



# Blood level of adipokines and nutritional status variables in adolescent pregnancy

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## Objective

To evaluate the serum levels of adiponectin and leptin and their relationship with nutritional variables during pregnancy in adolescents.

## Methods

This prospective cohort study evaluated eutrophic pregnant adolescents (body mass index [BMI], 18.5–24.9 kg/m<sup>2</sup>) during the 3 gestational trimesters (first, 10–14 weeks; second, 24–28 weeks; and third, 30–34 weeks). Serum adiponectin and leptin concentrations were measured using the enzyme-linked immunosorbent assay method. The relationship of these adipokines with the pre-gestational BMI, gestational weight gain, weight at the time of sample collection, and newborn weight were evaluated. Analysis of variance and the Kruskal-Wallis test were used for statistical analysis.

## Results

The study group comprised 62 pregnant adolescents. The serum concentration of adiponectin showed a significant difference between the first and third trimesters ( $P=0.003$ ), which decreased during pregnancy, but unrelated to nutritional variables. Serum leptin levels increased throughout the pregnancy ( $P<0.0001$ ) and showed a positive correlation with pre-gestational BMI, total weight gain, pregnancy weight at the time of sample collection, and newborns' weight.

## Conclusion

Serum levels of adiponectin and leptin vary inversely throughout pregnancy. This pattern in adolescents is similar to that observed in adults. Moreover, leptin concentrations increased throughout pregnancy, and they were positively correlated with all variables evaluated.

**Keywords:** Adiponectin; Leptin; Nutrition status; Pregnancy; Adolescents

## Introduction

Adolescent pregnancy is highly prevalent in the world despite the development level of the country [1]. The annual report published in 2013 by the United Nations Population Fund (UNFPA) on the situation of the world population cited that yearly, in developing countries, 7.3 million adolescent old have children, of which about 2 million were less than 14 years old [2].

During the gestational period, an inflammatory response develops in the maternal-fetal unit. Several mediators are involved in this process, which includes adipokines. These proteins' participation in local and systemic inflammatory

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reactions is recognized, but the influence of adipokines on the evolution of pregnancy is not fully understood yet [3]. Adiponectin has the function of regulating metabolism interfering with insulin resistance (IR) in the liver and muscles [4] besides presenting an anti-inflammatory, anti-hyperglycemic, and anti-atherogenic action [5]. During a healthy pregnancy, adiponectin levels progressively decrease, and IR develops, which returns to pre-pregnancy values after delivery [6]. Leptin works by modulating appetite and controlling levels of adipose tissue in eutrophic individuals. Blood leptin concentrations are high in women and even higher in pregnant women [7]. The placenta secretes leptin, and it has been associated with adverse maternal and perinatal outcomes [8].

Adiponectin and leptin present physiological actions, thus affecting patients' health according to their concentrations in the blood. However, few studies relate these two molecules in the evolution of pregnancy, especially in adolescents. To date, no studies have assessed the relationship between pre-gestational body mass index (BMI), maternal and newborn weight gain with the concentrations of these adipokines in adolescents. Therefore, this study aimed to assess serum levels of adiponectin and leptin and their relationship with nutritional variables throughout adolescent pregnancy. The results of this study will be useful for future investigations of the association between serum changes in adiponectin or leptin levels and adverse perinatal outcomes, such as miscarriage, pre-eclampsia, diabetes mellitus, preterm birth, fetal growth restriction, low birth weight, and inadequate gestational weight gain, among pregnant adolescents.

## Materials and methods

A prospective cohort study was conducted among eutrophic pregnant adolescents (BMI, 18.5–24.9 kg/m<sup>2</sup>) who attended the Adolescent Prenatal Sector of the Federal University of São Paulo (UNIFESP) from March 2015 to January 2017. The Research Ethics Committee from UNIFESP approved this study under the consubstantiated opinion No. 1514/11, and all pregnant women who agreed to participate voluntarily signed an informed consent form. The inclusion criterion was a single pregnancy in adolescents aged <20 years. Exclusion criteria were the use of medications (corticosteroids, antibiotics, immunosuppressants, and anti-inflammatories); maternal obstetric or chronic diseases (pre-eclampsia, gestational dia-

betes mellitus, chronic arterial hypertension, systemic lupus erythematosus, and asthma); and presence of obstetric complications (e.g., prematurity) at birth.

The BMI was calculated using the formula pre-gestational maternal weight (kg)/height (m<sup>2</sup>), with the former referred by the mother and the latter confirmed with measurement. The nutritional diagnosis was determined as recommended by the Institute of Medicine (IOM) [9].

Blood collection occurred during the 3 periods of pregnancy: first trimester (10 to 14 weeks), second trimester (24 to 28 weeks), and third trimester (30 to 34 weeks). An 8 mL volume of peripheral blood was collected by venipuncture in a dry tube. The samples were transported in thermal boxes, refrigerated at a temperature between 2°C and 8°C and processed within a maximum period of up to 4 hours after collection. The blood samples were centrifuged after clot retraction at 500 rpm for 15 minutes at room temperature. The serum obtained was aliquoted in sterile and dry microtubes and then stored at –80°C for future quantification of adiponectin and leptin serum concentrations.

Serum levels of adiponectin and leptin were assessed using the enzyme-linked immunosorbent assay (ELISA) method using the commercial kit Quantikine<sup>®</sup>-Human total Adiponectin/Acrp30 DuoSet (R&D Systems<sup>®</sup>, Minneapolis, MN, USA) and Quantikine<sup>®</sup> Human Leptin DuoSet (R&D Systems<sup>®</sup>). These kits are immunoenzymatic ELISA tests, which were performed according to the manufacturer's instructions. The test sensitivity was 0.0625 ng/mL for adiponectin and 32.25 pg/mL for leptin. The intra-assay and inter-assay CVs% for adiponectin were 3.5% and 6.5% and for leptin 3.2% and 4.4%, respectively.

For adiponectin and leptin dosage curves in pregnant adolescents in the first, second, and third trimester, tables containing minimum, maximum, mean, or median values and standard deviation (SD) or interquartile range were included. Normality tests such as Skewness and Kurtosis, Kolmogorov-Smirnov, and Shapiro-Wilk, were applied to assess the distribution of quantitative variables. Thus, analysis of variance (ANOVA) was applied for parametric distributions and the Kruskal-Wallis test for nonparametric distributions, followed by Tukey or Dunn post-tests, respectively. Pearson's coefficient (r) was used to assess the correlation between variables. Descriptive statistics were performed with minimum and maximum values, mean and SD for maternal age, BMI, and race. The level of statistical significance was established

at  $P < 0.05$ . Statistical analyzes were performed using the GraphPad Prism software version 6.0 (GraphPad Software, San Diego, CA, USA).

## Results

In this study, 62 eutrophic pregnant adolescents were included and evaluated in the three trimesters of pregnancy. Maternal age ranged from 12 to 19 years, with an average of  $16.45 \pm 1.87$  years. The mean of the pre-gestational BMI was  $21.8 \pm 2.23$  kg/m<sup>2</sup>. The average gestational age in the first,

second, and third trimesters was  $12.38 \pm 1.35$ ,  $25.36 \pm 2.62$ , and  $32.90 \pm 2.42$  weeks, respectively (Table 1).

We observed significant difference between the adiponectin values obtained in the first, second, and third trimesters. As pregnancy progressed, serum adiponectin levels gradually decreased (median 3,578 ng/mL, 2,774 ng/mL, 2,218 ng/mL, respectively). In Dunn post-tests, a statistically significant difference was observed when comparing the first and third trimesters ( $P = 0.003$ ). However, no statistically significant difference was found comparing the first and second trimesters and the second and third trimesters ( $P = 0.09$  and  $P = 0.82$ , respectively) (Table 2).

**Table 1.** Maternal and perinatal characteristics of pregnant adolescents

Characteristic	1 <sup>st</sup> trimester (n=62)	2 <sup>nd</sup> trimester (n=62)	3 <sup>rd</sup> trimester (n=62)
Age (yr)			
Minimum–Maximum		12–19	
Mean±SD		$16.45 \pm 1.87$	
BMI (kg/m <sup>2</sup> )			
Minimum–Maximum		18.5–24.9	
Mean±SD		$21.80 \pm 2.23$	
Race/ethnicity			
White		36 (58.06%)	
Mixed		26 (41.94%)	
Total weight gain (kg)			
Minimum–Maximum		6.50–30.00	
Mean±SD		$13.60 \pm 4.56$	
Newborn weight (g)			
Minimum–Maximum		2,115–4,200	
Mean±SD		$3,064.80 \pm 484.43$	
Gestational age at sample collection (wk)			
Minimum–Maximum	10.00–14.11	24.10–28.20	30.43–34.10
Mean±SD	$12.38 \pm 1.35$	$25.36 \pm 2.62$	$32.90 \pm 2.42$

BMI, body mass index; GA, gestational age; SD, standard deviation.

**Table 2.** Serum adiponectin and leptin concentrations in the three trimesters of adolescent pregnancies

Trimester (n=62)	Adiponectin (ng/mL)				Leptin (pg/mL)		
	Min–Max	Median	Interquartile range	$P^a$	Min–Max	Mean±SD	$P^b$
1 <sup>st</sup>	1,141–13,499	3,578	2,388–5,952	0.09	5,594–166,097	$36,807 \pm 31,946$	0.26
2 <sup>nd</sup>	1,205–16,035	2,774	1,578–4,336	0.003	1,401–96,912	$27,983 \pm 18,755$	0.005
3 <sup>rd</sup>	1,428–13,857	2,218	1,727–3,615	0.82	3,997–189,830	$54,435 \pm 39,226$	<0.0001
Kruskal-Wallis test, $P = 0.003$				One-way ANOVA, $P < 0.0001$			

SD, standard deviation; ANOVA, analysis of variance.

<sup>a</sup>Trimestral comparison using Dunn's test; <sup>b</sup>Trimestral comparison using Tukey's test

We did not observe any correlation between serum adiponectin levels and pre-pregnancy BMI, total pregnancy weight gain, maternal weight at the time of blood collection, and newborn weight (data not shown).

Serum leptin concentrations decreased in the second trimester; however, with the progression of pregnancy, there was a significant increase in blood levels ( $P < 0.0001$ ). In the third trimester, serum leptin levels were higher (mean,  $54,435 \pm 39,226$  pg/mL) when compared to the first (mean,  $36,807 \pm 31,946$  pg/mL) ( $P = 0.005$ ) and the second trimesters (mean,  $27,983 \pm 18,755$  pg/mL) ( $P < 0.0001$ ), when the Turkey's post-tests was performed (Table 2).

We observed a positive correlation between serum leptin levels and pre-pregnancy BMI, total weight gain during pregnancy, maternal weight at the time of blood collection, and newborn weight (Table 3).

## Discussion

In general, the vast majority of studies on adipokines are restricted to comparing the serum profile among pregnant

adults with and without obstetric complications or associated diseases [10-12]. Adipokines have been the subject of studies that seek to find and determine biomarkers to assist in prevention or prognosis of complications such as pre-eclampsia, gestational diabetes mellitus, restriction of intrauterine growth, and low birth weight [13,14].

In this study, serum adiponectin levels in pregnant adolescents decreased progressively throughout pregnancy. This result was also observed in adult women during healthy pregnancies [15,16]. A possible explanation would be due to the installation and/or change of IR, which tends to increase with the evolution of pregnancy and returns to pre-pregnancy values after delivery [17,18]. Thus, we found a negative correlation between gestational age and serum adiponectin concentration, which is also described in the literatures including other age groups [19,20].

Fuglsang et al. [21] compared the first with the third trimester of pregnancy and observed that plasma levels of adiponectin in adult pregnant women significantly decreased. This behavior was also observed in this study in pregnant adolescents and confirmed by other studies in pregnant women [22,23].

**Table 3.** Correlation between pre-gestational body mass index, total weight gain during pregnancy, weight at the time of blood collection and newborn weight, and serum leptin levels between the first, second, and third trimester of adolescent pregnancy

Variable	Leptin (pg/mL)		
	1 <sup>st</sup> Trimester (n=62)	2 <sup>nd</sup> Trimester (n=62)	3 <sup>rd</sup> Trimester (n=62)
Pre-gestational BMI (kg/m <sup>2</sup> )			
Pearson r	0.44	0.51	0.27
Confidence interval	0.21, 0.62	0.29, 0.67	0.02, 0.49
$P^a$	0.0003 <sup>b</sup>	<0.0001 <sup>b</sup>	0.03 <sup>b</sup>
Total weight gain (kg)			
Pearson r	0.22	0.26	0.32
Confidence interval	-0.02, 0.44	0.01, 0.48	0.07, 0.52
$P^a$	0.08	0.04 <sup>b</sup>	0.01 <sup>b</sup>
Weight at sample collection (kg)			
Pearson r	0.40	0.42	0.39
Confidence interval	0.17, 0.59	0.19, 0.61	0.15, 0.58
$P^a$	0.001 <sup>b</sup>	0.0007 <sup>b</sup>	0.001 <sup>b</sup>
Newborn weight (g)			
Pearson r	0.25	0.32	0.09
Confidence interval	0.003, 0.47	0.07, 0.53	-0.15, 0.33
$P^a$	0.04 <sup>b</sup>	0.01 <sup>b</sup>	0.44

<sup>a</sup>)Pearson's correlation coefficient; <sup>b</sup>) $P < 0.05$ .

In the second trimester, we observed a significant decrease in serum adiponectin concentrations, which may be related to the installation of IR, which occurs mainly during this period. Some studies correlated the concentration of adiponectin with IR indexes during pregnancy in adult women [24,25].

When assessing serum leptin levels, we observed significantly increased values from the first to the third trimester of pregnancy in adolescents. This result can also be observed in other studies involving pregnant women and adolescents with a methodology similar to ours [26,27].

Duarte et al. [28] studied serum leptin levels in the second and third trimesters of pregnant adolescents with and without pre-eclampsia, identifying a non-significant increase in this hormone between 21 and 30 weeks in both groups. They demonstrated that leptin concentrations tended to be higher in the last trimester of pregnancy with the presence of pre-eclampsia. Jones et al. [29] analyzed serum leptin concentrations between 28 and 32 weeks of pregnancy, dividing the adolescents into early (14 and 15 years) and late (16 to 18 years), and reduced leptin concentrations was observed only in the group of late teenagers.

We observed positive correlation between pre-pregnancy BMI and serum leptin levels. Thus, these results confirm the findings of a previous study that evaluated pregnant adolescents and young adults, in which those who had a higher concentration of leptin also had a higher pre-pregnancy BMI value and a lower level of adiponectin [30].

The higher concentrations of leptin that occur during pregnancy are related to weight gain, as well as changes in hormonal levels, which can stimulate leptin secretion [31]. We have identified a positive correlation between weight gain during pregnancy and serum leptin concentration in all trimesters of pregnancy.

In this study, the slight decline in serum leptin levels at second trimester was also attributed to the development of IR, which occurs during this period, as observed in other studies [11,27].

In a study conducted among 61 adult pregnant women, the authors observed that high serum leptin levels were found in those who gave birth to newborns with higher weight [30]. Similarly, in this study, a positive correlation was also observed between serum leptin concentration of pregnant adolescents and higher birth weight.

In summary, we observed that serum adiponectin levels in pregnant adolescents decreased throughout pregnancy;

however, pregnancy leptin levels increased, and they were positively correlated with all variables evaluated. Additionally, one of the strengths of this study was its originality. Our study had, as its main positive point, the prospective monitoring of serum concentrations of adiponectin and leptin during the pregnancy of adolescents, evaluating the same adolescent in all trimesters. Thus, this study was able to have more precise view of the molecules' behavior in the blood during this period, which can be used in the future to manufacture new biomarkers in order to prevent adverse perinatal outcomes, considering that this population is naturally classified as high risk.

In addition, we could not report some correlations of other variables related to maternal, neonatal body composition and leptin levels in the adolescent population, which is still little known.

## Conflict of interest

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

## Ethical approval

The study was approved by the Adolescent Prenatal Sector of the Federal University of São Paulo (UNIFESP) (No. 1514/11) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

## Patient consent

The patients provided written informed consent for the publication and the use of their images.

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