Effect of gestational weight gain on insulin resistance mediated by serum adipokine concentrations in advanced maternal age

Beibei Dong[1,2], Jinfang Sun[3], Mengmeng Zhi[1,2], Manman Han[1,2], Hao Lin[2,4], Hong Yu[5], Ling Li[1,2]

[1]Department of Endocrinology, ZhongDa Hospital, School of Medicine, Southeast University, Nanjing, China
[2]Pancreatic Research Institute, Southeast University, Nanjing, China
[3]Key Laboratory of Environmental Medicine Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, China
[4]Department of Clinical Science and Research, ZhongDa Hospital, Southeast University, Nanjing, China
[5]Department of Obstetrics and Gynecology, ZhongDa Hospital, School of Medicine, Southeast University, Nanjing, China

Corresponding authors:
Prof. Ling Li
Department
of Endocrinology
ZhongDa Hospital
School of Medicine
Southeast University
Nanjing 210009
Jiangsu Province, China
Phone: +86 025-83272012
Fax: +86 025-83272011
E-mail: li-ling76@hotmail.com

Prof. Hong Yu
Department
of Obstetrics
and Gynecology
ZhongDa Hospital
School of Medicine
Southeast University
Nanjing 210009
Jiangsu Province, China

Abstract

Introduction: With the implementation of the universal two-child policy, the number of pregnant women of advanced maternal age (AMA) will increase steadily. We aimed to investigate whether the effect of gestational weight gain on insulin resistance (IR) before delivery was mediated by serum adipokine concentrations in AMA.

Material and methods: This cross-sectional study included 80 pregnant women of AMA recruited consecutively before delivery from the Department of Obstetrics and Gynecology between August 2016 and July 2017. At delivery, maternal weight during the third trimester was recorded and serum adipokines were measured. IR was calculated using the homeostasis model assessment 2 (HOMA2) method.

Results: Weight gain (WG) during the third trimester was positively associated with serum leptin concentrations ($r = 0.34$, $p = 0.0018$) and HOMA-IR indices ($r = 0.25$, $p = 0.025$), but not related with serum concentration of adiponectin ($r = 0.12$, $p = 0.28$). WG during the third trimester and serum concentration of leptin were independently associated with the level of HOMA-IR by multivariate analysis. Subsequently, according to mediation analysis, the association between WG during the third trimester and HOMA-IR mediated by serum leptin concentrations was statistically significant ($z = 1.588$, $p < 0.05$).

Conclusions: Taken together, our findings suggest that the relationship between WG during the third trimester and IR was mediated by serum leptin concentrations in AMA, but not serum adiponectin concentrations.

Key words: gestational weight gain, leptin, adiponectin, insulin resistance, advanced maternal age.

Introduction

In October 2015, China completely relaxed the one-child policy, replaced by the universal two-child policy. The introduction of the universal two-child policy will stimulate the growth of the population in

AMS

Beibei Dong, Jinfang Sun, Mengmeng Zhi, Manman Han, Hao Lin, Hong Yu, Ling Li

comparison to the traditional one-child policy [1]. In addition, with the acceleration of social industrialization process, more women prefer to delay childbearing unless their education accomplished or professional careers achieved [2]. Therefore, the proportion of women delivering infants at the age of 35 years and above, usually defined as advanced maternal age, has increased steadily [3].

For decades, it has been indicated that pregnant women in advanced maternal age (AMA) will have higher risk of developing some adverse perinatal outcomes, including gestational diabetes mellitus (GDM), hereditary disease, pregnancy-induced hypertension and pre-eclampsia [4–6]. Among them, GDM is a common noncommunicable disease that can be prevented by appropriate lifestyle management [7]. It has been demonstrated that the distinguishing characteristic of GDM is increased insulin resistance (IR) progressively along with the increase of gestational week [8].

According to the Global Burden of Disease 2013 Study (GBD), up-to-date information indicated that levels and trends in overweight and obesity were steadily increasing along with the growth of age, especially for women in developing countries [9]. Moreover, previous studies have indicated that women with obesity before pregnancy were more prone to develop excessive weight gain (EWG) during pregnancy with reference to the Institute of Medicine (IOM) modified criteria (2009) [10, 11]. Numerous studies have demonstrated that people with obesity were more exposed to elevated circulating level of leptin [12–14] and low circulating level of adiponectin [15, 16], which may decrease the insulin secretion and sensitivity and further resulted in IR [17]. To date, there have been several studies that have demonstrated the effect of serum adipokines including leptin and adiponectin on the risk of GDM [17–21]. However, there are few studies focusing on whether the association between gestational weight gain (GWG) and IR is mediated by serum adipokines concentrations in AMA.

In the present study, we aimed to illustrate whether the association between GWG in the third trimester of pregnancy and IR was mediated by serum adipokines concentrations in AMA.

Material and methods

Study design

This cross-sectional study including 80 pregnant women with advanced maternal age and singleton pregnancy was conducted at the Zhong Da hospital, Southeast University, Nanjing in China. The sample size was calculated by the Power Analysis and Sample Size software (PASS2011) with α of 0.05 (two tailed), power ($1-\beta$) of 90%. The study was authorized by the ethics committee for human research in Zhong Da hospital and adhered to the STROBE guidelines for observational studies [22]. Written informed consent was obtained from all pregnant women prior to inclusion in the study. The pregnant women were recruited consecutively before delivery from the Department of Obstetrics and Gynecology between August 2016 and July 2017. If pregnant women were AMA, aged 35 years and above, with singleton pregnancy, they were eligible for the study. We excluded pregnant women with maternal age less than 35 years; diabetes and cardiovascular diseases before pregnancy; severe hepatic and renal disorders and autoimmune diseases prior to pregnancy. We collected demographic information including maternal age, education, pre-pregnancy body mass index (P-BMI), pregnancy and obstetrical history, last menstruation, history of macrosomia and GDM and family history of diabetes. GWG during late pregnancy was obtained by weight before delivery subtracted from weight at the beginning of the third trimester (28 week). Then, weight gain (WG) in the third trimester of pregnancy was stratified into non-EWG and EWG according to IOM 2009 [23].

Additionally, blood samples were collected after the participant had fasted for at least 8 h but not more than 12 h. Blood was centrifuged at 3000 rpm for 10 min within 4 h after collection. Subsequently, the serum was separated and glucose and lipid parameters including triglyceride (TG), total cholesterol (TC), and low- and high-density lipoprotein-cholesterol (LDL-C, HDL-C) were measured immediately. The remaining serum was stored at –80ºC for later analysis of insulin and adipokines including leptin and adiponectin.

Insulin was measured using electrochemiluminescence immunoassay (ECLI) in the Department of Clinical Laboratory, Zhong Da hospital affiliated Southeast University. Homeostasis model assessment-insulin resistance (HOMA-IR) indices, a safe method for pregnant women to evaluate the IR, were calculated using the updated HOMA2 method (based on the computer model and provided by the University of Oxford Diabetes Trial Unit) because it is more accurate than the original HOMA1 method [24].

Measurement of serum leptin and adiponectin concentrations

Serum leptin and adiponectin concentrations were measured by enzyme-linked immunosorbent assay (ELISA) kits (Millipore, Billerica, MA, USA and R&D Systems, Minneapolis, MN, USA, respectively). Average intra- and interassay coefficient of variation (CV) for internal controls for all assays were 3.7% and 4% for leptin, and 3.4% and 6.5%

for adiponectin. All assays were performed by operators blinded to sample identity.

Statistical analysis

Data were depicted as mean and standard deviation for continuous variables with normal distribution and median and interquartile range for continuous variables with skewed distribution. Categorical variables were represented as count and percentage. Mann-Whitney U test was conducted in continuous variables for group comparisons between non-EWG and EWG. Correlation analysis between WG in the third trimester of pregnancy and serum adipokines concentrations (and HOMA-IR) was performed using a Spearman rank correlation coefficient (r) and then multiple linear regression analysis was used for adjusting some confounder factors. Mediation analysis was achieved by a single-mediator model and the Sobel test was used to clarify the relation of WG in the third trimester of pregnancy to HOMA-IR adjusted for serum adipokines concentrations [25]. All statistical analysis was conducted using SPSS version 23.0. Two-sided p-values < 0.05 were considered for statistical significance [26].

Results

Baseline characteristics of study participants

A total of 80 pregnant women with AMA who had complete baseline characteristics were included in the study (Table I). Those pregnant women with AMA were normal weight before pregnancy; multiparous; had a high rate of miscarriage (≥ 1) and delivered in the third trimester of pregnancy (38.74 ±1.19 weeks). Clinical characteristics of the study participants before delivery are also described in Table I. Clinical information mostly comprised fasting blood glucose (FBG) and insulin concentrations; HOMA-IR; HOMA β-cell function (HOMA-β); HOMA-insulin sensitivity (HOMA-s %); TG and TC; LDL-c and HDL-c; and serum concentration of leptin and adiponectin. Further, according to IOM 2009, WG in the later pregnancy was stratified into non-EWG and EWG.

Relationship between weight gain in the third trimester of pregnancy and serum concentration of adipokines in advanced maternal age

Weight gain in the third trimester of pregnancy was positively correlated with serum concentration of leptin ($r = 0.34$, $p = 0.0018$, Figure 1 A), but not related with serum concentration of adiponectin ($r = 0.12$, $p = 0.28$, Figure 1 B). Compared with pregnant women of non-EWG in the third trimester of pregnancy, pregnant women with EWG had

significantly higher serum leptin concentrations ($p = 0.0002$, Figure 1 C). There was no significant difference in serum adiponectin concentrations between women with non-EWG and EWG ($p = 0.81$, Figure 1 D).

Relationship between weight gain in the third trimester of pregnancy and HOMA-IR indices in advanced maternal age

Weight gain in the third trimester of pregnancy was positively associated with HOMA-IR indices indicating the IR ($r = 0.25$, $p = 0.025$, Figure 2 A). There was a significant difference in the index of HOMA-IR between women in AMA with non-EWG and EWG during late pregnancy ($p = 0.005$, Figure 2 B). In multivariable analysis using stepwise

Table I. Baseline and clinical characteristics of the study population ($n = 80$)

Characteristics	Result
Age [years]	36 (35–38)
Education [years]	16 (14–16)
≤ 12	17 (21.30)
12–16	55 (68.80)
> 16	8 (10)
P-BMI [kg/m²]	23.07 ±2.51
Age at menarche [years]	14 (13–14)
Parity (numbers)	3 (2–4)
> 2	44 (55)
Abortion (numbers)	0.25 (1–2)
Gestational weeks [weeks]	38.74 ±1.19
Fasting blood glucose [mmol/l]	4.57 ±0.97
Fasting insulin [mIU/ml]	12.90 (7.86–19.80)
HOMA-IR	1.53 (0.92–2.32)
HOMA-β	1.65 (1.34–2.14)
HOMA-s%	0.67 (0.43–1.09)
TG [mmol/l]	3.80 (3.02–5.08)
TC [mmol/l]	6.30 ±1.17
LDL-C [mmol/l]	3.14 ±0.83
HDL-C [mmol/l]	1.99 ±0.35
Adiponectin [µg/ml]	5.05 (3.28–7.52)
Leptin [ng/ml]	28.24 (21.02–40.54)

Data are presented as n (%), mean ± SD or median (interquartile range) as appropriate. P-BMI – pre-pregnancy body mass index, HOMA-IR – HOMA-insulin resistance, HOMA-β – HOMA β-cell function, HOMA-s% – HOMA-insulin sensitivity, TG – triglyceride, TC – total cholesterol, LDL-C – low density lipoprotein-cholesterol, HDL-C – high density lipoprotein-cholesterol.
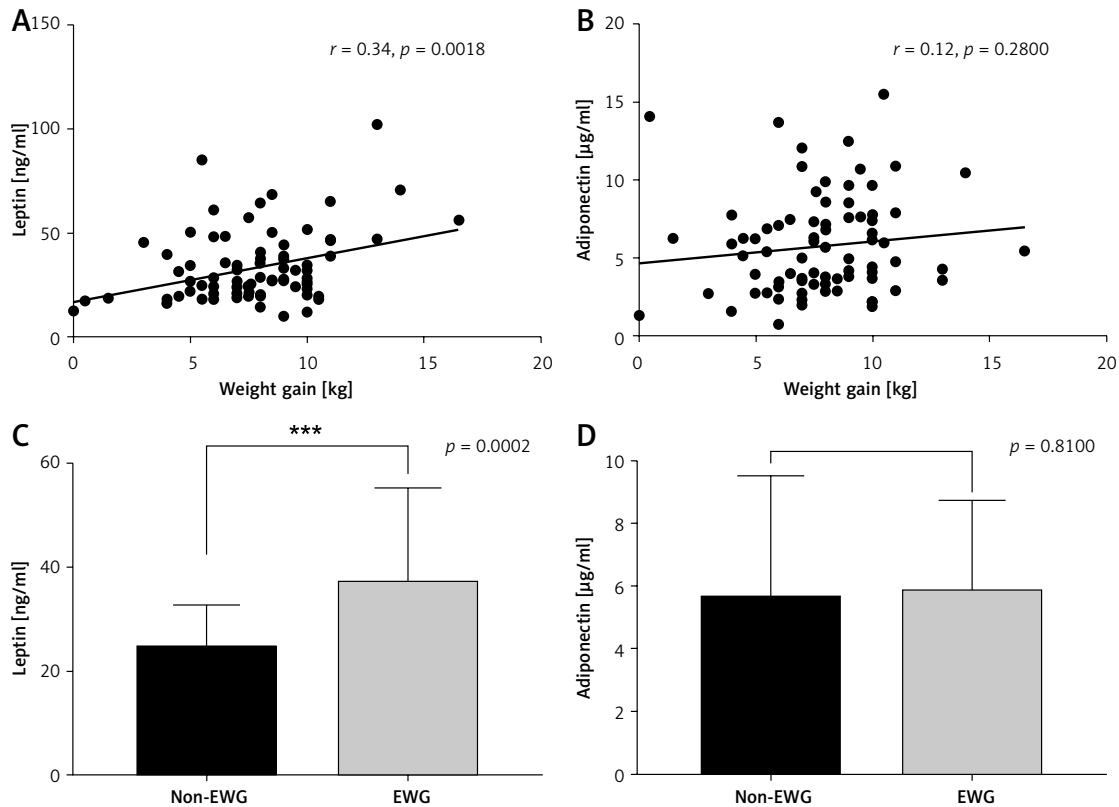
Beibei Dong, Jinfang Sun, Mengmeng Zhi, Manman Han, Hao Lin, Hong Yu, Ling Li

Figure 1. The correlation between serum concentration of leptin (**A**) and adiponectin (**B**) and weight gain in the late pregnancy. Subsequently, comparison of serum leptin (**C**) and adiponectin (**D**) levels in non-excessive weight gain (Non-EWG) and excessive weight gain (EWG) in the late pregnancy

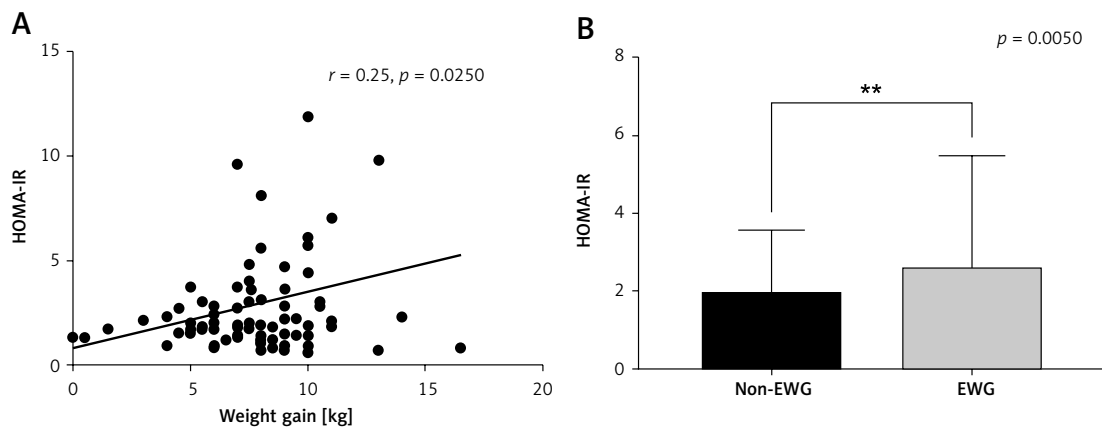****Statistically significant p-value < 0.001.*

Figure 2. A – Correlation between HOMA-IR indices and weight gain in the third trimester of pregnancy. **B** – Comparison of HOMA-IR levels in non-excessive weight gain (Non-EWG) and excessive weight gain (EWG) during the third trimester

regression (variables included maternal age, education, P-BMI, age at menarche, parity, abortion, WG in the third trimester of pregnancy, FBG, TG, TC, LDL-C, HDL-C and serum adipokines concentrations), age at menarche, parity, WG in the third trimester of pregnancy, serum concentration of leptin, FBG and HDL-C were significantly associated with the level of HOMA-IR ($\beta = -0.337$, $p < 0.001$; $\beta = 0.172$, $p = 0.021$; $\beta = 0.382$, $p < 0.001$; $\beta = 0.174$, $p = 0.023$; $\beta = 0.558$, $p < 0.001$ and $\beta = -0.162$, $p = 0.025$, respectively). However, serum adiponectin concentrations were not statistically related to the level of HOMA-IR in AMA (Table II).

Mediating effect of serum leptin concentrations between weight gain in the third trimester of pregnancy and the level of HOMA-IR in advanced maternal age

In the present study, WG in the third trimester of pregnancy was significantly associated with HOMA-IR indices ($c = 0.386$, $p < 0.001$, Figure 3).

Table II. Multiple linear regression among clinical parameters and the level of HOMA-IR

Variables	Unstandardized coefficients β	SE	Standardized coefficients β	t	P-value	95% CI for β
Age at menarche [years]	−0.637	0.141	−0.337	−4.507	< 0.001*	−0.919 to −0.355
Parity (numbers)	0.357	0.151	0.172	2.362	0.021	0.056 to 0.659
Weight gain [kg]	0.362	0.071	0.382	5.123	< 0.001*	0.221 to 0.503
Leptin [ng/ml]	0.027	0.012	0.174	2.328	0.023*	0.004 to 0.050
FBG [mmol/l]	1.514	0.189	0.558	7.998	< 0.001*	1.136 to 1.891
HDL-C [mmol/l]	−1.213	0.530	−0.162	−2.289	0.025*	−2.270 to −0.157
Constant	1.824	2.574	−	0.708	0.481*	−3.307 to 6.955

*Data were analyzed by multivariable analysis using stepwise regression. Variables included maternal age, education, pre-pregnancy body mass index, age at menarche, parity, abortion, weight gain, FBG, TG, TC, LDL-C, HDL-C and serum adipokines including leptin and adiponectin concentrations. Weight gain means weight gain in late pregnancy. FBG – fasting blood glucose, TG – triglyceride, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol. *Statistically significant p-value < 0.05.*

In addition, WG in the third trimester of pregnancy was significantly associated with serum leptin concentrations (a = 2.102, p = 0.002, Figure 3). However, the relation of serum leptin concentrations to the HOMA-IR adjusted for WG in the third trimester of pregnancy was not statistically significant (b = 0.031, p = 0.067, Figure 3). Subsequently, according to the Sobel test, the association between WG in the third trimester of pregnancy and HOMA-IR indices partly mediated by serum leptin concentrations was statistically significant (z = 1.588, p < 0.05, Table III).

Discussion

To our knowledge, the study is the first to demonstrate the effect of GWG during the third gestational trimester and IR in relation to serum adipokines concentrations mainly including leptin and adiponectin in AMA. We found that in AMA, serum concentration of leptin was positively associated with GWG during the third trimester and was significantly higher in women with EWG in comparison to women with non-EWG. Also, WG during the third trimester and serum concentration of leptin were independently associated with the level of HOMA-IR in multivariable analysis. However, there was no significant difference between serum adiponectin concentration and WG during the third trimester of pregnancy. Additionally, we found a mediated effect of serum leptin
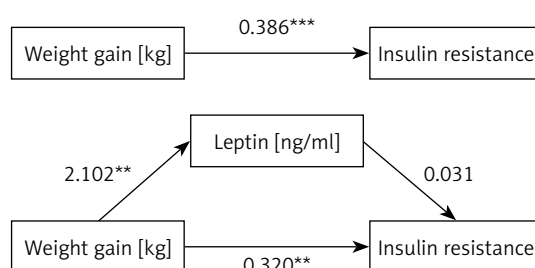
Figure 3. Single-mediator model among weight gain, leptin and insulin resistance (HOMA-IR)

concentrations between WG in late pregnancy and IR. Cumulatively, these findings suggest that the relationship between WG in late pregnancy and IR may be mediated by serum leptin concentrations in AMA.

Our study suggested that serum leptin concentration was positively related to WG during the third trimester of pregnancy (r = 0.34, p = 0.0018, Figure 1 A) and significantly higher in women with EWG in comparison with non-EWG (p = 0.0002, Figure 1 C). This association between serum leptin concentrations and GWG during late pregnancy was in accordance with those findings in other studies [11, 12, 27–29]. Recently, a cohort study demonstrated that leptin concentrations gradually increased during the third trimester of pregnancy (36 gestational week) and correlated with GWG in pregnant Mexican adolescents [30]. In another study, higher serum leptin concentra-

Table III. Mediating effect on serum concentration of leptin between weight gain and insulin resistance

Step	Dependent variable	Independent variable	Regression equation	t/z	P-value
1	Insulin resistance (Y)	Weight gain (X)	$Y = 0.386X + e_1$	3.931	< 0.001
2	Leptin (M)	Weight gain (X)	$M = 2.102X + e_2$	3.233	0.002
	Insulin resistance (Y)	Weight gain (X) Leptin (M)	$Y = 0.32X + 0.031M + e_3$	3.110 1.857	0.003 0.067
3	− (Sobel test)	− (Sobel test)	− (Sa = 0.65; Sb = 0.017)	1.588	< 0.05

tions in the second trimester was associated with greater subsequent GWG and its association remained significant after adjustment for BMI (β = 2.35; SE = 0.41; $p < 0.0001$) [31]. However, those findings only identified the relationship between serum leptin concentrations and GWG in general maternal age. Our novel finding was that in AMA, serum leptin concentrations were significantly associated with GWG and higher in women with EWG in comparison to those with non-EWG. The underlying metabolic effect of higher serum leptin concentrations may be due to leptin resistance to decrease its effect on target tissues along with the increase of GWG [12–14]. Leptin resistance in pregnant women with EWG prevents leptin from exerting its regulating effects on food intake, thus worsening the physiopathology of EWG and its metabolic complications [17]. On the other hand, several researchers have suggested that hyperleptinemia may be compensated for EWG by decreased food intake and increased energy expenditure [32].

In addition, we found that GWG was positively associated with IR as indicated by the HOMA-IR index ($r = 0.25$, $p = 0.025$, Figure 2 A) and HOMA-IR levels were higher in women with EWG compared with those with non-EWG ($p = 0.005$, Figure 2 B). In addition, the relationship between GWG and IR was still statistically significant in the multivariate analysis (β = 0.382, $p < 0.001$, Table II). Similarly, the ROLO study of 621 women without diabetes illustrated that EWG was associated with higher maternal HOMA-IR indices and higher maternal leptin concentrations [33]. We also observed that serum leptin concentrations were significantly associated with HOMA-IR levels after adjustment for some confounding factors including maternal age, education, P-BMI, age at menarche, parity, abortion, WG in the third trimester of pregnancy, FBG, TG, TC, LDL-C, HDL-C and serum adipokine concentrations. Physiologically, leptin is synthesized and secreted from placental trophoblasts into the maternal circulation in considerable amounts during pregnancy [34, 35] and the serum concentration of leptin was higher in pregnant women than that in nonpregnant women, with the peak occurring around the 28th week of gestation. At the same time, the trend of IR [36, 37] was similar to that of serum leptin concentration. It is possible that excessive gestational weight gain (EGWG) is associated primarily with maternal fat, but not lean body mass accrual, and may contribute to metabolic dysfunction including increased serum leptin concentration, IR and long-term obesity [38].

In the current study, we further investigated whether the effect of GWG on IR was mediated by serum adipokine concentrations using mediation analysis and the Sobel test ($z = 1.588$, $p < 0.05$, Table III). Consequently, we found that GWG was associated with IR partly mediated by serum leptin concentrations, but not serum adiponectin concentrations. Recently, a randomized controlled trial demonstrated that excess, compared with adequate WG was associated with significantly greater fat mass accrual. In addition, there was a strong positive correlation between the percentage of body fat and serum leptin concentrations ($r = 0.85$, $p < 0.001$), suggesting that most obese persons are insensitive to endogenous leptin production [39]. Thus, we speculated that EGWG may stimulate the secretion of serum leptin, which was associated with decreased appetite and increased energy expenditure compensating for WG. Moreover, it was demonstrated that women with EWG have higher levels of insulin in comparison to those with non-EWG [33]. In addition, large numbers of studies have illustrated that leptin was increased in pregnancy, especially in the third trimester, [30] and directly affected insulin sensitivity through regulating the efficiency of insulin-mediated glucose metabolism by skeletal muscle [40]. Accordingly, the effect of GWG on IR may be mediated by serum leptin concentrations in AMA.

A merit of this study is that it was the first to analyze whether serum adipokines concentrations mediated the relationship between WG and IR in AMA. Several studies have demonstrated the association between WG and serum adipokines concentrations [27, 28, 30, 31] or the IR [33]. However, there are few studies discussing the mechanism of the correlation between WG and IR in AMA. In addition, the design of this study for recruitment of participants decreased the incidence of selection bias.

Despite these strengths, several limitations should be noted in this study. First, women in AMA were recruited from a single hospital. Thus, the population in the study may not be representative of the entire population. Moreover, due to restriction of resources, we only detected the serum adipokines concentrations during late pregnancy thus the association between WG and IR mediated by serum leptin concentrations was not a cause-effect relationship. Another limitation was that we did not record information regarding diet and physical activity during late pregnancy and cannot assess their effects on GWG during late pregnancy and IR.

The study was the first to investigate the effect of GWG on IR which may be mediated by serum leptin concentrations in AMA, a noteworthy population. More cohort studies with large samples are needed to clarify the cause-effect of WG in the different trimesters of pregnancy on IR mediated by serum leptin adiponectin in AMA. In addition, further interventional studies in AMA are necessary to evaluate the effect of limiting EGWG through diet and exercise on the change of serum adipokine concentrations and improvement of IR.

In conclusion, this study demonstrated that the effect of GWG on IR was partly mediated by serum leptin concentrations in AMA. Whereas this association is on the basis of the background of this study, any causal interpretation of those relationships is not exact and restricted. Thus, well-designed and intervention studies are necessary to confirm whether the relationship between GWG and IR mediated by serum leptin concentrations in AMA from this study is generalizable to all pregnant women. Furthermore, this study also indicates that it is necessary to strengthen attention to the AMA to reduce the long-term health implication of higher IR before delivery influencing maternal and fetal health. Therefore, establishing potent strategies for prevention of EWG in the third trimester of pregnancy may bring far-reaching benefits. Obstetricians should pay more attention to provide guidance in monitoring and controlling GWG in the third trimester of pregnancy and more intervention studies are requested to confirm whether changes in WG during late pregnancy will lead to decreased IR coinciding with decreased serum leptin concentrations.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

References

1. Zeng Y, Hesketh T. The effects of China's universal two-child policy. Lancet 2016; 388: 1930-8.
2. Mathews TJ, Hamilton BE. Mean age of mother, 1970-2000. Natl Vital Stat Rep 2002; 51: 1-14.
3. Hsieh TT, Liou JD, Hsu JJ, Lo LM, Chen SF, Hung TH. Advanced maternal age and adverse perinatal outcomes in an Asian population. Eur J Obstet Gynecol Reprod Biol 2010; 148: 21-6.
4. Jolly M, Sebire N, Harris J, Robinson S, Regan L. The risks associated with pregnancy in women aged 35 years or older. Hum Reprod 2000; 15: 2433-7.
5. Jacobsson B, Ladfors L, Milsom I. Advanced maternal age and adverse perinatal outcome. Obstet Gynecol 2004; 104: 727-33.
6. Laopaiboon M, Lumbiganon P, Intarut N, et al.; WHO Multicountry Survey on Maternal Newborn Health Research Network. Advanced maternal age and pregnancy outcomes: a multicounty assessment. BJOG 2014; 121: 49-56.
7. Brown J, Alwan NA, West J, et al. Lifestyle interventions for the treatment of women with gestational diabetes. Cochrane Database Syst Rev 2017; 5: 1-132.
8. Farrar D. Hyperglycemia in pregnancy: prevalence, impact, and management challenges. Int J Womens Health 2016; 8: 519-27.
9. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 2014; 384: 766-81.
10. Li C, Liu YJ, Zhang WY. Joint and independent associations of gestational weight gain and pre-pregnancy body mass index with outcomes of pregnancy in chinese women: a retrospective cohort study. PLoS One 2015; 10: 1-15.
11. Ferraro ZM, Qiu Q, Gruslin A, Adamo KB. Excessive gestational weight gain and obesity contribute to altered expression of maternal insulin-like growth factor binding protein-3. Int J Womens Health 2013; 5: 657-65.
12. Kautzky-Willer A, Pacini G, Tura A, et al. Increased plasma leptin in gestational diabetes. Diabetologia 2001; 44: 164-72.
13. Maple-Brown L, Ye C, Hanley AJ, et al. Maternal pregravid weight is the primary determinant of serum leptin and its metabolic associations in pregnancy, irrespective of gestational glucose tolerance status. J Clin Endocrinol Metab 2012; 97: 4148-55.
14. Zhou YJ, Rui LY. Leptin signaling and leptin resistance. Front Med 2013; 7: 207-22.
15. Cnop M, Havel PJ, Utzschneider KM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003; 46: 459-69.
16. Ghoshal K, Bhattacharyya M. Adiponectin: probe of the molecular paradigm associating diabetes and obesity. World J Diabetes 2015; 6: 151-66.
17. Fasshauer M, Blüher M, Stumvoll M. Adipokines in gestational diabetes. Lancet Diabetes Endocrinol 2014; 2: 488-99.
18. Iliodromiti S, Sassarini J, Kelsey TW, Lindsay RS, Sattar N, Nelson SM. Accuracy of circulating adiponectin for predicting gestational diabetes: a systematic review and meta-analysis. Diabetologia 2016; 59: 692-9.
19. Horosz E, Bomba-Opon DA, Szymanska M, Wielgos M. Third trimester plasma adiponectin and leptin in gestational diabetes and normal pregnancies. Diabetes Res Clin Pract 2011; 93: 350-6.
20. Retnakaran R, Qi Y, Connelly PW, Sermer M, Hanley AJ, Zinman B. Low adiponectin concentration during pregnancy predicts postpartum insulin resistance, beta cell dysfunction and fasting glycaemia. Diabetologia 2010; 53: 268-76.
21. Park S, Kim MY, Baik SH, et al. Gestational diabetes is associated with high energy and saturated fat intakes and with low plasma visfatin and adiponectin levels independent of prepregnancy BMI. Eur J Clin Nutr 2013; 67: 196-201.
22. von Elm E, Altman DG, Egger M, et al.; STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Lancet 2007; 370: 1453-7.
23. Institute of Medicine (US) and National Research Council (US) Committee to Reexamine IOM Pregnancy

Beibei Dong, Jinfang Sun, Mengmeng Zhi, Manman Han, Hao Lin, Hong Yu, Ling Li

Weight Guidelines; Rasmussen KM, Yaktine AL, editors. Weight gain during the pregnancy reexamining the guidelines. The National Academies Press. Washington (DC): National Academies Press (US); 2009.

24. Caumo A, Perseghin G, Brunani A, Luzi L. New insights on the simultaneous assessment of insulin sensitivity and beta-cell function with the HOMA2 method. Diabetes Care 2006; 29: 2733-4.

25. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation analysis. Annu Rev Psychol 2007; 58: 593-614.

26. MacKinnon DP, Lockwood CM, Hoffman JM, West SG, Sheets V. A comparison of methods to test mediation and other intervening variable effects. Psychol Methods 2002; 7: 83-104.

27. Castellano Filho DS, do Amaral Correa JO, Dos Santos Ramos P, de Oliveira Montessi M, Aarestrup BJ, Aarestrup FM. Body weight gain and serum leptin levels of non-overweight and overweight/obese pregnant women. Med Sci Monit 2013; 19: 1043-9.

28. Franco-Sena AB, Rebelo F, Pinto T, et al. The effect of leptin concentrations and other maternal characteristics on gestational weight gain is different according to pre-gestational BMI: results from a prospective cohort. BJOG 2016; 123: 1804-13.

29. Rifas-Shiman SL, Fleisch A, Hivert MF, Mantzoros C, Gillman MW, Oken E. First and second trimester gestational weight gains are most strongly associated with cord blood levels of hormones at delivery important for glycemic control and somatic growth. Metabolism 2017; 69: 112-9.

30. Sámano R, Martínez-Rojano H, Chico-Barba G, et al. Serum concentration of leptin in pregnant adolescents correlated with gestational weight gain, postpartum weight retention and newborn weight/length. Nutrients 2017; 9: e1067.

31. Lacroix M, Battista MC, Doyon M, et al. Higher maternal leptin levels at second trimester are associated with subsequent greater gestational weight gain in late pregnancy. BMC Pregnancy Childbirth 2016; 16: 62.

32. Cui HX, López M, Rahmouni K. The cellular and molecular bases of leptin and ghrelin resistance in obesity. Nat Rev Endocrinal 2017; 13: 338-51.

33. Walsh JM, McGowan CA, Mahony RM, et al. Obstetric and metabolic implications of excessive gestational weight gain in pregnancy. Obesity (Silver Spring) 2014; 22: 1594-600.

34. Masuzaki H, Ogawa Y, Sagawa N, et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. Nat Med 1997; 3: 1029-33.

35. Cakir I, Uluhan M. Cardiotrophin-1 and leptin as cardiovascular risk markers in male patients with obstructive sleep apnea syndrome. Arch Med Sci Atheroscler Dis 2018; 3: e123-8.

36. Walsh JM, McGowan CA, Kilbane M, McKenna MJ, McAuliffe FM. The relationship between maternal and fetal vitamin D, insulin resistance, and fetal growth. Reprod Sci 2013; 20: 536-41.

37. Xue W, Li Y, Li J, Yan L, Yang F. Endothelium-dependent relaxation induced by etomidate in the aortas of insulin-resistant rats. Arch Med Sci 2018; 14: 1155-62.

38. Berggren EK, Groh-Wargo S, Presley L, Hauguel-de Mouzon S, Catalano PM. Maternal fat, but not lean, mass is increased among overweight/obese women with excess gestational weight gain. Am J Obstet Gynecol 2016; 214: 745.e1-5.

39. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334: 292-5.

40. Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. Science 1996; 274: 1185-8.