 30 pregnant women of normal weight, matched for both age and gestational week. Plasma samples were collected from all participants between the 18th and 28th weeks of pregnancy. The levels of LXA4, VEGFR-1, IL-6, and TNF-α were quantified using enzyme-linked immunosorbent assay.

Results: Plasma levels of LXA4 were notably lower in pregnant women with obesity, whereas levels of TNF-α and VEGFR1 were significantly higher ($p=0.041$, $p<0.001$, and $p<0.001$, respectively). There was no significant difference in IL-6 levels between groups ($p=0.072$). The binary logistic regression model revealed significant associations between obesity and the examined inflammatory mediators. Specifically, the results demonstrated that higher levels of LXA4 were linked to a reduced obesity risk, with each unit increase corresponding to a 0.926-fold decrease in the likelihood of obesity. Conversely, elevated levels of TNF-α and VEGFR1 were associated with an increased risk of obesity.

Conclusion: The study concluded that increased body mass index during pregnancy affects the levels of plasma lipoxin, cytokines, and angiogenesis-related factors. Although the exact mechanisms remain unclear, the observed changes suggest a disruption in the metabolic systems of women with obesity, which may influence physiological changes during pregnancy and lead to obesity-related pathological conditions.

Keywords: Angiogenic and antiangiogenic factors, inflammatory mediators, lipoxins, maternal obesity, pregnancy complications, vascular endothelial growth factor receptor 1

Öz

Amaç: Bu araştırmanın amacı, maternal obezite ile komplike gebelerde ikinci trimester maternal plazma lipoksin A4 (LXA4) düzeyleri ile interlökin-6 (IL-6) ve tümör nekroz faktör alfa (TNF-α) gibi proenflamatuvar moleküller ile birlikte antianjiyogenik faktör vasküler endotelyal büyüme faktörü reseptörü 1 (VEGFR-1) arasındaki ilişkiyi aydınlatmaktır.

Gereç ve Yöntemler: Çalışmaya obezite ile komplike 30 gebe ile yaş ve gebelik haftası açısından eşleştirilmiş 30 normal kilolu gebe dahil edildi. Katılımcılardan gebeliğin 18 ila 28. haftaları arasında plazma örnekleri toplandı. LXA4, VEGFR-1, IL-6 ve TNF-α seviyeleri, enzime bağlı immünosorbent testi kullanlarak ölçüldü.

PRECIS: Pregnant women with obesity show decreased plasma LXA4 levels and increased TNF-α and VEGFR-1 levels, indicating associations with maternal obesity risks and suggesting potential disruptions in metabolic systems during pregnancy.

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Bulgular: Obezite ile komplike gebe kadınların plasma LXA4 seviyeleri anlamlı derecede düşük, TNF-α ve VEGFR-1 plazma düzeyleri ise anlamlı derecede yüksek saptandı (sırasıyla p=0,041, p<0,001 ve p<0,001). Gruplar arasında IL-6 düzeyleri açısından anlamlı fark izlenmedi (p=0,072). Binary lojistik regresyon modeli, obezite ve incelenen enflamatuvar mediatörler arasında anlamlı ilişki olduğunu ortaya çıkardı. Spesifik olarak, sonuçlar, daha yüksek LXA4 seviyelerinin obezite riskinin azalmasıyla bağlantılı olduğunu ve her birim artışın obezite olasılığında 0,926 katlık bir azalmaya karşılık geldiğini gösterdi. Tersine, yüksek TNF-α ve VEGFR-1 düzeylerinin ikisi de obezite riskinin artmasıyla ilişkilendirildi.

Sonuç: Çalışma, gebelikte artan vücut kitle indeksinin plazma lipoksin, sitokin ve anjiyogenez ile ilişkili faktörleri etkilediği sonucuna varmıştır. Kesin mekanizmalar belirsizliğini korusa da, gözlemlenen değişikliklerin obezite ile komplike kadınların metabolik sistemlerinde, gebelik sırasındaki fizyolojik değişiklikleri etkileyebilecek ve obezite ile ilişkili patolojik durumlara yol açabilecek bozulma ile ilişkili olduğunu göstermektedir.

Anahtar Kelimeler: Anjiyojenik ve antianjiyogenik faktörler, enflamatuvar mediatörler, lipoksinler, maternal obezite, gebelik komplikasyonları, vasküler endotelyal büyüme faktörü reseptörü 1

Introduction

Pregnancy-associated obesity is a major public health concern that poses acute and chronic risks to both maternal and neonatal well-being[1]. Such obesity is characterized by a body mass index (BMI) greater than 30 kg/m^2 recorded at the initial antenatal assessment. Based on current data, approximately 30-70% of adults in Europe have excess weight, with 10-30% classified as having obesity[2]. Alarmingly, the global incidence of obesity is increasing rapidly and is approaching pandemic proportions. Evidence shows that maternal obesity increases both immediate health complications and mortality, and this risk profile extends to the long-term health prospects of both mothers and children. An estimated 24% of all pregnancy complications are due to maternal overweight or obesity[3]. In addition, excessive gestational weight gain is associated with one-third of all large-for-gestational-age (LGA) neonates. Pregestational obesity is associated with reduced fertility and a variety of pregnancy complications, including miscarriage, thromboembolism, gestational diabetes mellitus (GDM), hypertension, preeclampsia, congenital fetal anomalies, preterm birth, macrosomia, and postterm delivery. In addition, maternal obesity associated with pregnancy complications increases the long-term risk of obesity, diabetes, and cardiovascular disease in offspring[4]. These compelling statistics underscore the urgent need for a thorough understanding of the biological underpinnings that contribute to adverse perinatal outcomes in pregnancies complicated by obesity.

Although a limited number of studies have investigated maternal systemic inflammation in pregnant women with obesity, their findings are often conflicting. It has been postulated that physiological adaptations associated with pregnancy may mask the underlying inflammatory responses attributable to obesity[5]. Because of the significant involvement of inflammation in the development of both obesity and pregnancy, it is conceivable that collaborative interactions between maternal physiological adaptations and the inflammatory responses triggered by obesity could lead to an excessive amplification of inflammatory mediators. This, in turn, could contribute to an increase in both immediate and long-term morbidity in pregnant women with obesity. The expansion of adipose tissue contributes to the infiltration of macrophages and the release of inflammatory adipokines, including leptin, tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6)[6]. In obesity, there is an overexpression of TNF-α and IL-1β, which hampers insulin signaling in both animal and human adipose tissue. TNF-α, primarily a proinflammatory cytokine produced by myeloid cells, triggers the release of other inflammatory cytokines such as IL-6 and IL-1β. IL-6, primarily secreted by adipocytes, is linked to conditions such as hyperglycemia, insulin resistance, and obesity[7]. As with TNF-α, increases in body mass and waist circumference lead to increased production of IL-6 relative to free fatty acids. Within the context of maternal obesity, this condition manifests as an inflammatory metabolic disorder characterized by increased circulating proinflammatory cytokines and greater macrophage accumulation in adipose tissue. Inflammation also affects the placenta, creating an intrauterine environment that is prone to inflammation. In addition, research has found a positive correlation between maternal serum levels of IL-6 and fetal growth, linking the proinflammatory state of mothers with obesity to excessive intrauterine growth[8]. Although higher levels of cytokines such as leptin, C-reactive protein (CRP), IL-6, and intercellular adhesion molecule-1 have been found in pregnancies affected by obesity compared with similar pregnancies in individuals without obesity, these observations are not consistently replicated for all inflammatory markers[9]. The full picture of inflammatory mediators in pregnancies with obesity remains unclear and a subject of debate in the scientific community. Therefore, further research is needed to determine the medical relevance of these inflammatory molecules in the management of obesity in pregnancy.

Lipoxins (LXs) are bioactive lipid mediators synthesized from arachidonic acid with potent anti-inflammatory and immunoregulatory properties. LX, an endogenously produced eicosanoid, has anti-inflammatory, anabolic, and antifibrotic properties[10]. Lipoxins induce the inactivation of the major proinflammatory pathway and release of resoluble cytokines by downregulation proinflammatory cytokines and chemokines. Lipoxin A4 (LXA4) is a molecule that reduces adipose tissue inflammation and insulin resistance[11]. The natural lipid mediator LXA4 plays a crucial role in maintaining a healthy pregnancy by modulating factors related to inflammation, mast cells, and various other cellular components. It acts as a vital regulator in the complex biological processes of pregnancy,

contributing significantly to maintaining the delicate balance between inflammation and resolution necessary for a successful pregnancy[12]. Despite its pivotal role, there is a noticeable gap in the scientific literature regarding comprehensive investigations into the relationship between serum LXA4 levels and maternal obesity. Although it is well established that maternal obesity is linked to various adverse pregnancy outcomes and heightened inflammatory responses, the potential connections between these altered physiological and immunological states and variations in LXA4 levels remain poorly understood. This gap in understanding highlights the need for further investigation into this complex interplay of factors.

The successful achievement of an optimal pregnancy outcome relies on the establishment of the maternal– fetal vascular interface during early gestation and the continuous process of placentation throughout pregnancy. Disruptions in the placental production of angiogenic factors due to an imbalance in angiogenesis can lead to a range of adverse perinatal outcomes[13]. At the core of this vascular interface are the vascular endothelial growth factor (VEGF) and its receptor (VEGFR) system, which predominantly drives angiogenic activity within adipose tissue. VEGF family members bind to transmembrane tyrosine kinase receptors, specifically VEGFR1 (Flt-1), VEGFR2 (KDR/Flk-1), and VEGFR3 (Flt-4). Notably, VEGFR-1 and VEGFR-2 play significant roles as mediators of angiogenesis, whereas VEGFR-3 is involved in lymphangiogenesis regulation[14]. Imbalanced levels of certain antiangiogenic factors, including soluble fms-like tyrosine kinase 1 (sFlt1) and endoglin, have been strongly linked to various placental disorders such as preeclampsia, placental abruption, stillbirth, and intrauterine growth restriction. Recent studies have suggested that elevated maternal serum Flt1 levels may increase the risk of preterm birth, a risk that appears to be unrelated to pre-eclampsia[15]. It has been postulated that maternal obesity may induce an angiogenic imbalance via multiple adipokine-mediated pathways. However, our current understanding of the relationship between maternal obesity and the balance between angiogenic and antiangiogenic factors remains limited, particularly in the context of human pregnancy. Therefore, further investigation of this association is warranted to better understand its mechanistic basis and potential clinical implications.

Despite the literature documenting increased serum cytokine levels in pregnant women with obesity, there is a notable gap in our understanding of the relationship between serum LXA4 levels and maternal obesity. Therefore, this study aims to fill this knowledge gap. The primary objective of this research endeavor is to delineate the association between second trimester maternal plasma LXA4 levels, proinflammatory molecules IL-6 and TNF-α, and anti-angiogenic factor VEGFR-1 and obesity in pregnant women. The results of this investigation are expected to provide important insights into the dynamic interplay of these molecules in the context of maternal obesity.

Materials and Methods

The study encompassed all expectant mothers treated at the Inonu University Faculty of Medicine, Department of Obstetrics and Gynecology, during the period from May 01, 2021, to May 01, 2022, between the 18th and 28th weeks of gestation. Ethical approval was obtained from the Inonu University Clinical Research Ethics Committee (approval number: 2021/114, date: 31.03.2021). Adhering to the principles of the Declaration of Helsinki (2013 revision), the participants received comprehensive written and verbal details about the study, and their informed consent was duly obtained. Thirty pregnant women with a body mass index of 30 kg/m^2 and above formed the study group, whereas 30 normal-weight pregnant women with a body mass index between 18.5 and 24.9, matched for age and gestational weeks, constituted the control group. The gestational weeks of the pregnant women who participated in the study were confirmed based on the first trimester ultrasound measurements.

Participants were eligible for inclusion in the study if they met the following criteria:

- Women aged between 18 and 45 years.
- A singleton viable pregnancy.
- Normal obstetric and medical history with no history of any significant health issues.

Conversely, individuals were excluded from the study based on the following criteria:

- The presence of multiple pregnancies (twins, triplets, etc.).
- Evidence of coexisting systemic diseases in pregnant women, including chronic hypertension, dyslipidemia, asthma, chronic renal failure, malignancies, and any cardiac or pulmonary diseases.
- Detection of chromosomal abnormalities and fetal malformations.
- History of cigaret smoking and alcohol consumption during pregnancy.

Standard serum analyses were performed using the Abbott Architect C8000 system at the Biochemistry Laboratory of Inonu University School of Medicine. At the beginning of the study, 2 mL peripheral blood samples were collected from all participants as part of the routine laboratory procedure. These samples were collected in EDTA-anticoagulated tubes to prevent blood clotting. After collection, plasma was isolated by centrifugation at 3000 g for 15 min at room temperature and then stored at -80 °C to maintain sample integrity until analysis. After achieving the intended sample size, the frozen plasma samples were thawed. Quantitative levels of LXA4, VEGFR-1, IL-6, and TNF-α were determined using enzyme-linked immunosorbent assay (ELISA). Specifically, ELISA kits for LXA4, IL-6, TNF-α (catalog numbers E3155Hu, E2063Hu, and E0796Hu, respectively; manufactured by Sunredbio Corp, China), and VEGFR-1 (catalog number E3155Hu; manufactured by Cloude Clone Corp, China) were utilized, following the manufacturers' protocols. The measurement ranges for LXA4,

IL-6, TNF-α, and VEGFR-1 assays were 0.1-38.0, 1-400, 0.5-150, and 0.3-90.0 ng/mL, respectively. The inter- and intra-assay precision coefficients of variation consistently remained below 10% and 8%, respectively, ensuring the reliability and reproducibility of the results obtained from the ELISA kits used in this study.

In addition to the meticulous serum analyses, a comprehensive set of demographic, clinical, and biochemical parameters was systematically recorded for each participant.

Sample size calculation: The sample size calculation was based on a power analysis assuming that a 1.0 pg/mL decrease in the LXA4 ratio (equivalent to 1.7 standard deviations) in pregnant women with obesity would have a statistically significant effect. To detect such an effect with 80% power and a 5% significance level (two-tailed), a minimum of 30 participants would be needed in each study group.

Statistical Analysis

The statistical analysis was performed using SPSS software, version 22.0 (SPSS Inc, New York, USA). Baseline data for both the study and control groups are presented as medians with corresponding ranges and/or interquartile ranges for categorical variables. Continuous data are expressed as means, standard deviations, and minimum and maximum values. Data distribution was assessed using the Shapiro-Wilk test. Initial group comparisons involved two-sample t-tests for normally distributed data and Mann-Whitney U tests for non-normally distributed data. Categorical variables are indicated as counts and percentages, and comparisons were conducted using Pearson's exact chi-square and continuity-corrected chi-square tests. A binary logistic regression model was constructed, with group classification as the dependent variable and serum concentrations of LXA4 (ng/mL), IL-6 (ng/mL), TNF-α (ng/mL), and VEGFR1 (pg/mL) as independent variables. The "Enter" method was employed to simultaneously evaluate the significance of all variable coefficients in a single step. The goodness of fit of the model was assessed using the Hosmer-Lemeshow test, and a significance level of α=0.05 was considered for a two-tailed p-value.

Results

Clinical Characteristics of the Study Population

The statistical analysis revealed that there were no notable variations between the study and control groups in terms of age, gravidity, parity, gestational age at screening, gestational age at birth, mode of delivery, and birth weight (with p-values of 0.801, 0.079, 0.101, 0.250, 0.881, 0.639, and 0.115, respectively, as indicated in Table 1). Conversely, the obesity group exhibited significantly higher current and prepregnancy BMI levels, along with a higher occurrence of adverse perinatal outcomes compared with the control group (p-values of <0.001, <0.001, and 0.025, respectively, as presented in Table 1).

Evaluation of Plasma Levels of LXA4 (ng/mL), IL-6 (ng/mL), TNF-α (ng/mL) and VEGFR1 (pg/mL) in Pregnant Women with Obesity

Both pro- and anti-inflammatory mediators were assessed in the peripheral blood plasma of both the study and control groups. When compared with the control group, pregnant women with obesity showed a significant reduction in their plasma LXA4 concentration (p=0.041). No significant differences in IL-6 levels were observed between the two groups (p=0.072). In contrast, plasma levels of TNF-α and VEGFR1 were notably higher in the obesity group than in the control group (p<0.001 for both). Comprehensive information regarding the plasma concentrations of LXA4, IL-6, TNF-α, and VEGFR1 in both the study and control groups can be found in Table 2. Figure 1 illustrates the distribution of proinflammatory molecules in plasma samples from the study and control groups.

Predictive Value of Inflammatory Mediators in Obesity

To evaluate the capability of plasma proinflammatory molecules to predict obesity, we employed a binary logistic regression model. In this model, serum levels of LXA4, IL-6, TNF-α, and VEGFR-1 served as independent variables, whereas obesity (as opposed to control) was the dependent variable. The model's accuracy was confirmed through the Hosmer-Lemeshow test, indicating its capability to distinguish between the obesity and control groups (Hosmer-Lemeshow χ^2 =9.854, p=0.275>0.05). Our logistic regression model was statistically significant and reliable. In particular, an elevation of one unit (ng/mL) in LXA4 was associated with a 0.926-fold decrease in the likelihood of obesity. Likewise, a single unit increase in TNF-α corresponded to a 1.026-fold increase in the risk of obesity (ng/L), whereas an increment in VEGFR1 was linked to a 1.003-fold increase in the odds of obesity (ng/mL). Further details, such as parameter estimates (β), standard errors (se), Wald statistics (W), degrees of freedom (df), odds ratios [Exp (β)], and 95% confidence intervals, are available in Table 3.

Discussion

This study provides a substantial contribution to the scientific discourse surrounding the assessment of obesity during pregnancy, particularly in the context of its association with serum LXA4, TNF-α and VEGFR1 levels. Although obesity and inflammation have been frequently discussed in recent scientific discourse, there remains a notable gap in our knowledge of the behavior of inflammatory and anti-inflammatory molecules in normal-weight women compared with those characterized as overweight during pregnancy. Despite the increased predisposition of obese women to placental vascular dysfunction, investigations into the pathophysiological factors that may contribute to this vulnerability remain limited. Accumulating evidence suggests that the regulation and effects of metabolic systems in individuals with obesity differ markedly from those of their normal weight counterparts[16]. LXA4, a prominent lipoxin

Table 1. Clinical characteristics and birth outcomes of the pregnant women with obesity and the control group

Variable			Obesity (n=30)	Control (n=30)	p-value
Age (years)*			33.5 (21-43)	33.1 (20-43)	0.801
Gravidity*			3 (1-7)	2 (1-6)	0.079
Parity*			2 (0-4)	1 (0-4)	0.101
Weight (kg) *			92 (72.5-107)	63 (46-80)	**<0.001**
Height (cm)*			162 (151-170)	160 (145-176)	0.710
BMI (kg/m²)*			35.2 (29.04-41.62)	23.86 (20.31-28.3)	**<0.001**
Gestational age at screening (weeks)*			25 (18-28)	27 (18-28)	0.250
Pregravid weight (kg)*			85.5 (65-105)	54.5 (47-65)	**<0.001**
Pregravid BMI (kg/m²)*			32.42 (27.06-39.52)	21.92 (17.8-28.55)	**<0.001**
Adverse perinatal outcomes**	**FGR**		1 (3.3)	3 (10)	**0.025**
	GHT/Preeclampsia		5 (16.7)	2 (6.7)	
	Preterm birth		2 (6.7)	0 (0)	
	PPROM		1 (3.3)	0 (0)	
	GDM		5 (16.7)	0 (0)	
	LGA		2 (6.7)	0 (0)	
Gestational age at birth (weeks)*			37 (26-41)	38 (28-40)	0.881
Mode of delivery**	**Vaginal delivery**		8 (26.7)	11 (36.7)	0.639
	Cesarean section		22 (73.3)	19 (63.3)	
Birthweight (g)*			3020 (875-4800)	2770 (755-3725)	0.115
Gender**		**Female**	17 (56.7)	17 (56.7)	1.000
		Male	13 (43.3)	13 (43.3)	
Cord blood pH***			7.37±0.17	7.30±0.15	0.118
Cord blood base excess***			-4.47±3.35	-5.28±4.54	0.600

* median (minimum-maximum); ** n (%); *** mean ± standard deviation
BMI: Body mass index, FGR: Fetal growth restriction, GHT: Gestational hypertension, PPROM: Preterm premature rupture of membranes, GDM: Gestational diabetes mellitus, LGA: Large for gestational age
p-values marked with bold indicate statistically significant differences between the groups

Table 2. Comparison of plasma levels of LXA4, IL -6, TNF-α, and VEGFR1 between the study and control groups

Variable	Obesity (n=30)	Control (n=30)	p-value
LXA4 (ng/mL)*	30 (14.42-79.42)	39 (15.01-71.34)	**0.041**
IL-6 (ng/L)*	16.26 (11.12-26.75)	13.73 (10.06-44.34)	0.072
TNF- α (ng/L)*	148.23 (103.83-196.26)	83.12 (49.26-153.35)	**<0.001**
VEGFR1 (pg/mL)*	632.19 (490.46-847.41)	487.31 (406.17-569.52)	**<0.001**

* median (minimum–maximum), LXA4: Lipoxin A4, IL: Interleukin, TNF: Tumor necrosis factor, VEGFR-1: Vascular endothelial growth factor receptor 1.
p-values marked with bold indicate statistically significant differences between the groups

in mammals, plays a critical role in regulating inflammation during the menstrual cycle, endometrial neuroregulation, embryo implantation, pregnancy, and parturition. An increase in LXA4 in the first trimester is associated with normal pregnancy and placental development, a decrease in the second trimester indicates a healthy pregnancy, and an increase in the third trimester is associated with normal pregnancy and preparation for delivery[17]. Endogenous LXA4 is a key determinant of normal

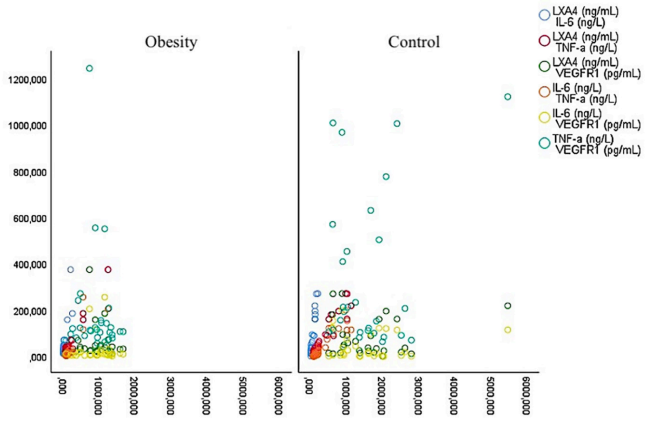

Figure 1. Binary distributions of parameters in the study and control groups

pregnancy outcomes by modulating mast cell migration and proinflammatory factors. Although a number of inflammatory markers have been analyzed previously, this study is the first to demonstrate significantly reduced plasma levels of LXA4 in the second trimester in pregnant women with obesity and is one of the first to demonstrate that factors associated with obesity may differentially influence aspects of inflammation and angiogenesis in overweight women compared with normal-weight women during pregnancy.

The receptor for LXA4, FPR2/ALX (N-formyl peptide receptor 2 and A lipoxin A), has been found to increase LXA4 levels in the third trimester of pregnancy relative to proinflammatory cytokines such as IL-1β and TNF-α[18]. Existing data on LXA4 levels during pregnancy are scarce. Previous studies have reported that LXA4 levels in women at 24 weeks of gestation exceed those in nonpregnant women[19]. Research by Perucci et al.[20] showed significantly elevated LXA4 levels in pregnant women with pre-eclampsia at or beyond 28 weeks gestation compared with normotensive pregnant women of the same gestational age. In this study, we found a significant difference in LXA4 levels in pregnant women between the obesity and control groups. The LXA4 levels observed in the pregnant women with obesity and the control group in our study are in line with the findings of Szczuko et al.[12], who documented variations in LXA4 levels throughout the weeks of pregnancy. Maternal obesity is an inflammatory metabolic disorder characterized by elevated circulating proinflammatory cytokines and increased macrophage infiltration within adipose tissue. This inflammation extends to the placenta, creating a proinflammatory intrauterine environment. Although there are documented cases of higher cytokine levels in pregnancies affected by obesity compared with pregnancies without obesity, these findings are not consistent across all inflammatory markers. The full picture of inflammatory mediators in pregnancies with obesity remains unclear and is the subject of ongoing debate within the scientific community. Therefore, further research is essential to determine the clinical significance of these inflammatory molecules in the management of obesity during pregnancy.

IL-6, a systemic adipokine, is secreted by adipose tissue and skeletal muscle in humans. Adipose tissue expansion is associated with an increase in the levels of proinflammatory adipokines, including TNF-α and IL-6[21]. In obesity, IL-6 has been implicated in the recruitment of macrophages into expanding adipose tissue, leading to chronic inflammation, impaired insulin sensitivity, and the potential development of type 2 diabetes[22]. TNF-α, secreted by macrophages in adipose tissue, is an inflammatory cytokine involved in the development and maintenance of insulin resistance[23]. In contrast to earlier stages of pregnancy, Friis et al.[24] found no significant variation in maternal IL-6 levels between BMI categories in pregnant women with obesity at 36-38 weeks of gestation and no significant difference in soluble tumor necrosis factor receptor II between BMI categories. Lodefalk et al.[25] reported a decrease in the expression of TNF, IL6, insulin-like growth factor (IGF)-1, and IGF2 in the placenta of pregnant women with obesity, which was inversely correlated with the duration of the pushing phase of labor. In pregnant patients with GDM and a BMI ≥33 kg/m^2, gene expression analysis of adipose tissue revealed significantly increased levels of TNF-α expression compared with controls[26]. Challier et al.[6] reported that plasma TNF-α levels in mothers with obesity were not significantly different from those in controls, whereas IL-6 and CRP levels were significantly elevated. In our study, no significant difference in

Table 3. Estimated values of the parameters in the model

Variables	β	SE	W	sd	p-value (sig)	Exp (β)	95% CI for Exp (β)	
							Lower limit	Upper limit
LXA4 (ng/mL)	-0.077	0.031	6.086	1	0.014	0.926	0.871	0.984
IL-6 (ng/L)	-0.002	0.018	0.014	1	0.907	0.998	0.964	1.034
TNF-α (ng/L)	0.026	0.009	9.032	1	0.003	1.026	1.009	1.044
VEGFR1 (pg/mL)	0.003	0.001	8.772	1	0.003	1.003	1.021	1.036
Constant	2.408	0.953	6.387	1	0.011	11.114		

β: parameter estimation, SE: standard error, W: Wald statistic, sd: degrees of freedom, Exp (β): odds ratio, 95% CI: confidence interval, LXA4: Lipoxin A4, IL: Interleukin, TNF: Tumor necrosis factor, VEGFR-1: Vascular endothelial growth factor receptor 1

IL-6 levels was observed between the obese and control groups, whereas a statistically significant increase in TNF-α levels was observed. Higher IL-6 levels in the early weeks of pregnancy in women with obesity probably reflect prepregnancy status rather than gestational weight gain[27]. Another study reported that IL-6 levels increased in the early weeks of pregnancy, decreased slightly in the middle of pregnancy, and increased again in the late weeks of pregnancy[28]. The lack of a significant difference in IL-6 levels in our study supports the conclusion that obesity in early pregnancy may have a greater influence on inflammation. The significantly elevated TNF-α levels in the group with obesity compared to normal TNF-α levels in pregnancy highlight the potentially pivotal role of TNF-α and LXA4 in modulating inflammatory processes within visceral adipose tissue.

Angiogenesis, which is associated with VEGF and placental growth factor levels, plays a critical role in the physiological and pathological conditions of embryonic development. VEGF mediates its effects through interaction with VEGFR-1/flt-1 and VEGFR-2/KDR receptors. In studies investigating conditions such as hypoxia, maternal sleep apnea, hyperglycemia, obesity, and abnormal fetal growth, changes in the expression levels of angiogenesis factors such as VEGF, VEGFR-1, and VEGFR-2 in plasma and placenta have been observed[29]. Dubova et al.[30] investigated placental VEGFR expression in both pregnancies with obesity and normal weight and found decreased VEGFR1 in the vascular endothelium, whereas VEGFR-2 and VEGFR-3 were increased in nonvillous cytotrophoblasts and endothelial cells of mature intermediate and terminal villous capillaries. In contrast, another study showed that preexisting obesity and diabetes had no significant effect on the expression or secretion of VEGF-A, VEGFR1, and VEGFR2 in pregnant women[31]. A 2021 study showed significantly higher protein and mRNA levels of VEGF and VEGFR-1 receptors in the placenta of physically active pregnant women than in their inactive counterparts[32]. Adipokines may play a pivotal role in obesity-related angiogenesis, with elevated leptin levels in obesity potentially enhancing angiogenesis via the upregulation of VEGFR expression and adhesion molecule expression. Therefore, it is plausible that increased secretion of angiogenic molecules in obesity could be mediated by increased adipokine expression. The increased serum VEGFR-1 levels in pregnant women with obesity compared with control pregnant women could be explained by the contribution of obesity to the release of VEGFR-1-mediated angiogenic molecules.

Study Limitations

Although this investigation sheds light on the interplay between LXA4 and inflammatory cytokines in maternal obesity, it is important to recognize several constraints in this study. The single-center approach limits population diversity, potentially restricting the applicability of the results to broader contexts. Despite the sample size being sufficient for the preliminary investigation of plasma LXA4 levels and inflammatory cytokines, its relatively small scale could weaken the statistical power and obscure specific potential effects. Replicating our findings using varied sample sizes and recruitment criteria can enhance their reliability. However, within our experimental framework, which is characterized by a moderate effect size, we achieved statistical significance and robust results. We should note that we only measured plasma LXA4 levels during the second trimester, thus missing the opportunity to monitor LXA4 fluctuations across different pregnancy stages. Nevertheless, this study has notable strengths. This study is the first to compare LXA4 levels in pregnant women with obesity with those in normal-weight pregnant women, offering valuable insights into the mechanisms driving obesity-related inflammation during pregnancy. The prospective cohort design permits real-time tracking of patient progress and identification of temporal relationships between variables. Consequently, this study not only contributes to existing scientific knowledge but also serves as a basis for future research. These future efforts could further clarify the pathophysiology of obesity-associated inflammation and potentially identify novel diagnostic markers and therapeutic interventions.

Conclusion

This study adds significantly to the body of scientific knowledge by demonstrating that increased BMI has potential effects on plasma lipoxin, cytokine, and angiogenesis-related factor levels during pregnancy. The precise mechanisms by which maternal obesity influences plasma LXA4, sFlt1, and cytokine levels remain incompletely understood. However, the observed differences between the two groups suggest a disruption of normal metabolic systems in women with obesity. Growing evidence suggests that these disruptions may significantly impact the body's normal changes during pregnancy and contribute to various health issues related to obesity. Consequently, changes in lipid balance during pregnancy may play a role in inadequate placental growth and compromised endothelial function. Given the growing global obesity epidemic, our findings highlight the urgent need for further investigation in this area. The physiological changes induced by obesity during pregnancy have both immediate and long-term health implications for the mother and developing fetus, and understanding these processes is critical to improving maternal and child health outcomes. By improving our understanding of these processes, we can better guide the development of targeted therapeutic strategies and preventive interventions, thereby ensuring healthier pregnancies and better health outcomes for both mothers and their offspring.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Inonu University Clinical Research Ethics Committee (approval number: 2021/114, date: 31.03.2021).

Informed Consent: The participants received comprehensive written and verbal details about the study, and their informed consent was duly obtained

Peer-review: Internally and externally peer-reviewed.
Authorship Contributions
Surgical and Medical Practices: Ö.O., Concept: R.M., Design: R.M., Ö.O., A.Ş.E., Data Collection or Processing: R.M., T.R.K., F.İ., Analysis or Interpretation: Ö.O., T.R.K., F.İ., Literature Search: Ö.O., Writing: R.M., Ö.O., T.R.K., F.İ., A.Ş.E.
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