Association of parity with insulin resistance early in pregnant women: ECLIPSES study

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Abstract

Context: Little is known about whether parity is associated with elevated early-pregnancy IR, or whether overweight/obesity contributes to increasing the possible effect.

Objective: We determined the associations between parity and glucose metabolism parameters in the first trimester of pregnancy in a Mediterranean pregnant population, and whether these associations are impacted by overweight/obesity.

Methods: Cross-sectional study of 264 healthy pregnant women from the ECLIPSES study who were recruited at 12 weeks of gestation. At baseline, details on socio-economic status, obstetric history (including parity, i.e., number of births), lifestyle factors, anthropometry, and blood samples were collected. Fasting serum glucose, insulin, and HOMA-IR index were assessed in the first trimester. Elevated IR was defined as the upper HOMA-IR tertile (≥1.58). Multivariable linear regression and Cox regression model with constant time were performed.

Results: Parity ranged from 0-4. After multivariable adjustment, the insulin levels (β (% change): 20.92, 95%CI: 4.08, 37.71) and HOMA-IR index (β (% change): 19.72, 95%CI: 2.43, 40.49) were positively associated with parity. Additionally, multiparous women, as compared to nulliparous, were more likely to have higher HOMA-IR levels (primiparous (one birth), β (% change): 16.88, 95%CI: -1.00, 37.99; multiparous (≥2 two births), β (% change): 32.18, 95%CI: 3.56, 68.71), and an increased relative risk (RR) of an elevated IR (primiparous (one birth), RR: 1.55, 95%CI: 1.03, 2.36; multiparous (≥2 two births), RR: 1.72, 95%CI: 1.05, 2.83). The combination of multiparity and overweight/obesity conferred a 3.04-fold increase in the RR of elevated IR, which increased proportionally to the number of parities.

Conclusions: This study demonstrates that parity may have a negative impact on early-pregnancy IR and that maternal overweight/obesity appears to further aggravate this relationship.

Introduction

Pregnancy is a time-limited condition, in which women undergo significant physiological and metabolic changes. These changes lead to maternal fat accumulation and a gradual increase in peripheral insulin resistance (IR) (1) throughout pregnancy to accommodate the growing fetus (2). However, it is important to note that excessive maternal hyperglycemia- and hyperinsulinemia-induced IR in early pregnancy is a hallmark of a metabolic disorder. This condition has recently emerged as the prominent cause of pregnancy complications and adverse perinatal outcomes, including gestational diabetes mellitus (GD) (3), preeclampsia (4), macrosomia, being large for gestational age, and cesarean delivery (5).

Moreover, although earlier studies (1,2,6,7) suggest that maternal IR returns to pre-pregnancy levels by 1 year postpartum, the recent literature casts some doubt on this premise (8,9). It has been hypothesized that recurrent maternal IR episodes due to repeated pregnancies lead to a progressive worsening of the glucose tolerance in each pregnancy, manifesting in GD or it may even permanently disturb glucose homeostasis in women in later life (8–11). Nevertheless, the underlying mechanisms for parity-related IR during pregnancy are largely unknown, complex and most likely reflect a range of factors, such as placental hormones, lifestyle modifications, and genetic and epigenetic contributions (12).

In this regard, the effect of the multiparity on the GD or recurrent GD during later pregnancies has been a topic of research for the last few years (11,13). Nonetheless, the results are not completely convincing, i.e., the high rate of GD among multiparous women could be due to the confounding effect of higher maternal age or body adiposity (14,15). In addition, the diagnosis of GD is not made until the latter half of pregnancy (at 24–28 weeks) (16) which might be too late to completely reverse the intrauterine hyperglycemia-induced adverse effects on offspring that can occur in the early stages of pregnancy (17). Alternatively, maternal IR in the first trimester, assessed by the homeostasis model assessment of the IR index (HOMA-IR), has been proposed as a reliable marker to predict subsequent GD (3,18). In this context, there are few studies examining whether repeated parity could be associated with an increased risk of IR in very early pregnancy. This is possibly because IR is not routinely assessed during prenatal examinations.

To the best of our knowledge, only three studies have researched this relationship; however, their findings are inconsistent (19–21). Two studies have found a positive association between IR in middle pregnancy (20-30 weeks gestation) and parity (20,21), which was not confirmed by the study by Seghieri et al. (19). In this last study, the authors (19) found that parity is not directly linked to insulin sensitivity/secretion during the last trimester in pregnant women at high risk of GD; however, high pre-gestational body mass index (BMI) was strongly related to a significant impairment in insulin sensitivity during this period. In accordance with these findings, at least in part, we recently found that maternal BMI in early pregnancy is more relevant than gestational weight gain for predicting cardiometabolic risk during pregnancy (22).

Nevertheless, it remains undetermined whether parity confers an independent effect on early-pregnancy IR, and particularly in a healthy Mediterranean pregnant population, in which the socio-demographic and Mediterranean lifestyle traits of women can be regarded as protective factors against IR. It is also necessary to determine whether parity contributes, in combination with high maternal BMI, to a worse IR. Thus, the aim of the present study is to assess how parity is related to glucose metabolism parameters measured early in pregnancy in a pregnant population from a Mediterranean region of northern Spain, and assess whether these associations vary according to overweight/obesity status.

Materials and Methods

Study design and participants

We conducted a retrospective study analyzing data from 264 healthy pregnant women with singleton pregnancies and without previous history of diabetes. These women had data available on parity history, fasting serum glucose, and insulin concentrations in the first trimester (approximately at 12 weeks gestation). Women participated in the ECLIPSES (*Ensayo CLInico Para Suplementar con hierro a EmbarazadaS*) study, in which a total of 791 pregnant women were recruited during their first antenatal visit in 12 sexual and reproductive health care services (ASSIR) of the Catalan Institute of Health (Catalonia, Spain) between 2013-2017. Briefly, the ECLIPSES aimed to determine the highest level of effectiveness of iron supplementation based on hemoglobin (Hb) levels in early pregnancy to optimize maternal and child health. Details of

the study's protocol, as well as inclusion/exclusion criteria, have been described elsewhere (23). The ECLIPSES study was registered at www.clinicaltrialsregister.eu (ID: EUCTR-2012-005480-28) and at www.clinicaltrials.gov (ID: NCT03196882). Ethical approval for the study was obtained from the Jordi Gol Institute for Primary Care Research and the Pere Virgili Institute for Health Research. The research complies with the tenets of the Helsinki Declaration. Participants provided written informed consent.

Data collection

Midwives and nutritionists collected data on demographic characteristics (age, socioeconomic information, and education level), health behaviors (physical activity [PA], smoking, and diet), medical and obstetric history (planned pregnancy [yes, no]), as well as anthropometric measurements in the first trimester of pregnancy (at week 12). Familiar socioeconomic status (SES) was calculated by combining information on occupational status, classified according to the Catalan classification of occupations (CCO-2011), and educational level. It was then classified as low, middle, or high. The women's educational level was classified into three groups: low (primary school or less), medium (secondary studies), and high (university studies or more).

PA was measured using the short version of the International Physical Activity Questionnaire (IPAQ-S) (24). This was derived from total metabolic equivalents (METs-min/week) values based on frequency and duration of walking and moderate and vigorous-intensity activity and divided into tertiles (T1:<1070, T2: 1070-3336, T3: ≥3336 METs-min/week).

Smoking was assessed with the Fagerström questionnaire (Fagerström_Q) (25) and women were classified into three groups: current, former, and never smokers. Eating habits were assessed by dietitians using a 45-item self-administered food frequency questionnaire (FFQ) validated in our population (26). Herein, we focused on women's overall diet quality assessed by using a relative Mediterranean Diet (rMedDiet) score, that has been used in the previous ECLIPSES studies (27). For this study, based on the participant distributions, the continuous rMedDiet score (ranging from 0 to 18 points) was categorized into tertiles (T1:<9, T2: 9-12, T3:≥12 points). Alcohol consumption was assessed as yes or no.

Maternal weight (kg) and height (cm) were also measured. Early pregnancy BMI (ep-BMI) (i.e., at week 12) was calculated from these measurements (weight(kg)/height(m)^2), and women were

classified into two groups: normal weight (NW, ep-BMI <25.0 kg/m^2) and overweight/obesity (OWO, ep-BMI ≥25.0 kg/m^2) for this analysis.

Parity assessment

Parity was defined as the number of singleton pregnancies of at least 20 weeks (regardless of whether the child was liveborn or not) as reported by women on the interviewer-administered questionnaire. For this analysis, pregnant women were classified as nulliparous (no prior viable pregnancies) or multiparous (given birth to 1-4). To more accurately examine the effect of parity, in secondary analyses women were categorized as having no children (nulliparous), 1 child, and ≥2 children.

Outcome ascertainment

Blood samples were collected from pregnant women in the first trimester of pregnancy (12 weeks). Serum was separated from blood cells by centrifugation and stored in Biobank at -80℃ until analysis of the fasting glucose and insulin. We only included in the analysis women who underwent blood tests after an overnight fast. Fasting glucose concentrations were measured using standard automated enzymatic methods. The coefficient of variation (CV) was of 1.74%. Fasting insulin level was measured by chemiluminescence immunoassay on an ADVIA Centaur analyzer using a commercial kit (ADVIA Centaur IRI, Siemens Healthcare Diagnostics Inc., Tarrytown, USA). The lower and upper limits were 0.5 and 300 mUI/L, respectively, and CV was 4.88%. All measurements were conducted at the ICS Camp de Tarragona-Terres de l'Ebre accredited laboratory, Joan XXIII University Hospital in Tarragona.

IR was assessed according to the HOMA-IR index, calculated according to the following equation: HOMA-IR = [fasting glucose (mmol/L) x fasting insulin (μIU/ml)]/22.5. The HOMA-IR index was analyzed as a continuous variable, with a larger HOMA-IR value indicating more severe IR. In addition, to classify the group at higher risk, HOMA-IR was categorized in tertiles (cutoff points were 1.04 and 1.58), and, while considering statistical power, IR was defined as being in the upper tertile. This HOMA-IR threshold of 1.58 is within the range of the cutoff values of HOMA-IR (1.51 to 2.31) in the first trimester for predicting GD (18).

Statistical analysis

Data were analyzed using STATA (15.0, Stata Corp LP, TX, USA). Descriptive data are expressed as mean±SD for quantitative variables and number (%) for categorical variables. Between-group comparisons were performed with Student's T-test, Chi-square, or ANOVA test, as appropriate. Since insulin and HOMA-IR were right-skewed, as expected, both were log-transformed to improve normality prior to analysis.

Unadjusted and multivariable-adjusted linear regression models were fitted to estimate the associations of parity, as the main exposure variable, separately with each outcome (fasting glucose, insulin, and HOMA-IR levels). Parity was analyzed using two different approaches: as a binary categorical explanatory variable (nulliparous: reference and multiparous), and as an ordinal categorical explanatory variable with three categories (0 (nulliparous): reference, 1 child, and >2 previous children). An additional separate linear regression analysis was also performed to evaluate the joint association of parity and ep-BMI-based weight status in two groups (NW and OWO) as predictor, with each outcome. For this analysis, women were grouped into four categories: nulliparous+NW (reference), multiparous+NW, nulliparous+OWO, and multiparous+OWO. Based on previously known or association/risk factors for either the exposure, the outcome, or both (12,22,28), we considered the following *a priori* selected covariates as possible confounders: age (<25: reference, 25-29, ≥30 years), ep-BMI categories (NW: reference, OWO; except in the parity+ep-BMI analysis, where it was integrated into the composite explanatory variable itself), educational level (low (primary school or less)/medium (secondary studies): reference, high (university studies or more)), smoking status (never smoker: reference, current/former smoker), alcohol consumption (no: reference, yes), planned pregnancy (no: reference, yes), physical activity (as tertiles (T): T1≤1070: reference, T2 1071-3335, T3 ≥3336 METs-min/week), and rMedDiet score (as tertiles (T): T1≤8: reference, T2 9-11, T3 ≥12 points). Because dietary covariates (MedDiet score and alcohol intake, both 3.2%, n=9) had missing values, we employed multiple imputation (MI) with the chained-equations method to impute missing data (29) based on the correlation of missing variables with other participant characteristics such as maternal age and BMI. For each analysis, we created 20 imputed datasets and pooled the results using the "mi" command in Stata. Estimates were presented as β coefficients (β) with 95% confidence intervals (CIs). In the case of log-transformed outcomes

(insulin and HOMA-IR), estimates were reported as percent changes calculated using the equation: (e^β - 1) × 100%). The multicollinearity test was carried out by looking at the tolerance (1/VIF) values and variance inflation factors (VIFs). All tolerance values were >0.10 and all VIFs were <2.0, so there was no multicollinearity.

In addition, separate multivariable-adjusted Cox regression model with constant follow-up time set at t=1 for all individuals (given the cross-sectional design) and robust variance rather than logistic regression was applied to estimate the relative risks (RR) and 95% confidence interval (CI) (30) for elevated HOMA-IR index (≥1.58 points) according to parity (nulliparous as reference) and parity+ep-BMI categories (nulliparous+NW as reference) (in separate models); and included the same covariates as the linear models. A test for linear trend was calculated by treating ordinal categorical exposure variable as continuous variable.

Finally, supplementary subgroup multivariable analyses, examining the association of parity with each outcome, were performed stratified by ep-BMI-based weight status (NW and OWO). The interaction between weight status and parity in these associations was assessed by calculating the likelihood ratio test between the fully adjusted model and the same model, including the interaction product-term, in the complete dataset (n=255), as it cannot be performed with multiply imputed data. The level of statistical significance was set to two-sided *p* values <0.05.

Results

The characteristics of the study participants are shown in **Table 1**. The average age of our sample was 29.6±4.7 years, and a large percentage of the women were over 30 years old (57%). The mean ep-BMI was 24.1±3.5 kg/m^2, which falls within the normal classification (18.5–24.9 kg/m^2), but the prevalence of overweight/obesity was 36.0% at early pregnancy. The analyzed cohort of women did not exhibit significant differences in sociodemographic and lifestyle characteristics when compared to non-participants, except for age and ep-BMI, which was lower (both p <0.05) (data not shown). The parity of our population ranged from 0 to 4, 57.6% of women were multiparous women. The multiparous women were significantly older, less educated, and more likely to be from a higher SES, than nulliparae (**Table 1**).

The average fasting glucose, insulin, and HOMA-IR levels were 70.2±10.7 mg/dL, 7.77±1.76 mU/L, and 1.32±1.85 points, respectively, for the total study population (**Table 2**). We observed that multiparous women had higher insulin and HOMA-IR levels than nulliparous women. Similarly, the HOMA-IR index was significantly higher in overweight/obese women compared to normal-weight peers. This gradually increased according to parity and ep-BMI categories, and the mean HOMA-IR was significantly higher in the multiparous+OWO group compared with the nulliparous+NW group (**Table 2**). The fasting glucose concentrations were similar in all groups. We further tested for possible associations between parity and each glucose metabolism parameter separately with a univariate and multivariable-adjusted linear regression model (**Table 3**). In the univariate model, multiparity was significantly associated with higher fasting insulin levels (β coefficient for % change: 22.14, 95%CI: 6.72, 40.49; p=0.006) and a higher HOMA-IR index (β (% change): 23.37, 95%CI: 6.18, 43.33; p=0.011). These variables increased significantly from 1 to ≥2 parities (p-for-trend >0.007). In the fully adjusted main effects model, these associations did not change appreciably from the unadjusted associations. Multiparity was consistently related to the insulin levels (β (% change): 20.92, 95%CI: 4.08, 37.71; p=0.016) and the HOMA-IR index (β (% change): 19.72, 95%CI: 2.43, 40.49; p=0.03) independently of confounding factors such as maternal ep-BMI modeled either categorically or in its continuous form (data not shown). We observed similar positive trends between parity and insulin and the HOMA-IR index in overweight/obese and non-overweight/obese women (**Supplementary Table 1** (31)). Although the association of parity≥2 with the HOMA-IR index, compared to being in the nulliparous group, seemed to be stronger in overweight/obese women. However, cross-product terms between weight status and parity for their associations with each outcome (glucose, insulin, and HOMA-IR) were not significant (p>0.14 for all interactions, as shown in **Supplementary Table 1** (31)). When the parity number was increased in combination with being overweight/obese, that is, when analyzed together, the positive relationships with insulin and HOMA-IR gradually and significantly increased (**Table 3**). Specifically, in the full model, the group of multiparous+OWO women was associated with a greater increase in mean insulin levels of 64.87% (95%CI: 34.99, 101.38; p<0.001) and in the mean HOMA-IR index of 68.20% (95%CI: 35.12, 110.01; p<0.001) compared with those in the nulliparous+NW group (**Table 3**). The multivariate-adjusted

geometric means of the HOMA-IR according to combined parity+maternal ep-BMI categories are shown in **Figure 1**.

According to the HOMA-IR cut-off point ≥1.58, the prevalence of elevated IR was 41.0% and 49.0% for multiparous women and overweight/obese women, respectively. Among the multiparous and overweight/obese women, the prevalence increased to 57.6% (**Figure 2**). After adjustment for confounders, multiparity was associated with a 1.59-fold (95%CI: 1.07, 2.36; p=0.021) increase in the relative risk of elevated IR and, with a trend (p-for-trend=0.018) towards increased parity-associated risk of elevated IR as parity increased (parity =1, RR: 1.55, 95%CI: 1.03, 2.36; p=0.036 and parity≥2, RR: 1.72, 95%CI: 1.05, 2.83; p=0.031). Interestingly, multiparous women with overweight/obesity were nearly two times as likely to have elevated IR than the total multiparous population (RR: 3.04, 95%CI: 1.70, 5.47; p<0.001). The biggest difference in the RR was between the parity≥2 children+OWO group and the nulliparous+NW group (RR: 3.85, 95%CI: 1.98, 7.76; p<0.001). Results are shown in detail in **Figure 2**.

Discussion

This study researched the relationship between parity and glucose metabolism parameters in the first trimester of pregnancy among healthy pregnant women from a Mediterranean region. We found a significant positive association between multiparity and IR as assessed by HOMA-IR index, even after considering several traditional confounding factors. Women with one or more parity also had a 1.59-fold increased relative risk of having elevated IR compared with nulliparous women, which increased proportionally to the number of parities. This positive relationship between parity and greater relative risk of IR was more robust in combination with being overweight/obese.

The results of our study highlight the clinical relevance of early assessment for IR in pregnant women who have had multiple pregnancies, particularly in pregnant women with overweight or obesity. According to our selected HOMA-IR threshold of 1.58, the prevalence rate of early-pregnancy IR among multiparous pregnant women was 41.0%, about 50% among the overweight/obese group, and 57.6% among women who met both conditions. Although there is no standardized cutoff value of HOMA-IR for identifying IR in pregnancy (32), it is worth noting

that, Cohen et al. (33) validated the use of HOMA-IR as a surrogate to the hyperinsulinemic-euglycemic clamp technique "gold-standard" as a measure of IR in early pregnancy, even in obese women. In our study, the decision to define the upper tertile of HOMA-IR as the threshold for IR could be viewed as somewhat arbitrary; however, it is not inconsistent with published data on other measures of IR that identify insulin-resistant individuals as being in the top tertile (34,35). Furthermore, maternal IR in early pregnancy, at HOMA-IR cut-off levels within the range from 1.51 to 2.31, has been reported as a reliable marker of subsequent GD (18,36). Our HOMA-IR threshold of 1.58 is well within this range. In this context, previous researchers have also indicated that women with high HOMA-IR in early to mid-pregnancy have an increased risk of subsequent preeclampsia, excessive weight gain during pregnancy, and of giving birth to macrosomic and large for gestational age neonates (4,5,37). Thus, exposure in early pregnancy to elevated maternal IR, especially in multiparous women, should not be ignored.

Regarding glucose homeostasis disorders in pregnancy related to repeat pregnancies, extensive research has been carried out to investigate the relationship between multiparity and GD or recurrent GD during later pregnancies (11,13). However, few studies have focused on maternal IR in early pregnancy in healthy pregnant women without previous history of diabetes.

Supporting our results in the first trimester of pregnancy (~12 weeks), an earlier study conducted by Abdelsalam et al. (20) which involved 300 pregnant Sudanese women aged 17–35 years in Khartoum state (Sudan), reported that both maternal fasting insulin levels and HOMA-IR in middle-pregnancy (~20-30 weeks gestation) increased significantly with higher parity compared to nulliparity. The highest levels were observed in grand multiparity (>5 times). Similarly, Jinlan et al. (21), analyzing the data of 208 Chinese pregnant women aged 25-35 years, in Huzhou region, Zhejiang Province (Southeast China), stated that women with a second pregnancy were more likely to have higher glucose intolerance and HOMA-IR–based IR during the second-third trimester compared to primiparas (first-time pregnancy). In another study conducted by Seghieri et al. (19) in a selected group of 1880 third-trimester pregnant women at higher risk for GD (i.e., all women had glucose intolerance, overweight/obesity, and advanced age, over 29 years), in the Pistoia area (Tuscany, Italy), it was found that ISI_{OGTT}–based insulin sensitivity during the third trimester decreased significantly only in pregnant women with parity>3 compared to nulliparous.

However, contrary to our findings, the earlier parity-to-insulin sensitivity relationship disappeared after adjustment for age, pregestational BMI, and weight gain, which are relevant confounders that are strongly related to worsening insulin sensitivity. These data could lead us to speculate on whether there is a true effect on this relationship or whether this could arise solely from confounding. Importantly, adjustment of our analyses for these factors simultaneously with other behavioral factors (including educational level, smoking, physical activity and eating habits) did not change the associations observed. Therefore, we can state that our results are robust under different modeling approaches. The apparent disagreement with the above findings could be due to differences in study design, population characteristics, and the methodologies used for evaluating IR, as well as the fact that in our study, the assessment period was in the first trimester. Interestingly, supporting our results at least in part, the study by Seghieri et al. (19) found that in comparison with the initial pregnancy, insulin sensitivity significantly decreased in the subsequent pregnancy. Similarly, Skajaa et al. (6) studying pregnant women with type 1 diabetes reported that daily insulin requirements from week to week increased significantly throughout pregnancy with increasing parity after adjusting for BMI, age, and pre-pregnancy HbA1c. This last study also revealed that the individual total insulin requirement in the woman's first pregnancy increased with each pregnancy during pregnancy but did not in the non-pregnant status. This reaffirms, in part, our results and further strengthens the hypothesis that parity *per se* negatively affects insulin sensitivity during pregnancy, thereby increasing insulin requirement.

In addition, our findings are also consistent with the evidence from published studies indicating a relationship between multiparity and GD (11,13), and most researchers report increased risk of diabetes in the later life of women with high parity (8,9). However, it has been suggested that a woman would need to have at least four pregnancies for the risk of diabetes to be affected (8,9). Unfortunately, our data does not permit us to test the effect of having more than three (n=3) or four (n=1) children on first-trimester IR due to the small number of women with these pregnancies; however, it is worth examining in future research.

It is well known that being overweight or obese before and during pregnancy predisposes the woman to a higher metabolic dysregulation in pregnancy (38) including early IR, as observed in our study. According to our results, overweight/obese women were more insulin resistant at the

start of their pregnancy compared with normal-weight women regardless of parity. This finding is in accordance with previous reports (22,38). An original aspect of this study that deserves attention is the association of the combination of parity and overweight/obesity with IR in early pregnancy, which has not been previously researched. According to our study findings, nulliparous pregnant women with an ep-BMI≥25.0 kg/m^2 had an almost three times higher risk of IR compared to normal weight nulliparous women. It is interesting to note that overweight/obese multiparous pregnant women had a six times higher risk of IR. Moreover, in this vulnerable group, the risk of IR showed an increasing trend with increasing parity. The above data suggest that the combination of parity and maternal overweight/obesity may result in additive adverse effects that contribute to a worse IR early in pregnancy.

The relationship between parity and maternal IR early in pregnancy is complex and still not fully understood. This probably reflects a combination of sociodemographic/environmental factors and placental hormone changes due to repeated pregnancies that result indirectly or directly in a progressive worsening of IR. We found, for example, that multiparous pregnant women were older and were less likely to have a university education and more likely to belong to a higher social class than nulliparae. In the present study, the last two factors were significantly related to higher ep-BMI, which in turn were independently and positively associated with early IR.

Multiparity is also an independent predictor for obesity (39). About three-quarters of women are unable to reach their pre-pregnancy weight one year after delivery (40), this leads to inter-pregnancy accumulation of adipose tissue, thereby contributing to IR in successive pregnancies and beyond (41). Nevertheless, our data does not allow us to draw conclusions about this issue. It has also been argued that, just like outside pregnancy, obesity in pregnancy promotes a high production of pro-inflammatory factors and oxidative stress due to the accumulation of adipose tissue macrophages, which induces IR (39,42). Thus, obese multiparous pregnant women could have even greater metabolic consequences, including IR, as confirmed in our study. This is probably explained by either the greater adiposity, repeated episodes of inflammatory response, or a combination of these. Furthermore, regardless of obesity, an increase in the production of placental hormones, such as progesterone, estrogen, and placental lactogens, in pregnancy to

accommodate the growing fetus (43) leads to a relative IR. This effect can accumulate, especially for multiparous women (39).

Taken together, our study offers new knowledge about the role of multiparity in maternal IR in subsequent pregnancies, which provides an opportunity for an early targeted intervention. However, it is necessary to consider several limitations when interpreting our findings. First, our population consisted of healthy pregnant women living in the Mediterranean region with specific socio-demographic and healthy Mediterranean lifestyle traits, which prevents the generalization of the findings to other populations. In addition, due to limited resources, it was not feasible to use the "gold standard" hyperinsulinemic-euglycemic clamp test in early pregnancy to quantify IR (33). However, the HOMA-IR is a non-invasive, cost-effective, and simple surrogate measure of this parameter widely used in large epidemiologic studies and has also been validated in studies with pregnant women (44). Moreover, the fact that we did not have information on stillbirths due to early miscarriages and intrauterine fetal demise could have induced a classification bias. Lastly, previous findings support the significance of other potential factors mediating the recurrence of GD, such as the inter-pregnancy interval and percentage of body fat or fat distribution pattern through successive pregnancies (45) not having this data is, therefore, another potential weakness.

Conclusion

In summary, our study suggests that multiparity may have a negative impact on maternal IR, which is already detectable during the early stages of pregnancy, and that overweight/obesity appears to further aggravate this relationship. Therefore, it is strongly recommended that all pregnant women who have given birth previously, especially those with overweight or obesity, undergo early IR screening in subsequent pregnancies. Undoubtedly, identifying elevated IR at a very early stage in these high-risk pregnant women would make it possible to implement earlier interventions involving lifestyle modifications and/or pharmacological treatment to avoid future complications that could affect both the mother and baby.

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Author Contributions

VA designed and conducted the research. VA performed data curation. AD and EM analyzed the data and wrote the article. All authors revised the manuscript for important intellectual content and read and approved the final version. The corresponding author attests that all listed authors meet authorship criteria and that no one who meets the criteria has been omitted. VA is the guarantor of this work, as such, she has had full access to all study data and takes responsibility for their integrity and for the accuracy of the data analysis.

Data Availability and materials

The datasets generated and/or analyzed during the current study are not publicly available due to subject confidentiality; however, they are available from the corresponding author on reasonable request.

Abbreviations

ASSIR: Sexual and reproductive health care services

CIs: Confidence intervals

Ep-BMI: Early pregnancy BMI

ECLIPSES: Ensayo CLInico Para Suplementar con hierro a EmbarazadaS

FFQ: Food frequency questionnaire

GD: Gestational diabetes mellitus

HOMAR: Homeostasis model assessment of the IR

Hb: Hemoglobin

IR: Insulin resistance

IPAQ-S: International Physical Activity Questionnaire

MI: Multiple imputation

METs: Metabolic equivalents

NW: Normal weight

OWO: Overweight/obesity

PA: Physical activity

rMedDiet: Relative Mediterranean Diet

SES: Socioeconomic status

VIFs: Variance inflation factors

References

1. Abhari FR, Ghanbari Andarieh M, Farokhfar A, Ahmady S. Estimating rate of insulin resistance in patients with preeclampsia using HOMA-IR index and comparison with nonpreeclampsia pregnant women. Biomed Res Int. 2014;2014:140851.
2. Das S, Behera MK, Misra S, Baliarsihna AK. Beta-cell function and insulin resistance in pregnancy and their relation to fetal development. Metab Syndr Relat Disord. 2010;8(1):25–32.
3. Song S, Zhang Y, Qiao X, Duo Y, Xu J, Peng Z, et al. HOMA-IR as a risk factor of gestational diabetes mellitus and a novel simple surrogate index in early pregnancy. Int J Gynaecol Obstet. 2022;157(3):694–701.
4. Wolf M, Sandler L, Muñoz K, Hsu K, Ecker JL, Thadhani R. First trimester insulin resistance and subsequent preeclampsia: a prospective study. J Clin Endocrinol Metab. 2002;87(4):1563–8.
5. Yamashita H, Yasuhi I, Fukuda M, Kugishima Y, Yamauchi Y, Kuzume A, et al. The association between maternal insulin resistance in mid-pregnancy and neonatal birthweight in uncomplicated pregnancies. Endocr J. 2014;61(10):1019–24.
6. Skajaa GO, Fuglsang J, Kampmann U, Ovesen PG. Parity Increases Insulin Requirements in Pregnant Women with Type 1 Diabetes. J Clin Endocrinol Metab. 2018;103(6):2302–8.

7. Berggren EK, Presley L, Amini SB, Hauguel-de Mouzon S, Catalano PM. Are the metabolic changes of pregnancy reversible in the first year postpartum? Diabetologia. 2015;58(7):1561–8.
8. Guo P, Zhou Q, Ren L, Chen Y, Hui Y. Higher parity is associated with increased risk of Type 2 diabetes mellitus in women: A linear dose–response meta-analysis of cohort studies. J Diabetes Complications. 2017 Jan 1;31(1):58–66.
9. Li P, Shan Z, Zhou L, Xie M, Bao W, Zhang Y, et al. Parity and risk of type 2 diabetes: A systematic review and dose-response meta-analysis. Eur J Endocrinol. 2016;175(5):R231–45.
10. Kritz-Silverstein D, Barrett-Connor E, Wingard DL. The Effect of Parity on the Later Development of Non-Insulin-Dependent Diabetes Mellitus or Impaired Glucose Tolerance. N Engl J Med. 1989;321(18):1214–9.
11. Moses RG. The recurrence rate of gestational diabetes in subsequent pregnancies. Diabetes Care. 1996;19(12):1348–50.
12. Kampmann U, Knorr S, Fuglsang J, Ovesen P. Determinants of Maternal Insulin Resistance during Pregnancy: An Updated Overview. J Diabetes Res. 2019;2019: 5320156.
13. Schwartz N, Nachum Z, Green MS. The prevalence of gestational diabetes mellitus recurrence - Effect of ethnicity and parity: A metaanalysis. Am J Obstet Gynecol. 2015;213(3):310–7.
14. Al-Rowaily MA, Abolfotouh MA. Predictors of gestational diabetes mellitus in a high-parity community in Saudi. East Mediterr Health J. 2010;16(6):636–41.
15. Laine MK, Kautiainen H, Gissler M, Raina M, Aahos I, Järvinen K, et al. Gestational diabetes in primiparous women–impact of age and adiposity: a register-based cohort study. Acta Obstet Gynecol Scand. 2018;97(2):187–94.
16. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41(Suppl 1):S13–27.

17. Catalano PM, Kirwan JP, Haugel-De Mouzon S, King J. Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. J Nutr. 2003;133(5 Suppl 2):1674S-1683S.
18. Duo Y, Song S, Zhang Y, Qiao X, Xu J, Zhang J, et al. Predictability of HOMA-IR for Gestational Diabetes Mellitus in Early Pregnancy Based on Different First Trimester BMI Values. J Pers Med. 2022;13(1):60.
19. Seghieri G, de Bellis A, Anichini R, Alviggi L, Franconi F, Breschi MC. Does parity increase insulin resistance during pregnancy? Diabetic Medicine. 2005;22(11):1574–80.
20. Abdelsalam KEA, Elamin AAM. Influence of grand multiparity on the levels of insulin, glucose and HOMA-IR in comparison with nulliparity and primiparity. Pak J Biol Sci. 2017;20(1):42–6.
21. YAO Jinlan, Meimei M, Yu Y, Xiaofan F, Xiufang D. Influence of parity on blood glucose levels and insulin resistance in pregnant women. Chinese Journal of Integrative Nursing. 2019;5(10):10–2.
22. Motevalizadeh E, Díaz-López A, Martín-Luján F, Basora J, Arija V. Prenatal Factors Associated with Maternal Cardiometabolic Risk Markers during Pregnancy: The ECLIPSES Study. Nutrients. 2023;15(5):1135.
23. Arija V, Fargas F, March G, Abajo S, Basora J, Canals J, et al. Adapting iron dose supplementation in pregnancy for greater effectiveness on mother and child health: Protocol of the ECLIPSES randomized clinical trial. BMC Pregnancy Childbirth. 2014;14(1):33.
24. Hinkle SN, Zhang C, Grantz KL, Sciscione A, Wing DA, Grobman WA, et al. Nutrition during Pregnancy: Findings from the National Institute of Child Health and Human Development (NICHD) Fetal Growth Studies–Singleton Cohort. Curr Dev Nutr. 2021;5(1):1–16.
25. Fagerström KO. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addictive Behaviors. 1978;3(3–4):235–41.

26. Rodríguez IT, Ballart JF, Pastor GC, Jordà EB, Val VA. [Validation of a short questionnaire on frequency of dietary intake: reproducibility and validity]. Nutr Hosp. 2008;23(3):242–52.

27. Díaz-López A, Díaz-Torres S, Martín-Luján F, Basora J, Arija V. Prenatal adherence to the Mediterranean diet decreases the risk of having a small-for-gestational-age baby, ECLIPSES study. Sci Rep. 2022;12(1):13794.

28. Solanke BL. Maternal socio-demographic factors associated with low parity and grand multiparity in Nigeria. Women Health. 2019;59(7):730–47.

29. Seaman SR, Bartlett JW, White IR. Multiple imputation of missing covariates with non-linear effects and interactions: an evaluation of statistical methods. BMC Med Res Methodol. 2012;12.

30. Barros AJD, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. BMC Med Res Methodol. 2003;3:1–13.

31. Motevalizadeh E, Díaz-López A, Martín F, Basora J, Arija V. Supplementary data for "Association of parity with insulin resistance early in pregnant women: ECLIPSES study." Figshare. Deposited September 26, 2023. https://figshare.com/s/2d8605799c24f57ba034.

32. Tang Q, Li X, Song P, Xu L. Optimal cut-off values for the homeostasis model assessment of insulin resistance (HOMA-IR) and pre-diabetes screening: Developments in research and prospects for the future. Drug Discov Ther. 2015;9(6):380–5.

33. Cohen O, Epstein GS, Weisz B, Homko CJ, Sivan E. Longitudinal assessment of insulin sensitivity in pregnancy. Validation of the homeostasis model assessment. Clin Endocrinol (Oxf). 2006;64(6):640–4.

34. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. Ann Intern Med. 2003;139(10):802–9.

35. Cheal KL, Abbasi F, Lamendola C, McLaughlin T, Reaven GM, Ford ES. Relationship to insulin resistance of the adult treatment panel III diagnostic criteria for identification of the metabolic syndrome. Diabetes. 2004;53(5):1195–200.

36. Kırlangıç MM, Arıcı Halıcı BN. Evaluation of First Trimester Fasting Blood Glucose, HOMA-IR and HbA1c in Prediction of Gestational Diabetes Mellitus in Non-Obese Pregnant Women: A Retrospective Study. Turk J Diab Obes. 2022;6(3):267–73.
37. Li J, Leng J, Li W, Zhang C, Feng L, Wang P, et al. Roles of insulin resistance and beta cell dysfunction in macrosomia among Chinese women with gestational diabetes mellitus. Prim Care Diabetes. 2018;12(6):565–73.
38. Catalano PM. Obesity, insulin resistance, and pregnancy outcome. Reproduction. 2010;140(3):365–71.
39. Rebholz SL, Jones T, Burke KT, Jaeschke A, Tso P, D'Alessio DA, et al. Multiparity leads to obesity and inflammation in mothers and obesity in male offspring. Am J Physiol Endocrinol Metab. 2012;302(4):449–57.
40. Balsarkar G. Clinical Practice Guidelines for Weight Management in Postpartum Women: An AIIMS-DST Initiative in Association with FOGSI. J Obstet Gynaecol India. 2022;72(2):99–103.
41. Gunderson EP, Murtaugh MA, Lewis CE, Quesenberry CP, West DS, Sidney S. Excess gains in weight and waist circumference associated with childbearing: The Coronary Artery Risk Development in Young Adults Study (CARDIA). Int J Obes Relat Metab Disord. 2004;28(4):525–35.
42. Challier JC, Basu S, Bintein T, Minium J, Hotmire K, Catalano PM, et al. Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta. Placenta. 2008;29(3):274–81.
43. Mouzon SH De, Lassance L. Endocrine and metabolic adaptations to pregnancy; impact of obesity. Horm Mol Biol Clin Investig. 2015;24(1):65–72.
44. Gutch M, Kumar S, Razi SM, Gupta K, Gupta A. Assessment of insulin sensitivity/resistance. Indian J Endocrinol Metab. 2015;19(1):160–4.
45. Schwartz N, Green MS, Yefet E, Nachum Z. Modifiable risk factors for gestational diabetes recurrence. Endocrine. 2016;54(3):714–22.

TABLES AND FIGURE LEGENDS

Table 1. Sociodemographic and lifestyle characteristics of pregnant women.

Table 2. Comparison of glucose metabolism parameters by parity, maternal ep-BMI-based weight status, and combined parity + ep-BMI-based weight status categories in the first trimester of pregnancy

Table 3. Linear regression models of the associations of parity (modeled separately as nulliparous vs. multiparous and as parity categories (0: nulliparous, 1, and ≥2 children)) and combined parity + ep-BMI categories with glucose metabolism parameters.

Figure 1.

Title: Multivariable-adjusted geometric means and 95% CIs of HOMA-IR index in the first trimester of pregnancy according to parity and maternal early-pregnancy BMI categories (n=264).

Footnotes: The adjusted means were calculated by analysis of covariance (ANCOVA). The models were mutually adjusted for age categories (<25 (ref.), 25-29, ≥30 years), educational level (low/medium (ref.), high), smoking status (never smoker (ref.), current/former smoker), alcohol consumption (no (ref.), yes), planned pregnancy (no (ref.), yes), physical activity tertile (T1: ≤1070 (ref.), T2:1071-3335, T3: ≥3336 METs-min/week), and Mediterranean diet score tertile (T1: ≤8 (ref.), T2: 9-11, T3: ≥12 points). *p-value<0.05, and **p-value<0.001 compared with the reference category (nulliparous + NW). The points represent adjusted geometric means, and the whisker plots represent 95% CIs. The horizontal lines (blue) represent the overall geometric means. Abbreviations: BMI, body mass index; NW, normal weight (BMI<25 kg/m2); OWO, overweight/obesity (BMI≥25 kg/m2); HOMA-IR, homeostasis model assessment-insulin resistance.

Figure 2.

Title: Multivariable-adjusted RR (95% CI) for elevated HOMA-IR according to parity and combined parity + ep-BMI categories.

Footnotes: Multivariable-adjusted Cox regression models with constant follow-up time set at t=1 for all individuals and robust variance were used to calculate the RR and 95% confidence interval (95% CI). †The models were mutually adjusted for age categories (<25 (ref.), 25-29, ≥30 years),

BMI categories (NW (ref.), OWO; not for adjusted in the parity+ep-BMI categories analysis, where it was integrated into the composite explanatory variable itself), educational level (low/medium (ref.), high), smoking status (never smoker (ref.), current/former smoker), alcohol consumption (no (ref.), yes), planned pregnancy (no (ref.), yes), physical activity tertile (T1: ≤1070 (ref.), T2:1071-3335, T3: ≥3336 METs-min/week), and Mediterranean diet score tertile (T1: ≤8 (ref.), T2: 9-11, T3: ≥12 points). The significance of the numbers in bold as p-value < 0.05. The diamonds represent RR and the whisker plots represent 95% CIs. Abbreviations: Relative Risk, RR; BMI, body mass index; ep-BMI, early-pregnancy BMI; NW, normal weight (BMI<25 kg/m2); OWO, overweight/obesity (BMI≥25 kg/m2); HOMA-IR, homeostasis model assessment-insulin resistance; Ref., reference. *The top tertile of HOMA-IR (≥ 1.58). P for trend was calculated by treating ordinal categorical exposure variable as continuous variable.

600 **Table 1.** Sociodemographic and lifestyle characteristics of pregnant women.

General Characteristics	Overall (n=264)	Parity		*p*-value†
		Nulliparous women (n=112)	Multiparous women (n=152)	
Age (years), mean ± SD	29.6 ± 4.7	28.6 ± 4.5	30.6 ± 4.7	**<0.001****
Age categories (years), n (%)				
<25	40 (15)	25 (22)	15 (10)	
25-29	73 (28)	34 (30)	39 (26)	**0.005***
≥30	152 (57)	53 (47)	98 (64)	
Weight (kg), mean ± SD	63.3 ± 9.6	62.9 ± 9.9	63.5 ± 9.5	0.57
Ep-BMI (kg/m^2), mean ± SD	24.1 ± 3.5	23.9 ± 3.7	24.3 ± 3.4	0.48
Ep-BMI categories, n (%)				
Normal weight (BMI<25 kg/m^2)	169 (64)	76 (68)	93 (61)	
Overweight/obesity (BMI ≥ 25 kg/m^2)	95 (36)	36 (32)	59 (39)	0.264
Educational level, n (%)				
Low (primary or less)	83 (31)	28 (25)	55 (36)	
Medium (secondary)	97 (37)	36 (32)	61 (40)	**0.004***
High (university or more)	84 (32)	48 (43)	36 (24)	
Familiar SES, n (%)				
Low	35 (13)	7 (6)	28 (18)	
Medium	180 (68)	77 (69)	103 (68)	**0.003***
High	49 (19)	28 (25)	21 (14)	
Smoking status, n (%)				
Never smoker	185 (70)	74 (66)	111 (73)	
Former smoker	42 (16)	23 (20)	19 (13)	0.21
Current smoker	37 (14)	15 (13)	22 (14)	
Alcohol consumption				
No	222 (84)	95 (85)	127 (84)	0.30
Yes	33 (13)	11 (10)	22 (14)	
Missing	9 (3)	6 (5)	3 (2)	
Physical Activity (METs-min/week)				
T1 (≤1070)	87 (33)	36 (32)	51 (34)	
T2 (1071-3335)	117 (44)	51 (45)	66 (43)	0.94
T3 (≥3336)	60 (23)	25 (22)	35 (23)	
rMedDiet score (point)				
T1 (≤8)	92 (35)	37 (33)	55 (36)	
T2 (9-11)	107 (41)	41 (37)	66 (44)	0.34
T3 (≥12)	56 (21)	28 (25)	28 (18)	
Missing	9 (3)	6 (5)	3 (2)	
Planned pregnancy				
No	62 (23)	22 (20)	40 (26)	0.21
Yes	202 (77)	90 (80)	112 (74)	

601 Values are expressed in means ± SD (standard deviation) or number (%, percentage).
602 Abbreviations: BMI, body mass index; SES, socioeconomic status; METs, metabolic equivalents;
603 T, tertile; rMedDiet, Mediterranean diet. †*p*-value for the differences across primiparous vs.
604 multiparous women as derived from Student's t-test or Chi-square test, as appropriate. **p*-
605 value<0.05, and ***p*-value<0.001 compared with the reference category.
606

607 **Table 2.** Comparison of glucose metabolism parameters by parity, maternal ep-BMI-based
608 weight status, and combined parity + ep-BMI-based weight status categories in the first
609 trimester of pregnancy.

Variables	N	Glucose (mg/dL)	Insulin (mU/L)†	HOMA-IR†
		Mean ± SD	Mean ± SD	Mean ± SD
Overall	264	70.2 ± 10.7	7.77 ± 1.76	1.32 ± 1.85
Parity				
Nulliparous	112	69.8 ± 9.4	6.89 ± 1.75	1.18 ± 1.82
Multiparous	152	70.4 ± 11.5	**8.41 ± 1.78****	**1.44 ± 1.87****
Ep-BMI categories				
Normal weight (BMI<25 kg/m^2)	169	69.5 ± 10.4	6.89 ± 1.70	1.17 ± 1.84
Overweight/obesity (BMI≥25 kg/m^2)	95	71.5 ± 11.4	**9.49 ± 1.70****	**1.65 ± 1.79****
Parity + ep-BMI categories				
Nulliparous + NW	76	68.5 ± 9.3	6.35 ± 1.71	1.07 ± 1.78
Multiparous + NW	93	70.4 ± 11.3	7.39 ± 1.78	1.26 ± 1.88
Nulliparous + OWO	36	72.8 ± 9.4	8.16 ± 1.77	1.46 ± 1.84
Multiparous +OWO	59	70.7 ± 12.2	**10.48 ± 1.65****	**1.80 ± 1.75****

610 Values are expressed in means ± SD (standard deviation). Abbreviations: BMI, body mass index;
611 NW, normal weight (BMI<25 kg/m^2); OWO, overweight/obesity (BMI≥25 kg/m^2); HOMA-IR,
612 homeostasis model assessment-insulin resistance; ep-BMI, early-pregnancy BMI. †Geometric
613 means of log-transformed. The significance of the numbers in bold is *p-value < 0.05, and
614 **p<0.001 compared with the first category as derived from Student's t-test or ANOVA, as
615 appropriate.
616

Table 3. Linear regression models of the associations of parity (modeled separately as nulliparous vs. multiparous and as parity categories (0: nulliparous, 1, and ≥2 children)) and combined parity + ep-BMI categories with glucose metabolism parameters.

	N	Glucose (mg/dL)		Insulin (mU/L) (% change)†		HOMA-IR index (% change)†	
		Model 1 (n=264) β (95% CI)	Model 2 (n=264) β (95% CI)	Model 1 (n=264) β (95% CI)	Model 2 (n=264) β (95% CI)	Model 1 (n=264) β (95% CI)	Model 2 (n=264) β (95% CI)
Parity							
Nulliparous	112	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Multiparous	152	0.60 (-2.03, 3.24)	0.15 (-2.68, 3.00)	**22.14 (6.72, 40.49)***	**20.92 (4.08, 37.71)***	**23.37 (6.18, 43.33)***	**19.72 (2.43, 40.49)***
Nulliparous	112	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
1 previous child	115	0.35 (-2.46, 3.16)	-0.24 (-3.21, 2.72)	**19.72 (3.67, 39.10)***	**18.06 (1.41, 37.44)***	**19.94 (2.12, 40.78)***	16.88 (-1.00, 37.99)
≥2 previous children	37	1.38 (-2.63, 5.40)	1.71 (-2.67, 6.11)	**30.47 (5.65, 61.28)***	**29.18 (3.28, 61.61)***	**32.45 (5.34, 66.70)***	**32.18 (3.56, 68.71)***
p for trend‡		0.52	0.58	**0.007**	**0.010**	**0.011**	**0.014**
Parity + ep-BMI categories							
Nulliparous + NW	76	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Multiparous + NW	93	1.87 (-1.39, 5.12)	1.53 (-1.94, 5.01)	15.03 (-2.47, 35.93)	15.49 (-2.96, 37.71)	17.47 (-1.98, 40.49)	17.35 (-3.25, 42.62)
Nulliparous + OWO	36	**4.31 (0.04, 8.57)***	3.95 (-0.39, 8.28)	**28.27 (3.15, 59.36)***	**27.51 (2.12, 59.20)***	**36.48 (7.79, 72.98)***	**34.99 (6.18, 72.29)***
Multiparous + OWO	59	2.18 (-1.48, 5.83)	1.64 (-2.29, 5.58)	**66.70 (38.40, 99.37)****	**64.87 (34.99, 101.38)****	**69.89 (39.10, 109.59)****	**68.20 (35.12, 110.01)****
p for trend‡		0.152	0.265	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Parity + ep-BMI categories							
Nulliparous + NW	76	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
1 previous child + NW	71	2.09 (-1.39, 5.57)	1.57 (-2.07, 5.20)	13.88 (-4.88, 34.99)	15.03 (-4.88, 39.10)	16.18 (-4.30, 40.49)	16.18 (-4.88, 42.76)
≥2 previous children + NW	22	1.13 (-3.96, 6.24)	1.56 (-4.02, 7.15)	20.92 (-6.76, 56.83)	18.53 (-10.42, 58.41)	22.14 (-8.61, 61.61)	22.14 (-10.42, 66.53)
Nulliparous + OWO	36	**4.30 (0.04, 8.57)***	3.97 (-0.36, 8.31)	**28.40 (3.05, 60.00)***	**28.40 (2.22, 60.01)***	**36.34 (7.25, 73.33)***	**35.53 (6.18, 71.60)***
1 previous child + OWO	44	1.15 (-2.83, 5.15)	0.37 (-3.91, 4.64)	**63.23 (32.31, 99.37)****	**58.41 (27.12, 99.37)****	**63.23 (31.00, 105.44)****	**59.36 (24.61, 101.38)****
≥2 previous children OWO	15	5.16 (-0.79, 11.13)	5.44 (-0.86, 11.45)	**78.60 (31.00, 141.09)****	**85.89 (33.64, 156.00)****	**91.55 (37.71, 166.45)****	**99.37 (40.48, 185.38)****
p for trend‡		0.129	0.192	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Linear regression models were used to calculate the β coefficient (β) and 95% confidence interval (95% CI). Model 1: unadjusted model. Model 2: model adjusted for age categories (<25 (ref.), 25-29, ≥30 years), BMI categories (NW (ref.), OWO; not for adjusted in the parity+ep-BMI categories analysis, where it was integrated into the composite explanatory variable itself), educational level (low/medium (ref.), high), smoking status (never smoker (ref.), current/former smoker), alcohol consumption (no (ref.), yes), planned pregnancy (no (ref.), yes), physical activity tertile (T1: ≤1070 (ref.), T2:1071-3335, T3: ≥3336 METs-min/week), and

Mediterranean diet score tertile (T1: ≤8 (ref.), T2: 9-11, T3: ≥12 points). The significance of the numbers in bold as *p-value < 0.05, and **p-value<0.001 compared with the reference category. Abbreviations: BMI, body mass index; ep-BMI, early-pregnancy BMI; NW, normal weight (BMI<25 kg/m^2); OWO, overweight/obesity (BMI≥25 kg/m^2); HOMA-IR, homeostasis model assessment-insulin resistance; Ref., reference. †Natural log-transformed; parameter estimates (β coefficient) have been back transformed to reflect the percent change in each outcome (insulin and HOMA-IR) associated with a 1-unit increase in each exposure variable. ‡p-value for trend was calculated by treating ordinal categorical exposure variable as continuous variable

Figure 1

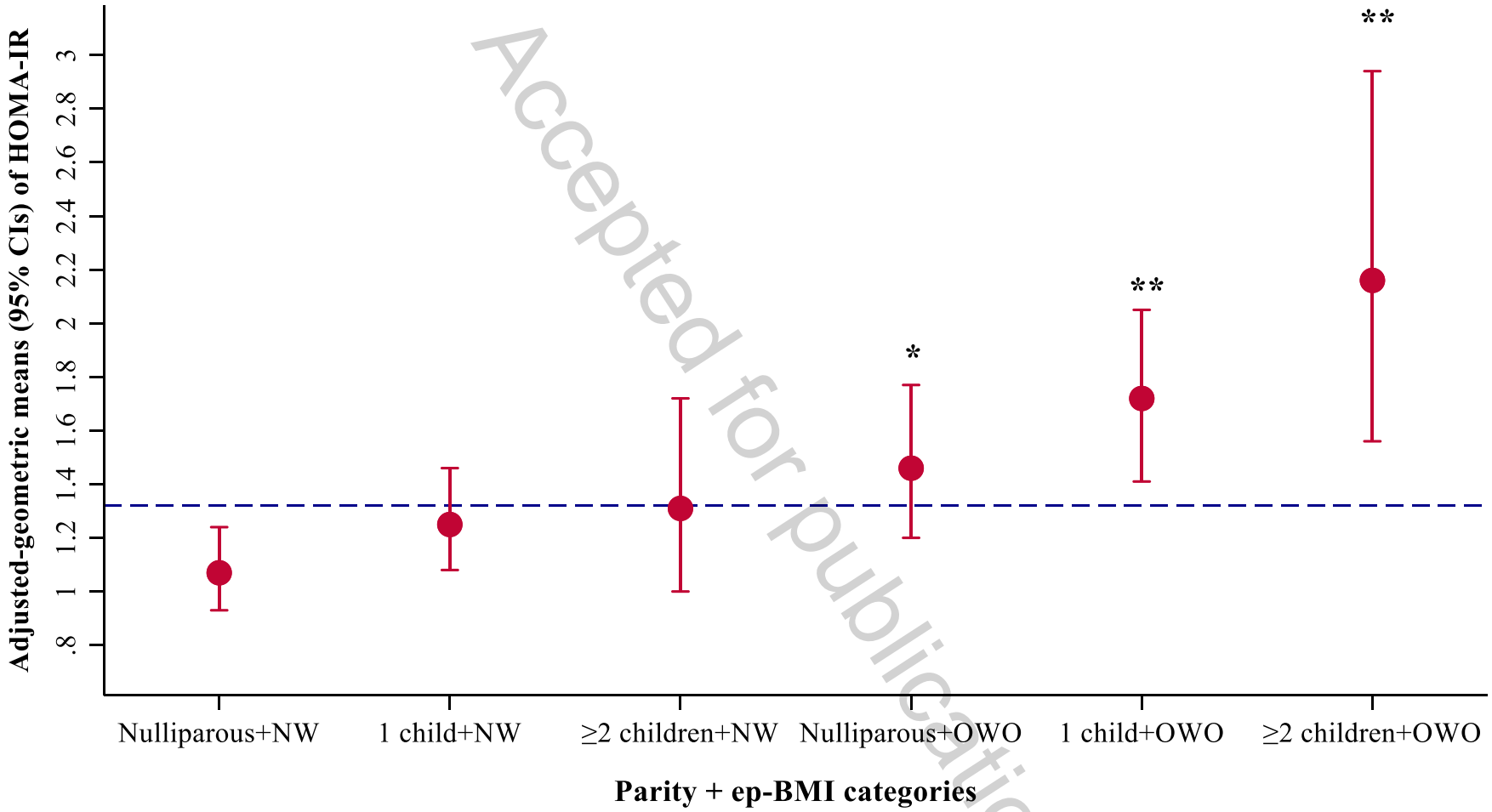
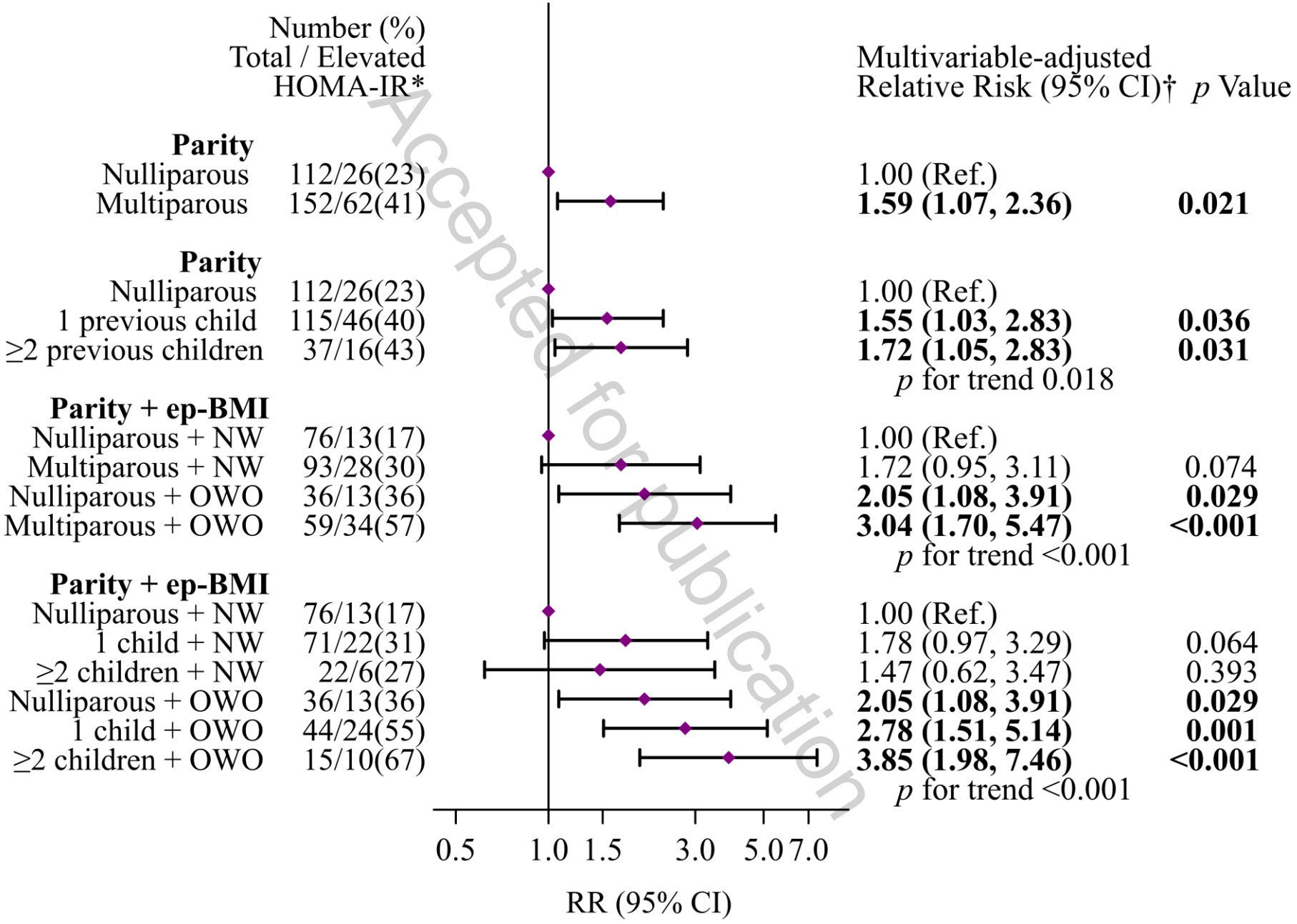


Figure 2



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