BMJ Open

Association between parity and persistent weight gain at age 40-60 years: a longitudinal prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024279
Article Type:	Research
Date Submitted by the Author:	18-May-2018
Complete List of Authors:	Zoet, Gerbrand; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Paauw, Nina; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Groenhof, Katrien; Universitair Medisch Centrum Utrecht, Julius Center for Health Sciences and Primary Care Franx, Arie; University Medical Center Utrecht, Obstetrics & Gynaecology Gansevoort, Ron T.; University Medical Center Groningen, Division of Nephrology Groen, Henk; University Medical Centre Groningen, Department of Epidemiology Van Rijn, Bas; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Lely, Titia; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology
Keywords:	Pregnancy, Parity, Cardiovascular risk factors, BMI, HDL cholesterol, Hypertension < CARDIOLOGY

Association between parity and persistent weight gain at age 40-60 years: a longitudinal prospective cohort study

Gerbrand A. Zoet, MD[a]; Nina D. Paauw, MD[a]; T. Katrien J. Groenhof, MD[b], Arie Franx, MD PhD[a]; Ron T. Gansevoort, MD, PhD[c]; Henk Groen[d], MD, PhD; Bas B. van Rijn, MD, PhD[a,e], A.Titia Lely, MD, PhD[a].

[a] Wilhelmina Children's Hospital Birth Center, University Medical Center Utrecht, Lundlaan 6, 3508 AB, Utrecht, the Netherlands

[b] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands

[c] Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands

[d] Department of Epidemiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands

[e] Academic Unit of Human Development and Health, University of Southampton, Princess Anne Hospital, Coxford Road, Southampton SO16 5YA, United Kingdom

Corresponding author at: G.A. Zoet, University Medical Center Utrecht, Room KE 02.510.2, Lundlaan 6, PO Box 85090, 3508 AB, Utrecht, The Netherlands. Tel.: +31 88 7557526, e-mail: g.zoet@umcutrecht.nl

Short title: Parity and persistent weight gain

Total word count: 3080 (excluding title page, abstract, references, figures and tables)

Abstract

Objectives: Physiological metabolic adaptations occur in the pregnant woman. These may persist postpartum and thereby contribute to an unfavorable cardiovascular disease (CVD) risk profile in parous women. The aim of the current study is to assess time-dependent changes of cardiometabolic health in parous women compared to nulliparous women.

Design and setting: We studied data of 2459 women who participated in the PREVEND study, a population-based prospective longitudinal cohort for assessment of CVD and renal disease in the general population.

Participants: We selected women ≥40 years at the first visit, who reported no new pregnancies during the 4 follow-up visits. All women were categorized in parity groups, and stratified for age.

Outcome measures: We compared BMI, high-density lipoprotein (HDL) cholesterol, blood pressure as continuous measurements and as clinical relevant CVD risk factors among parity groups over the course of six years using generalized estimating equation (GEE) models adjusted for age.

Results: The BMI was significantly higher in women para 2 or more in all age categories: per child, the BMI was 0.6 kg/m^2 higher. corresponding with 1.5–2.0 kg weight gain per child. HDL cholesterol was significantly lower in women para 2 or more aged 40-49 and 50-59 years: per child, the HDL cholesterol was up to 0.09 mmol/L lower. Blood pressure did not differ among parity groups in any of the age categories.

Conclusions: Higher parity is associated with higher BMI, lower HDL cholesterol and a higher prevalence of cardiovascular risk factors, which is constant over time. These findings warrant for prospective research assessing determinants of cardiometabolic health at earlier age to understand the role of pregnancy in the development of cardiovascular disease in women.

Keywords: Pregnancy; parity; cardiovascular risk factors; BMI; HDL cholesterol; hypertension.

Study summary: Strengths and limitations of this study

- This longitudinal cohort comprised a large, well-phenotyped cohort with uniform assessment of all measurements during a median follow-up of 6 years.
- The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects.
- Since parity itself was not assessed in this cohort, we used the number of children as a proxy for the number of childbirths.
- Women para > 2 were older, less often used oral contraceptives and more often used antihypertensive medication which might have resulted in a slightly different metabolic profile.
- Age at first delivery, inter-pregnancy interval and lactation have not been assessed in this cohort and therefore, adjustment of the analyses for these factors was not possible.

Funding: The PREVEND study was supported by Dutch Kidney Foundation (Grant E.033), The Groningen University Medical Center (Beleidsruimte) and Dade Behring, Ausam, Roche, Abbott and Gentian who financed laboratory equipment and reagents for various laboratory determinations. Gerbrand Zoet is supported by the Dutch Heart Foundation (grant number 2013T083). Titia Lely is supported by the ZonMW Clinical Fellowship (40-000703-97-12463).

Introduction

Pregnancy is associated with major alterations to the cardiovascular system and metabolic profile.[1–4] Increases in weight, lipid levels, blood plasma volume and cardiac output are needed to maintain a healthy, physiological pregnancy. Postpartum, this maternal adaptation reverses to its pre-pregnancy state, although several changes, (e.g. increased body weight and hypercholesterolemia) may persist for several months or longer.[5] Possibly, these persisting changes contribute to an increased prevalence of metabolic syndrome (MetS) and an unfavorable cardiometabolic profile in parous women.[6–8] The amount of gestational weight gain (GWG) affects postpartum weight retention.[9] At long-term follow up, excessive gestational weight gain is associated with an increased BMI, up to a 3–4 kg/m^2 21 years after pregnancy.[10–12]

Previous studies assessing the relation between parity and cardiometabolic health showed conflicting results and even the association between parity and obesity is questioned in some studies.[13–16] Long-term effects have only scarcely been investigated and most studies had a follow-up of only 1 to 3 years postpartum.[17,18] A recent large cross-sectional study among Hispanic women in the US demonstrated that multiparity (i.e. more than 4 or more than 6 children) was associated with obesity, low HDL cholesterol and elevated fasting glucose, also after adjustment for sociodemographic and lifestyle factors.[8] In addition, results from the cross-sectional Rotterdam study showed an lower HDL cholesterol and higher total cholesterol and glucose/insulin ratios with higher parity in Caucasian women at 70 years of age.[6]

Studies on the development of cardiovascular risk factors over time and the quantification of this effect per childbirth are conflicting. Some studies suggested a linear association between number of children and an unfavorable cardiometabolic profile, while others stated that only multiparity is associated with an increased cardiovascular disease risk.[6–8,19,20]

Some studies even showed a 'J-shaped' association in which women with two children had the lowest prevalence of coronary heart disease.[6–8]

The aim of the current study was to assess time-dependent changes of cardiometabolic health in parous women, stratified for number of children, as compared with nulliparous controls. This study was performed in a well-defined longitudinal prospective cohort study that primarily assessed development of CVD, albuminuria and renal disease.[21]

Methods

Participants

The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a longitudinal cohort follow-up study for assessment of cardiovascular and renal disease in the general population. Details of this study have previously been published elsewhere.[22,23] In summary, all inhabitants of the city of Groningen, the Netherlands, from 1997 to 1998 at age 28–75 years (n = 85,421) were invited to participate. All that applied, filled out a questionnaire and collected a first-morning void urine sample. Pregnant women and subjects with type 1 diabetes mellitus were excluded. The urinary albumin concentration was assessed in a total of 40,856 (47.8%) responders. A total of 6,000 participants were enrolled out of 7,768 subjects with a urinary albumin concentration ≥ 10mg/L. In addition, 2,592 participants were enrolled out of 3,394 subjects with a urinary albumin concentration < 10mg/L. Thereby, the PREVEND study was enriched by subjects with an elevated albumin excretion.

In total, 4,301 women were enrolled in the PREVEND study (**Figure 1**). For the current analysis, only women aged 40 years or older at the first visit and who reported no new pregnancies during the follow-up visits were included. Women who reported no children were categorized as nulliparous (n = 464; 18.9%). Women who reported one child, two children or more than two children, were categorized as para 1 (n = 277; 11.3%), para 2 (n = 1021; 41.5%) and para > 2 (n = 697; 28.3%). The PREVEND study has

been approved by the medical ethics committee of the University Medical Centre Groningen. Written informed consent was obtained from all participants.

Measurements and visits

Between 1997 and 2012, the screening visits took place every 2-4 years. Participants completed questionnaires and underwent physical examinations. Blood samples and 24-hour urine samples were taken. The questionnaires included questions regarding parity. Participants reported their number of children, which was used as a proxy for the number of childbirths. Details of clinical and laboratory measurements have previously been described elsewhere.[22] Prescription data from pharmacies was used to assess the use of antihypertensive and lipid lowering medication. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg or the use of blood pressure lowering medication. Type 2 diabetes mellitus (T2DM) was defined as a fasting plasma glucose ≥ 7.0 mmol/L, random sample plasma glucose ≥ 11.1 mmol/L, self-reported physician diagnosis of type 2 diabetes mellitus, and/or the use of glucose-lowering medication.[24] Obesity was defined as BMI ≥ 30 kg/m^2.

Data selection for analyses was based on a fixed median time interval of six years between the visits.

Statistical analysis

Data was arranged per patient per visit. Continuous variables with a normal distribution are presented as mean ± standard deviation (SD) and analyzed using Student *t*-test or One-Way ANOVA followed by Tukey post-hoc analysis. Continuous variables with a skewed distribution were expressed as median with 25th–75th percentile and analyzed using Mann-Whitney U test or Kruskall Wallis. Categorical variables were analyzed using Pearson Chi square test or Fisher's exact test, where appropriate.

For longitudinal assessment (time factor) of the outcome measures among the different parity groups (group factor), a generalized estimating equations (GEE) analysis was performed, including the interaction term group*visit (interaction factor). All analyses were performed using an autoregressive correlation matrix structure. This assumes a variable correlation between measurements depending on the time between measurements, as was expected in the current analysis. For GEE analyses of continuous dependent variables (BMI, HDL cholesterol and MAP), participants were further stratified in three age categories (40 – 49 years old, 50 – 59 years old, and ≥ 60 years old). In addition, we performed a stepwise correction strategy, correcting for age (model 1), age and education (model 2), age and oral contraceptive use (model 3) and age, education and oral contraceptive use (model 4).

Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad Software Inc. San Diego, CA, USA). In all analyses, a p-value <0.05 was considered statistically significant.

Results

Study population

Table 1 provides an overview of the baseline characteristics of women who were nulliparous, para 1, para 2 and para > 2 at the first PREVEND visit. The mean age was significantly higher in women who were para > 2. The majority of all women were Caucasian. The median follow-up time was 6 years in all groups. The cardiovascular risk profile among the parity groups differed significantly on BMI, blood pressure, smoking and use of alcohol. Unfavorable blood glucose measurements and cholesterol profiles were related to higher parity. The use of blood pressure lowering medication was higher in women who were para > 2 compared to the other groups, but the use of glucose and lipid lowering medication did not differ among the groups. Women who were para > 2 less often used oral contraceptives compared to women who were nulliparous, para 1 or para 2.

Cardiometabolic profile in relation to parity and age

During the 6-year study period, there was a constant, significant difference in BMI among the parity groups at all age categories (**Figure 2A**). The BMI was higher with every increase of parity at all age categories: per child, the BMI was 0.6kg/m2 higher, equal to 1.5-2.0 kg. In women para > 2, the BMI was 0.7–2.0 kg/m^2 higher compared to the other parity groups. Over time, BMI increased significantly in all age groups (p_{time}<0.001), the change in BMI over time was similar among all parity groups ($p_{interaction}$=0.662–0.947). In a stepwise correction model, correction for age alone and correction for age and oral contraceptive use did not influence the differences in BMI among parity groups at all age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use, differences among parity groups were not statistically significant anymore at age 50-59 years only.

The prevalence of obesity increased with increasing parity at entry ($p_{for\ trend}$<0.001) and at 6 year follow up ($p_{for\ trend}$<0.001; **Figure 3**). At visit one, 15% of the nulliparous women was obese, compared to 26% of the women para >2. After the course of six years, this was increased to 16% of the nulliparous women compared to 30% of the para >2. The increase in prevalence over time was similar among the groups (p=0.450). In a stepwise correction model, correction for age alone and correction for age and oral contraceptive use did not influence the differences in HDL cholesterol among parity groups at all age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use, differences among parity groups were not statistically significant anymore at all age groups.

HDL cholesterol differed among the groups, except for participants aged ≥ 60 years (**Figure 2B**). The HDL cholesterol was lower with every increase of parity, except for participants older than 60 years: per child, the HDL decreased with 0.05 mmol/L. Women para 2 and women para > 2 had

significant lower HDL cholesterol at both measurements compared to nulliparous women aged 40–49 years (0.08–0.12 mmol/L) and 50–59 years (0.10–0.17 mmol/L). Over time, HDL cholesterol increased significantly in all age groups (p_{time}=0.001–0.007) and the change in HDL cholesterol over time was similar among all parity groups ($p_{interaction}$=0.163–0.530).

Low HDL cholesterol (<1.29 mmol/L) prevalence differed among the groups at visit one ($p_{for\ trend}$<0.001) and at follow up visit ($p_{for\ trend}$=0.006); low HDL cholesterol was more common when parity increased (**Figure 3**). Low HDL cholesterol prevalence inclined similar in all groups over time (p=0.160).

There were no differences among the parity groups over time in MAP at all ages, although MAP increased in all groups at age 40–49 years and 50–59 years and decreased in all groups at age ≥ 60 years (**Figure 2C**). The change in MAP over time was similar among all parity groups ($p_{interaction}$=0.348–0.815). Prevalence of hypertension increased with parity both at entry visit ($p_{for\ trend}$<0.001) and at follow up ($p_{for\ trend}$<0.001). Hypertension prevalence increased similar in all groups over time by 4–10% (p=0.761; **Figure 3**).

Occurrence of T2DM did not differ among the groups at entry ($p_{for\ trend}$=0.094), although a positive association was found between T2DM prevalence and parity after six years ($p_{for\ trend}$=0.018). The increase in T2DM over time was comparable at all groups (p=0.336). T2DM prevalence was < 10% at all groups at both visits (**Figure 3**).

Discussion

In this longitudinal cohort study, having 2 children or more was associated with a higher BMI in all age categories and with a lower HDL cholesterol, but not with changes in blood pressure. A higher parity was associated with higher prevalence of obesity, low HDL cholesterol and a higher prevalence of

hypertension. These associations were was constant over time. As analyses were stratified and/or adjusted for age, these results suggest a direct association of parity itself with BMI, HDL cholesterol levels and cardiovascular risk factor prevalence. Other factors attributing to parity, i.e. socio-economic status and oral contraceptive use, might have contributed to these differences.

Especially the effect of parity on BMI is of great interest, since BMI appears to be one of the most important cardiometabolic risk factors. This is not only due to the direct effect on cardiovascular disease onset, but also due to its adverse effect on lipid profile and blood pressure.[25–28] Results from a population-based cohort study among 4699 women suggested that weight or weight chances might be an important mediator in the effect of parity on metabolic syndrome, thus confirming the importance of BMI in regard to cardiometabolic health.[19] In our study, roughly each extra child is associated with 1.5–2.0 kg weight gain. Similar, each extra child is associated with 0.05 mmol/L decrease in HDL cholesterol, which might be the result of the increased BMI.

Parallel to these metabolic differences in continuous measurements among the groups, occurrence of several clinical relevant cardiovascular risk factors, such as obesity and hypertension, differed among the groups as well. This is in line with cross-sectional studies assessing metabolic profile in relation to the number of children.[6–8,19] However, some studies could not confirm the relation between parity and metabolic health, especially after adjustment for covariates such as lifestyle.[14,15,19,29] In the stepwise correction, we found no or minimal influence of age, age and education level or age and oral contraceptive use on our results. Only after full correction for age, education level and oral contraceptive use, the statistical significance among parity groups diminished. Consequently, our findings should be interpreted with caution, as these factors and others, such as lifestyle changes following childbirth, might influence cardiovascular health next to parity itself.[29] Moreover, lifestyle

effects of family life and the protective effect of lactation could explain the influence of parity on cardiometabolic health.[30–32]

Another possible explanation behind the mechanism of this relationship between parity and cardiovascular risk factors could be found in the effect of disturbances by pregnancy itself that continue to last postpartum. For example, breastfeeding is associated with a lower risk of cardiovascular risk factors, i.e. T2DM, although the role of breastfeeding remains controversial and a recent meta-analysis showed no significant effect of breastfeeding on postpartum weight retention.[33–36] Other factors involved in the relationship between parity and cardiovascular risk factors might be found in circulation markers. An example might be found in the ovarian peptide hormone relaxin, produced by the corpus luteum or placental throughout pregnancy, has emerged as cardiovascular modulator involved in vasodilatation and inducing angiogenesis.[37,38] Moreover, relaxin was positively associated with insulin sensitivity and lipid profile in women with type 2 diabetes mellitus as well.[39]

Previous cohort studies showed a 'J-shaped' association between parity and coronary heart disease, with lowest prevalence in women with two children, or stated that only multiparity (i.e. more than four or five children) was associated with increased cardiovascular disease risk.[6–8] However, our results indicate a stepwise effect of parity on cardiometabolic health and having two children or more than two children already affects BMI, HDL cholesterol levels and cardiovascular risk factor prevalence.

Our paper is the first study providing detailed assessment of cardiometabolic health development over time in relation to parity. This longitudinal study comprised a large, well-phenotyped cohort with uniform assessment of all measurements during a median follow-up of 6 years. The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects. However, several limitations need to be discussed. The mean age of women para > 2 was significantly

higher than women who were nulliparous, para 1 or para 2. In addition, women para > 2 less often used oral contraceptives and more often used antihypertensive medication. This might result in a slightly different metabolic profile although correction of the GEE-analysis for age and oral contraceptive use did not significantly change the results. Despite extensive phenotyping, age at first delivery, inter-pregnancy interval and lactation have not been assessed in the PREVEND study and therefore, adjustment of the analyses for these factors was not possible. Since parity itself was not assessed in the PREVEND, we used the number of children as a proxy for the number of childbirths. This might lead to inaccurate estimates of parity numbers, as the possibility of surrogacy or twin pregnancies is not taken into account. Additionally, no information was available regarding subfertility and several pregnancy complications, which leads to a lower number of children in these women and might reflect influence the cardiometabolic profile in later life as well. Lastly, pre-pregnancy BMI and gestational weight gain have not been assessed in the PREVEND study either, although their role on postpartum weight retention seemed limited in a recent publication.[9,18]

The PREVEND study was enriched with subjects with an elevated albumin excretion, which mostly results in an unfavorable cardiovascular risk profile compared to the general population. However, albuminuria did not significantly differ among the groups within our analyses. In addition, adjustment for albuminuria did not change the results (data not shown). Although our findings suggest an effect of parity itself on metabolic parameters, it should be noted that causality cannot be determined in our study. Therefore, one could argue that the relationship is reversed, e.g. women with higher BMI or lower HDL cholesterol are more fertile and therefore have more children. Prospective research assessing pre-pregnancy determinants of cardiometabolic health are warranted to further assess the possible causal effect of pregnancy itself.

Conclusion

In this longitudinal cohort study, higher parity is associated with higher BMI and lower HDL cholesterol. This difference among parity groups is constant over time. Furthermore, higher parity is associated with a higher prevalence of cardiovascular risk factors among the parity groups over time. These findings warrant for prospective research assessing determinants of cardiometabolic health at earlier age to understand the role of pregnancy in the development of cardiovascular disease in women.

References

1. Bartels a., O'Donoghue K. Cholesterol in pregnancy: a review of knowns and unknowns. *Obstet Med*. 2011;4(4):147-151. doi:10.1258/om.2011.110003.
2. Tan EK, Tan EL. Alterations in physiology and anatomy during pregnancy. *Best Pract Res Clin Obstet Gynaecol*. 2013;27(6):791-802. doi:10.1016/j.bpobgyn.2013.08.0011.
3. Jebeile H, Mijatovic J, Louie JCY, Prvan T, Brand-Miller JC. A systematic review and metaanalysis of energy intake and weight gain in pregnancy. *Am J Obstet Gynecol*. 2016;214(4):465-483. doi:10.1016/j.ajog.2015.12.049.
4. de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J, Spaanderman ME. Physiologic adaptation of plasma volume during pregnancy: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2016;d(November 2016):177-187. doi:10.1002/uog.17360.
5. Berge L, Armesen E, Forsdahl A. Pregnancy related changes in some cardiovascular risk factors. *Acta Obstet Gynecol Scand*. 1996;75(5):439-442.
6. Humphries KH, Westendorp ICD, Bots ML, et al. Parity and Carotid Artery Atherosclerosis in Elderly Women: The Rotterdam Study. *Stroke*. 2001;32:2259-2264.
7. Lawlor D a., Emberson JR, Ebrahim S, et al. Is the association between parity and coronary heart disease due to biological effects of pregnancy or adverse lifestyle risk factors associated with child-rearing? Findings from the British women's heart and health study and the British regional heart st. *Circulation*. 2003;107(9):1260-1264. doi:10.1161/01.CIR.0000053441.43495.1A.
8. Vladutiu CJ, Siega-Riz AM, Sotres-Alvarez D, et al. Parity and components of the metabolic syndrome among US hispanic/latina women: Results from the hispanic community health study/study of latinos. *Circ Cardiovasc Qual Outcomes*. 2016;9(2_suppl_1):S62-S69. doi:10.1161/CIRCOUTCOMES.115.002464.
9. Rong K, Yu K, Han X, et al. Pre-pregnancy BMI, gestational weight gain and postpartum weight retention: a meta-analysis of observational studies. *Public Health Nutr*. 2015;18(12):2172-2182. doi:10.1017/S1368980014002523.
10. Amorim AR, Rössner S, Neovius M, Lourenço PM, Linné Y. Does excess pregnancy weight gain constitute a major risk for increasing long-term BMI? *Obesity (Silver Spring)*. 2007;15(5):1278-1286. doi:10.1038/oby.2007.149.
11. Al Mamun a. Associations of excess weight gain during pregnancy with long-term maternal obesity, hypertension and diabetes: Evidence from 21 years postpartum follow-up. *Obes Rev*. 2010;11:51. doi:http://dx.doi.org/10.1111/j.1467-789X.2010.00763-4.x.

12. Fraser a, Tilling K, Macdonald C, Hughes R, Sattar N. Associations of gestational weight gain with maternal body mass index, waist circumference, and blood pressure measured 16 y after pregnancy: the Avon Longitudinal Study of Parents and Children. *Am J Clin Nutr*. 2011;93(6):1-4. doi:10.3945/ajcn.110.008326.1.

13. Steenland K, Lally C, Thun Mi. Parity and COronary Heart Disease among Women in the American Cancer Society CPS II Population. *Epidemiology*. 1996;7:641-643.

14. de Kleijn MJ, van der Schouw YT, van der Graaf Y. Reproductive history and cardiovascular disease risk in postmenopausal women: a review of the literature. *Maturitas*. 1999;33(1):7-36.

15. Chung E, Kim Y, Usen O. Associations Between Parity, Obesity, and Cardiovascular Risk Factors Among Middle-Aged Women. *J Women's Heal*. 2016;25(8):818-825. doi:10.1089/jwh.2015.5581.

16. Robinson WR, Cheng MM, Hoggatt KJ, Stürmer T, Siega-Riz AM. Childbearing is not associated with young women's long-term obesity risk. *Obesity*. 2014;22(4):1126-1132. doi:10.1002/oby.20593.

17. Schmitt NM, Nicholson WK, Schmitt J. The association of pregnancy and the development of obesity - results of a systematic review and meta-analysis on the natural history of postpartum weight retention. *Int J Obes (Lond)*. 2007;31(11):1642-1651. doi:10.1038/sj.ijo.0803655.

18. Nehring I, Schmoll S, Beyerlein A, Hauner H, Von Kries R. Gestational weight gain and long-term postpartum weight retention: A meta-analysis. *Am J Clin Nutr*. 2011;94(5):1225-1231. doi:10.3945/ajcn.111.015289.

19. Cohen A, Pieper CF, Brown AJ, Bastian L a. Number of children and risk of metabolic syndrome in women. *J Womens Health (Larchmt)*. 2006;15(6):763-773. doi:10.1089/jwh.2006.15.763.

20. Ness RB, Harris T, Cobb J, et al. Number of pregnancies and the subsequent risk of cardiovascular disease. *N Engl J Med*. 1993;328:1528-1533.

21. Pinto-Sietsma S-J, Mulder J, Janssen WMT, Hillege HL, Zeeuw D De, De Jong PE. Smoking Is Related to Albuminuria and Abnormal Renal Function in Nondiabetic Persons. *Ann Intern Med*. 2000;(133):585-591.

22. Hillege HL, Janssen WMT, Bak a. a a, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med*. 2001;249(6):519-526. doi:10.1046/j.1365-2796.2001.00833.x.

23. Joosten MM, Gansevoort RT, Mukamal KJ, Harst P Van Der, Geleijnse JM, Feskens EJM. Urinary and plasma magnesium and risk of ischemic heart disease 1 – 3. *October*. 2013;97(13):1299-1306. doi:10.3945/ajcn.112.054114.INTRODUCTION.

24. Abbasi A, Corpeleijn E, Gansevoort RT, et al. Role of HDL cholesterol and estimates of HDL particle composition in future development of type 2 diabetes in the general population: The PREVEND study. *J Clin Endocrinol Metab*. 2013;98(8):1352-1359. doi:10.1210/jc.2013-1680.

25. Lamon-Fava S, Wilson PWF, Schaefer EJ. Impact of Body Mass Index on Coronary Heart Disease Risk Factors in Men and Women: The Framingham Offspring Study. *Arterioscler Thromb Vasc Biol*. 1996;16:1509-15151.

26. Wilson PWF, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch Intern Med*. 2002;162(16):1867-1872. doi:10.1001/archinte.162.16.1867.

27. Bogers R, Bemelmans W, Hoogenveen R, et al. Association of overweight with increased risk of coronary heart disease partly independent of blood pressure and cholesterol levels. *Arch Intern Med*. 2007;167(16):1720-1728. doi:10.1001/archinte.167.16.1720.

28. Mongraw-Chaffin ML, Peters S a E, Huxley RR, Woodward M. The sex-specific relationship between body mass index

and coronary heart disease: A systematic review and meta-analysis of 95 cohorts with 1.2 million participants. *Lancet Diabetes Endocrinol*. 2015;3(6):437-449. doi:10.1002/aur.1474.Replication.

29. Hardy R, Lawlor D a., Black S, Wadsworth MEJ, Kuh D. Number of children and coronary heart disease risk factors in men and women from a British birth cohort. *BJOG An Int J Obstet Gynaecol*. 2007;114(6):721-730. doi:10.1111/j.1471-0528.2007.01324.x.

30. Gunderson EP, Jacobs DR, Chiang V, et al. Childbearing is associated with higher incidence of the metabolic syndrome among women of reproductive age controlling for measurements before pregnancy: the CARDIA study. *Am J Obstet Gynecol*. 2009;201(2):177.e1-177.e9. doi:10.1016/j.ajog.2009.03.031.

31. McClure CK, Catov JM, Ness RB, Schwarz EB. Lactation and maternal subclinical cardiovascular disease among premenopausal women. *Am J Obstet Gynecol*. 2012;207(1):1-15. doi:10.1016/j.ajog.2012.04.030.

32. Natland Fagerhaug T, Forsmo S, Jacobsen GW, Midthjell K, Andersen LF, Ivar Lund Nilsen T. A prospective population-based cohort study of lactation and cardiovascular disease mortality: the HUNT study. *BMC Public Health*. 2013;13(1):1070. doi:10.1186/1471-2458-13-1070.

33. Baker JL, Gamborg M, Heitmann BL, Lissner L, Sørensen TI a, Rasmussen KM. Breastfeeding reduces postpartum weight retention. *Am J Clin Nutr*. 2008;88(6):1543-1551. doi:10.3945/ajcn.2008.26379.

34. Jarlenski MP, Bennett WL, Bleich SN, Barry CL, Stuart E a. Effects of breastfeeding on postpartum weight loss among U.S. women. *Prev Med*. 14AD;69:146-150. doi:10.1016/j.ypmed.2014.09.018.Effects.

35. Britz SE, McDermott KC, Pierce CB, Blomquist JL, Handa VL. Changes in maternal weight 5-10 years after a first delivery. *Womens Health (Lond Engl)*. 2012;8(5):513-519. doi:10.2217/whe.12.35.

36. He X, Zhu M, Hu C, et al. Breast-feeding and postpartum weight retention: a systematic review and meta-analysis. *Public Health Nutr*. 2015;18(18):3308-3316. doi:10.1017/S1368980015000828.

37. Sherwood OD. Relaxin's physiological roles and other diverse actions. *Endocr Rev*. 2004;25(2):205-234. doi:10.1210/er.2003-0013.

38. Bani D. Relaxin as a natural agent for vascular health. *Vasc Health Risk Manag*. 2008;4(3):515-524.

39. Szepietowska B, Gorska M, Szelachowska M. Plasma relaxin concentration is related to beta-cell function and insulin sensitivity in women with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2008;79(3):2007-2009. doi:10.1016/j.diabres.2007.10.017.

Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work; no relationships with companies that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

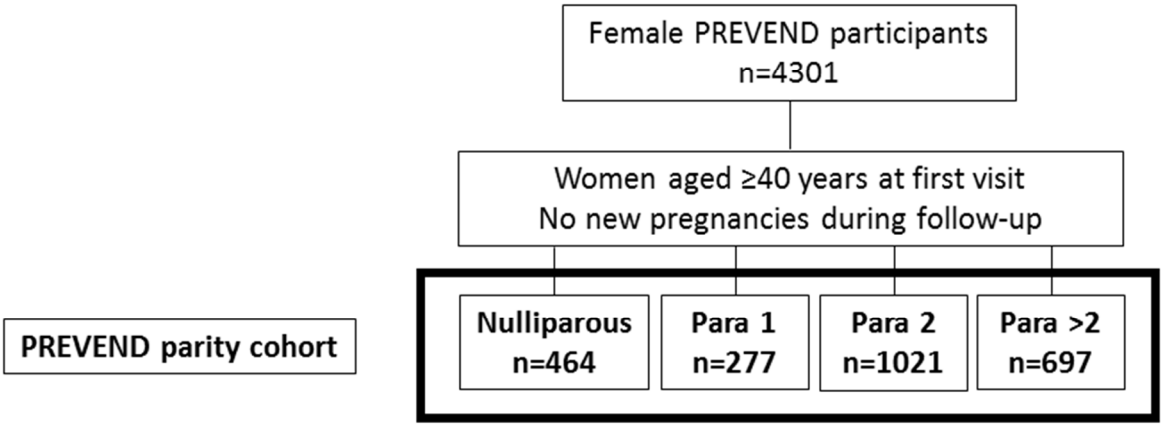
Author's contributions

GAZ, NDP, KJG, RTG, HG and ATL were involved in conception and design of the study. GAZ, KJG and ATL drafted the manuscript. All authors edited the manuscript; all authors read and approved the final manuscript.

Data sharing statement

Data sharing: patient level data and full dataset and technical appendix and statistical code are available from the corresponding author (g.zoet@umcutrecht.nl). Informed consent was not obtained but the presented data are anonymized and the risk of identification is low.

Figure 1: Flowchart



403 **Table 1: at entry table PREVEND stratified for parity**

	No children (N=464) ‡	One child (N=277) ‡	Two children (N=1021) ‡	More than two children (N=697) ‡	*P-value*
General characteristics					
Age (years)	52.2 (10.1)	52.2 (9.0)	52.3 (8.4)	56.9 (9.6)	**<0.001**
Follow-up time (years)	11 (11-12)	11 (11-12)	11 (11-12)	11 (11-12)	0.71
Caucasian (n (%))	445 (96.9%)	264 (96.4%)	995 (98.0%)	663 (95.7%)	**0.04**
Job (n (%))	315 (68.3%)	127 (47.4%)	467 (46.5%)	264 (38.6%)	**<0.001**
Cardiovascular risk profile					
BMI (kg/m²)	24.6 (22.5-27.7)	25.3 (22.5-28.6)	25.8 (23.4-28.8)	26.9 (24.0-30.2)	**<0.001**
SBP (mmHg)	120 (109-138)	121 (110-140)	122 (111-138)	130 (115-146)	**<0.001**
DBP (mmHg)	71 (65-78)	71 (67-77)	71 (66-78)	73 (67-79)	**0.004**
Current smoker (n (%))	139 (30.0%)	105 (37.9%)	326 (31.9%)	195 (28.0%)	**0.02**
Alcohol use (n (%))	203 (44.0%)	137 (49.6%)	503 (49.4%)	378 (54.5%)	**0.007**
Cardiovascular comorbidity (n (%))	11 (2.4%)	11 (4.1%)	24 (2.4%)	24 (3.6%)	0.30
Renal disease requiring dialysis (n (%))	1 (0.2%)	0 (0%)	3 (0.3%)	1 (0.1%)	0.78
Laboratory results					
Glucose (mmol/L)	4.6 (4.3-5.0)	4.7 (4.3-5.1)	4.7 (4.3-5.0)	4.8 (4.4-5.2)	**0.002**
Insulin (mmol/L)	7.0 (4.9-10.7)	7.6 (5.1-11.5)	7.8 (5.6-11.2)	8.5 (6.0-13.4)	**<0.001**
HOMAir	1.4 (1.0-2.4)	1.6 (1.0-2.5)	1.6 (1.1-2.4)	1.8 (1.2-3.0)	**<0.001**
Total Cholesterol (mmol/L)	5.7 (5.0-6.4)	5.7 (5.1-6.5)	5.8 (5.0-6.7)	6.0 (5.3-6.7)	**<0.001**
HDL Cholesterol (mmol/L)	1.5 (1.2-1.8)	1.5 (1.2-1.8)	1.4 (1.2-1.7)	1.4 (1.2-1.7)	**<0.001**
Triglycerides (mmol/L)	1.0 (0.8-1.5)	1.1 (0.9-1.7)	1.1 (0.9-1.6)	1.2 (0.9-1.6)	**<0.001**
Medication use					
Total blood pressure lowering (n (%))	65 (16.4%)	39 (16.5%)	140 (15.6%)	151 (24.4%)	**<0.001**
ACEi/ARB (n (%))	19 (4.7%)	8 (3.3%)	41 (4.5%)	44 (7.0%)	0.07
Glucose lowering (n (%))	7 (1.7%)	2 (0.8%)	12 (1.3%)	12 (1.9%)	0.63
Lipid lowering (n (%))	19 (4.7%)	15 (6.3%)	32 (3.5%)	37 (5.9%)	0.11
Oral contraceptive use (n (%))	79 (18.2%)	50 (19.5%)	198 (21.1%)	90 (14.6%)	**0.01**

404

405 Data are presented as median (25^{th} – 75^{th} percentile) unless otherwise stated. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic
406 blood pressure; ACEi/ARB: angiotensin converting enzyme inhibitor and/or angiotensin receptor blocker; HOMAir: homeostatic model
407 assessment index.

408 ‡ Total number that participated at visit 1 of the study, not all variables were available for each participant at baseline

Figure 2: Development over time of BMI (A), HDL cholesterol (B) and MAP (C), stratified for parity

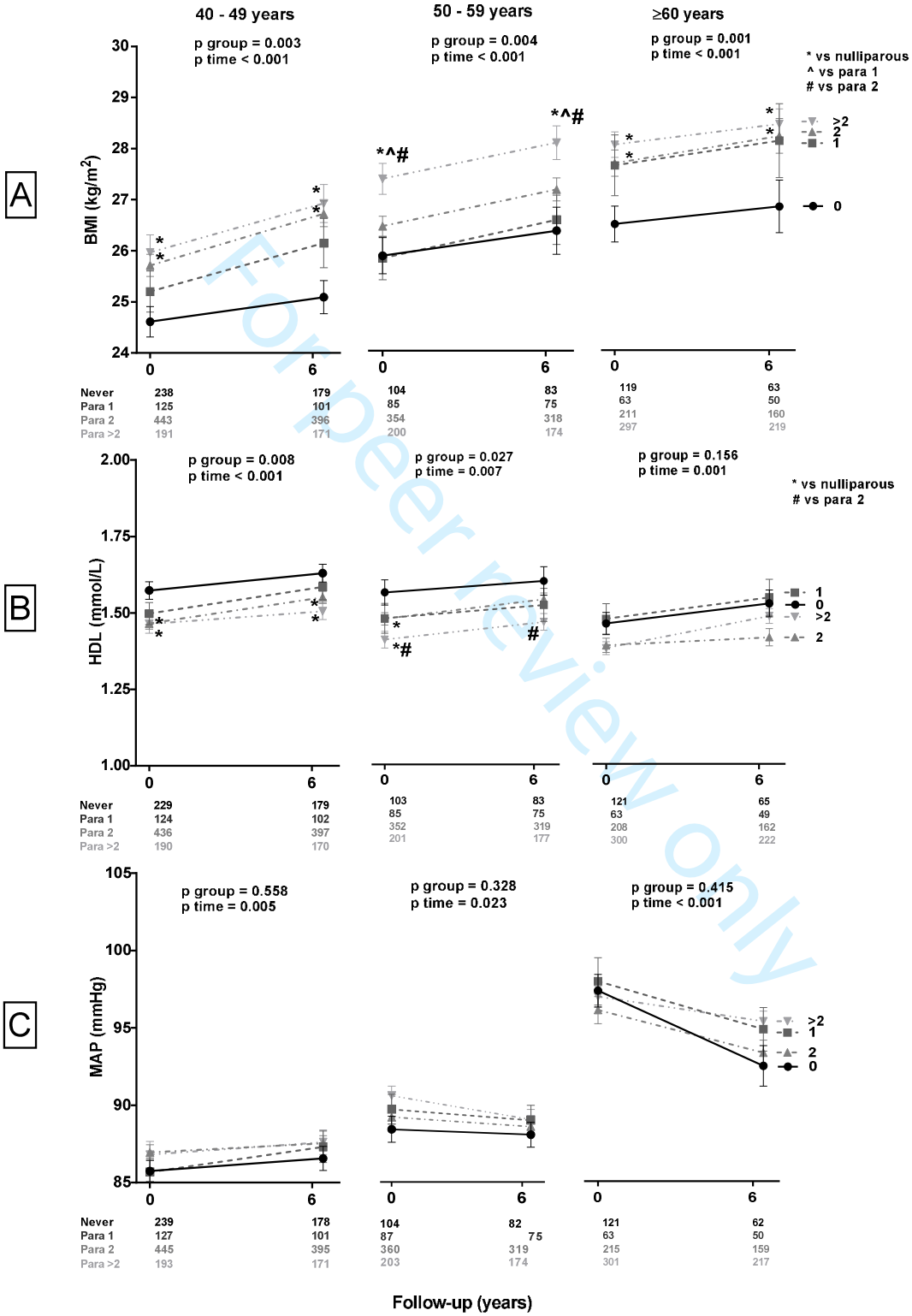
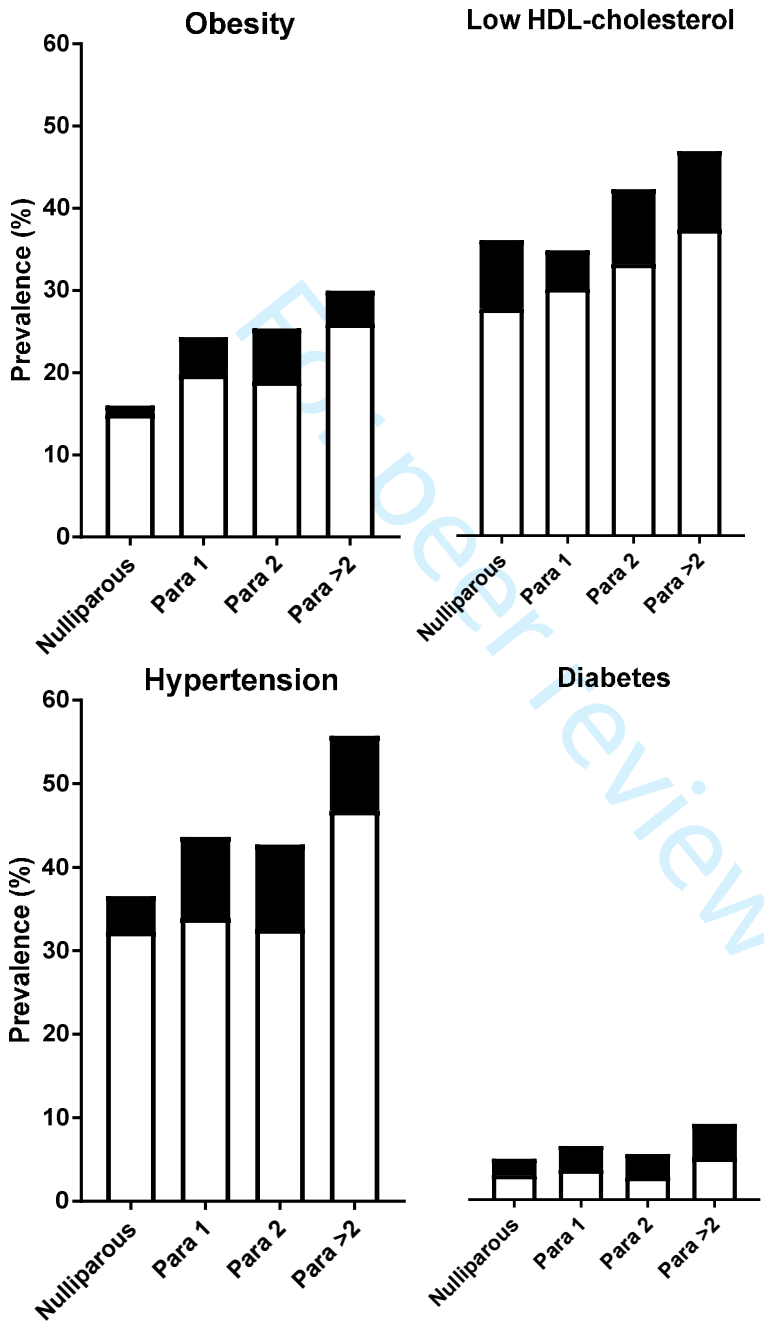


Figure 3: CVD risk factors at entry



Legend: Hypertension = RR ≥140/90 mm/Hg and/or use of antihypertensive medication;
Obesity = BMI ≥30kg/m2; Low HDL cholesterol = HDL cholesterol <1.29 mmol/L;
Diabetes = fasting plasma glucose ≥7.0 mmol/L, self-report of a physician diagnosis and/or use of glucose-lowering medication.
□ = first visit; ■ = follow-up visit

Supplemental table 1: stepwise correction for GEE-analysis

Table 1A: Correction models for GEE-analysis, stratified at age 40 – 50 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & SES**			**Adjusted for age & OCC**			**Adjusted for age & SES & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.003	<0.001	0.667	0.011	0.938	0.694	0.323	0.552	0.685	0.010	0.910	0.627	0.342	0.64	0.628
HDL-cholesterol	0.008	<0.001	0.530	0.003	0.737	0.520	0.047	0.472	0.519	0.006	0.666	0.570	0.080	0.438	0.565
MAP	0.558	0.005	0.815	0.866	<0.001	0.779	0.871	<0.001	0.777	0.874	<0.001	0.875	0.904	<0.001	0.871

Abbreviations: SES, Socio-economic status; OCC, oral contraceptives

Table 1B: Correction models for GEE-analysis, stratified at age 50 – 60 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & SES**			**Adjusted for age & OCC**			**Adjusted for age & SES & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.004	<0.001	0.947	0.006	0.197	0.945	0.009	0.031	0.920	0.011	0.269	0.849	0.017	0.048	0.812
HDL-cholesterol	0.027	0.007	0.184	0.031	0.040	0.171	0.051	0.152	0.151	0.085	0.035	0.055	0.118	0.126	0.045
MAP	0.328	0.023	0.494	0.411	<0.001	0.478	0.522	0.005	0.457	0.637	0.003	0.802	0.669	0.025	0.786

Abbreviations: SES, Socio-economic status; OCC, oral contraceptives

Table 1C: Correction models for GEE-analysis, stratified at age > 60 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & SES**			**Adjusted for age & OCC**			**Adjusted for age & SES & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.001	<0.001	0.662	0.001	0.116	0.662	0.006	0.087	0.663	0.002	0.011	0.625	0.006	0.009	0.626
HDL-cholesterol	0.156	0.001	0.163	0.155	0.114	0.191	0.314	0.142	0.201	0.162	0.102	0.358	0.299	0.132	0.384
MAP	0.415	<0.001	0.348	0.646	<0.001	0.407	0.649	<0.001	0.407	0.667	<0.001	0.508	0.678	<0.001	0.507

Abbreviations: SES, Socio-economic status; OCC, oral contraceptives

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohort reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	5, 6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	5

	#6b	For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6
Bias	#9	Describe any efforts to address potential sources of bias	5, 6, 7
Study size	#10	Explain how the study size was arrived at	5
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	5, 7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6, 7
	#12b	Describe any methods used to examine subgroups and interactions	6, 7
	#12c	Explain how missing data were addressed	6, 7
	#12d	If applicable, explain how loss to follow-up was addressed	6, 7
	#12e	Describe any sensitivity analyses	na
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	5
	#13b	Give reasons for non-participation at each stage	5
	#13c	Consider use of a flow diagram	figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential	7, table 1

		confounders. Give information separately for exposed and unexposed groups if applicable.	
	#14b	Indicate number of participants with missing data for each variable of interest	Figure 2
	#14c	Summarise follow-up time (eg, average and total amount)	6, 8, 9
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	7, 8, 9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7, 8, 9
	#16b	Report category boundaries when continuous variables were categorized	7, 8, 9
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	na
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	na
Key results	#18	Summarise key results with reference to study objectives	9, 10
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11, 12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	See note 1
Generalisability	#21	Discuss the generalisability (external validity) of the study results	See note 2
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

Author notes

1. 10, 11, 12, 13

2. 10, 11, 12, 13

BMJ Open

Association between parity and persistent weight gain at age 40-60 years: a longitudinal prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024279.R1
Article Type:	Research
Date Submitted by the Author:	02-Oct-2018
Complete List of Authors:	Zoet, Gerbrand; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Paauw, Nina; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Groenhof, Katrien; Universitair Medisch Centrum Utrecht, Julius Center for Health Sciences and Primary Care Franx, Arie; University Medical Center Utrecht, Obstetrics & Gynaecology Gansevoort, Ron T.; University Medical Center Groningen, Division of Nephrology Groen, Henk; University Medical Centre Groningen, Department of Epidemiology Van Rijn, Bas; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Lely, Titia; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Cardiovascular medicine, Epidemiology
Keywords:	Pregnancy, Parity, Cardiovascular risk factors, BMI, HDL cholesterol, Hypertension < CARDIOLOGY

Association between parity and persistent weight gain at age 40-60 years: a longitudinal prospective cohort study

Gerbrand A. Zoet, MD[a]; Nina D. Paauw, MD[a]; T. Katrien J. Groenhof, MD[b], Arie Franx, MD PhD[a]; Ron T. Gansevoort, MD, PhD[c]; Henk Groen[d], MD, PhD; Bas B. van Rijn, MD, PhD[a,e], A.Titia Lely, MD, PhD[a].

[a] Wilhelmina Children's Hospital Birth Center, University Medical Center Utrecht, Lundlaan 6, 3508 AB, Utrecht, the Netherlands

[b] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands

[c] Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands

[d] Department of Epidemiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands

[e] Academic Unit of Human Development and Health, University of Southampton, Princess Anne Hospital, Coxford Road, Southampton SO16 5YA, United Kingdom

Corresponding author at: G.A. Zoet, University Medical Center Utrecht, Room KE 02.510.2, Lundlaan 6, PO Box 85090, 3508 AB, Utrecht, The Netherlands. Tel.: +31 88 7557526, e-mail: gzoet@umcutrecht.nl

Short title: Parity and persistent weight gain

Total word count: 3080 (excluding title page, abstract, references, figures and tables)

Abstract

Objectives: Physiological metabolic adaptations occur in the pregnant woman. These may persist postpartum and thereby contribute to an unfavorable cardiovascular disease (CVD) risk profile in parous women. The aim of the current study is to assess time-dependent changes of cardiometabolic health in parous women compared to nulliparous women.

Design and setting: We studied data of 2459 women who participated in the PREVEND study, a population-based prospective longitudinal cohort for assessment of CVD and renal disease in the general population.

Participants: We selected women ≥40 years at the first visit, who reported no new pregnancies during the 4 follow-up visits. All women were categorized in parity groups, and stratified for age.

Outcome measures: We compared BMI, high-density lipoprotein (HDL) cholesterol, blood pressure as continuous measurements and as clinical relevant CVD risk factors among parity groups over the course of six years using generalized estimating equation (GEE) models adjusted for age.

Results: The BMI was significantly higher in women para 2 or more in all age categories: per child, the BMI was 0.6 kg/m^2 higher. corresponding with 1.5–2.0 kg weight gain per child. HDL cholesterol was significantly lower in women para 2 or more aged 40-49 and 50-59 years: per child, the HDL cholesterol was up to 0.09 mmol/L lower. Blood pressure did not differ among parity groups in any of the age categories.

Conclusions: Higher parity is associated with higher BMI, lower HDL cholesterol and a higher prevalence of cardiovascular risk factors, which is constant over time. These findings warrant for prospective research assessing determinants of cardiometabolic health at earlier age to understand the role of pregnancy in the development of cardiovascular disease in women.

Keywords: Pregnancy; parity; cardiovascular risk factors; BMI; HDL cholesterol; hypertension.

Study summary: Strengths and limitations of this study

- This longitudinal cohort comprised a large, well-phenotyped cohort with uniform assessment of all measurements during a median follow-up of 6 years.
- The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects.
- Since parity itself was not assessed in this cohort, we used the number of children as a proxy for the number of childbirths.
- Women para > 2 were older, less often used oral contraceptives and more often used antihypertensive medication which might have resulted in a slightly different metabolic profile.
- Age at first delivery, inter-pregnancy interval and lactation have not been assessed in this cohort and therefore, adjustment of the analyses for these factors was not possible.

Funding: The PREVEND study was supported by Dutch Kidney Foundation (Grant E.033), The Groningen University Medical Center (Beleidsruimte) and Dade Behring, Ausam, Roche, Abbott and Gentian who financed laboratory equipment and reagents for various laboratory determinations. Gerbrand Zoet is supported by the Dutch Heart Foundation (grant number 2013T083). Titia Lely is supported by the ZonMW Clinical Fellowship (40-000703-97-12463).

Introduction

Pregnancy is associated with major alterations to the cardiovascular system and metabolic profile.[1–4] Increases in weight, lipid levels, blood plasma volume and cardiac output are needed to maintain a healthy, physiological pregnancy. Postpartum, this maternal adaptation reverses to its pre-pregnancy state, although several changes, (e.g. increased body weight and hypercholesterolemia) may persist for several months or longer.[5] Possibly, these persisting changes contribute to an increased prevalence of metabolic syndrome (MetS) and an unfavorable cardiometabolic profile in parous women.[6–8] The amount of gestational weight gain (GWG) affects postpartum weight retention.[9] At long-term follow up, excessive gestational weight gain is associated with an increased BMI, up to a 3–4 kg/m^2 21 years after pregnancy.[10–12]

Previous studies assessing the relation between parity and cardiometabolic health showed conflicting results and even the association between parity and obesity is questioned in some studies.[13–16] Long-term effects have only scarcely been investigated and most studies had a follow-up of only 1 to 3 years postpartum.[17,18] A recent large cross-sectional study among Hispanic women in the US demonstrated that multiparity (i.e. more than 4 or more than 6 children) was associated with obesity, low HDL cholesterol and elevated fasting glucose, also after adjustment for sociodemographic and lifestyle factors.[8] In addition, results from the cross-sectional Rotterdam study showed an lower HDL cholesterol and higher total cholesterol and glucose/insulin ratios with higher parity in Caucasian women at 70 years of age.[6]

Studies on the development of cardiovascular risk factors over time and the quantification of this effect per childbirth are conflicting. Some studies suggested a linear association between number of children and an unfavorable cardiometabolic profile, while others stated that only multiparity is associated with an increased cardiovascular disease risk.[6–8,19,20]

Some studies even showed a 'J-shaped' association in which women with two children had the lowest prevalence of coronary heart disease.[6–8]

The aim of the current study was to assess time-dependent changes of cardiometabolic health in parous women, stratified for number of children, as compared with nulliparous controls. This study was performed in a well-defined longitudinal prospective cohort study that primarily assessed development of CVD, albuminuria and renal disease.[21]

Methods

Participants

The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a longitudinal cohort follow-up study for assessment of cardiovascular and renal disease in the general population. Details of this study have previously been published elsewhere.[22,23] In summary, all inhabitants of the city of Groningen, the Netherlands, from 1997 to 1998 at age 28–75 years (n = 85,421) were invited to participate. All that applied, filled out a questionnaire and collected a first-morning void urine sample. Pregnant women and subjects with type 1 diabetes mellitus were excluded. The urinary albumin concentration was assessed in a total of 40,856 (47.8%) responders. A total of 6,000 participants were enrolled out of 7,768 subjects with a urinary albumin concentration ≥ 10mg/L. In addition, 2,592 participants were enrolled out of 3,394 subjects with a urinary albumin concentration < 10mg/L. Thereby, the PREVEND study was enriched by subjects with an elevated albumin excretion.

In total, 4,301 women were enrolled in the PREVEND study (**Figure 1**). For the current analysis, only women aged 40 years or older at the first visit and who reported no new pregnancies during the follow-up visits were included. Women who reported no children were categorized as nulliparous (n = 464; 18.9%). Women who reported one child, two children or more than two children, were categorized as para 1 (n = 277; 11.3%), para 2 (n = 1021; 41.5%) and para > 2 (n = 697; 28.3%). The PREVEND study has

been approved by the medical ethics committee of the University Medical Centre Groningen. Written informed consent was obtained from all participants.

Patient and Public Involvement

No participants were involved with setting out the research question, developing the outcome measures or planning the study design. The results of study results will be disseminated by the newsletter and the study website.

Measurements and visits

Between 1997 and 2012, the screening visits took place every 2-4 years. Participants completed questionnaires and underwent physical examinations. Blood samples and 24-hour urine samples were taken. The questionnaires included questions regarding parity. Participants reported their number of children, which was used as a proxy for the number of childbirths. In addition, education level, current alcohol use and current smoking were assessed in these questionnaires. Details of clinical and laboratory measurements have previously been described elsewhere.[22] Prescription data from pharmacies was used to assess the use of antihypertensive and lipid lowering medication. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg or the use of blood pressure lowering medication. Type 2 diabetes mellitus (T2DM) was defined as a fasting plasma glucose ≥ 7.0 mmol/L, random sample plasma glucose ≥ 11.1 mmol/L, self-reported physician diagnosis of type 2 diabetes mellitus, and/or the use of glucose-lowering medication.[24] Obesity was defined as BMI ≥ 30 kg/m^2.

Data selection for analyses was based on a fixed time interval of six years between the visits.

Statistical analysis

Data was arranged per patient per visit. Continuous variables with a normal distribution are presented as mean ± standard deviation (SD) and analyzed using Student *t*-test or One-Way ANOVA followed by Tukey post-hoc analysis. Continuous variables with a skewed distribution were expressed as median with 25^{th}–75^{th} percentile and analyzed using Mann-Whitney U test or Kruskall Wallis. Categorical variables were analyzed using Pearson Chi square test or Fisher's exact test, where appropriate.

For longitudinal assessment (time factor) of the outcome measures among the different parity groups (group factor), a generalized estimating equations (GEE) analysis was performed, including the interaction term group*visit (interaction factor). All analyses were performed using an autoregressive correlation matrix structure. This assumes a variable correlation between measurements depending on the time between measurements, as was expected in the current analysis. For GEE analyses of continuous dependent variables (BMI, HDL cholesterol and MAP), participants were further stratified in three age categories (40 – 49 years old, 50 – 59 years old, and ≥ 60 years old). In addition, we performed a stepwise correction strategy, correcting for age (model 1), age and education (model 2), age and oral contraceptive use (model 3) and age, education and oral contraceptive use (model 4).

Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad Software Inc. San Diego, CA, USA). In all analyses, a p-value <0.05 was considered statistically significant.

Results

Study population

Table 1 provides an overview of the baseline characteristics of women who were nulliparous, para 1, para 2 and para > 2 at the first PREVEND visit. The mean age was significantly higher in women who were para > 2. The majority of all women were Caucasian. The median follow-up time was 6 years in all groups. The cardiovascular risk profile among the parity groups differed significantly on BMI, blood pressure, smoking and use of alcohol. Unfavorable blood glucose measurements and cholesterol profiles

were related to higher parity. The use of blood pressure lowering medication was higher in women who were para > 2 compared to the other groups, but the use of glucose and lipid lowering medication did not differ among the groups. Women who were para > 2 less often used oral contraceptives compared to women who were nulliparous, para 1 or para 2.

Cardiometabolic profile in relation to parity and age

During the 6-year study period, there was a constant, significant difference in BMI among the parity groups at all age categories (**Figure 2A**). The BMI was higher with every increase of parity at all age categories: per child, the BMI was 0.6kg/m2 higher, equal to 1.5-2.0 kg. In women para > 2, the BMI was 0.7–2.0 kg/m^2 higher compared to the other parity groups. Over time, BMI increased significantly in all age groups (p_{time}<0.001), the change in BMI over time was similar among all parity groups ($p_{interaction}$=0.662–0.947). In a stepwise correction model, correction for age alone and correction for age and oral contraceptive use did not influence the differences in BMI among parity groups at all age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use, differences among parity groups were not statistically significant anymore at age 50-59 years only.

The prevalence of obesity increased with increasing parity at entry ($p_{for\ trend}$<0.001) and at 6 year follow up ($p_{for\ trend}$<0.001; **Figure 3**). At visit one, 15% of the nulliparous women was obese, compared to 26% of the women para >2. After the course of six years, this was increased to 16% of the nulliparous women compared to 30% of the para >2. The increase in prevalence over time was similar among the groups (p=0.450). In a stepwise correction model, correction for age alone and correction for age and oral contraceptive use did not influence the differences in HDL cholesterol among parity groups at all age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use, differences among parity groups were not statistically significant anymore at all age groups.

HDL cholesterol differed among the groups, except for participants aged ≥ 60 years (**Figure 2B**). The HDL cholesterol was lower with every increase of parity, except for participants older than 60 years: per child, the HDL decreased with 0.05 mmol/L. Women para 2 and women para > 2 had significant lower HDL cholesterol at both measurements compared to nulliparous women aged 40–49 years (0.08–0.12 mmol/L) and 50–59 years (0.10–0.17 mmol/L). Over time, HDL cholesterol increased significantly in all age groups (p_{time}=0.001–0.007) and the change in HDL cholesterol over time was similar among all parity groups ($p_{interaction}$=0.163–0.530).

Low HDL cholesterol (<1.29 mmol/L) prevalence differed among the groups at visit one ($p_{for\ trend}$<0.001) and at follow up visit ($p_{for\ trend}$=0.006); low HDL cholesterol was more common when parity increased (**Figure 3**). Low HDL cholesterol prevalence inclined similar in all groups over time (p=0.160).

There were no differences among the parity groups over time in MAP at all ages, although MAP increased in all groups at age 40–49 years and 50–59 years and decreased in all groups at age ≥ 60 years (**Figure 2C**). The change in MAP over time was similar among all parity groups ($p_{interaction}$=0.348–0.815). Prevalence of hypertension increased with parity both at entry visit ($p_{for\ trend}$<0.001) and at follow up ($p_{for\ trend}$<0.001). Hypertension prevalence increased similar in all groups over time by 4–10% (p=0.761; **Figure 3**).

Occurrence of T2DM did not differ among the groups at entry ($p_{for\ trend}$=0.094), although a positive association was found between T2DM prevalence and parity after six years ($p_{for\ trend}$=0.018). The increase in T2DM over time was comparable at all groups (p=0.336). T2DM prevalence was < 10% at all groups at both visits (**Figure 3**).

Discussion

In this longitudinal cohort study, having 2 children or more was associated with a higher BMI in all age categories and with a lower HDL cholesterol, but not with changes in blood pressure. A higher parity was associated with higher prevalence of obesity, low HDL cholesterol and a higher prevalence of hypertension. These associations were constant over time. As analyses were stratified and/or adjusted for age, these results suggest a direct association of parity itself with BMI, HDL cholesterol levels and cardiovascular risk factor prevalence. Other factors attributing to parity, i.e. socio-economic status and oral contraceptive use, might have contributed to these differences.

BMI appears to be one of the most important cardiometabolic risk factors because it has direct effect on cardiovascular disease onset, but also due to its adverse effect on lipid profile and blood pressure[25–28] and therefore the influence of parity on BMI is of great interest. Results from a population-based cohort study among 4699 women suggested that weight or weight changes might be an important mediator in the effect of parity on metabolic syndrome, thus confirming the importance of BMI in regard to cardiometabolic health.[19] In our study, roughly each extra child is associated with 1.5–2.0 kg weight gain. Similar, each extra child is associated with 0.05 mmol/L decrease in HDL cholesterol, which might be the result of the increased BMI.

Specific BMI measures, HDL cholesterol levels and MAP measures differed among the three age groups. Because women from all different ages were seen throughout all screening visits, we expect this to be an effect of age itself, thereby reflecting the growing influence of age on cardiometabolic health with increasing age.

Parallel to these metabolic differences in continuous measurements among the groups, occurrence of several clinical relevant cardiovascular risk factors, such as obesity and hypertension, differed among

the groups as well. This is in line with cross-sectional studies assessing metabolic profile in relation to the number of children.[6–8,19] However, some studies could not confirm the relation between parity and metabolic health, especially after adjustment for covariates such as lifestyle.[14,15,19,29] In the stepwise correction, we found no or minimal influence of age, age and education level or age and oral contraceptive use on our results. Only after full correction for age, education level and oral contraceptive use, the statistical significance among parity groups diminished. Consequently, our findings should be interpreted with caution, as these factors and others, such as lifestyle changes following childbirth, might influence cardiovascular health next to parity itself.[29] Moreover, lifestyle effects of family life and the protective effect of lactation could explain the influence of parity on cardiometabolic health.[30–32]

Another possible explanation behind the mechanism of this relationship between parity and cardiovascular risk factors could be found in the effect of disturbances by pregnancy itself that continue to last postpartum. For example, breastfeeding is associated with a lower risk of cardiovascular risk factors, i.e. T2DM, although the role of breastfeeding remains controversial and a recent meta-analysis showed no significant effect of breastfeeding on postpartum weight retention.[33–36] Other factors involved in the relationship between parity and cardiovascular risk factors might be found in circulation markers. An example might be found in the ovarian peptide hormone relaxin, produced by the corpus luteum or placental throughout pregnancy, has emerged as cardiovascular modulator involved in vasodilatation and inducing angiogenesis.[37,38] Moreover, relaxin was positively associated with insulin sensitivity and lipid profile in women with type 2 diabetes mellitus as well.[39]

Previous cohort studies showed a 'J-shaped' association between parity and coronary heart disease, with lowest prevalence in women with two children, or stated that only multiparity (i.e. more than four or five children) was associated with increased cardiovascular disease risk.[6–8] However, our results

indicate a stepwise effect of parity on cardiometabolic health and having two children or more than two children already affects BMI, HDL cholesterol levels and cardiovascular risk factor prevalence.

Our paper is the first study providing detailed assessment of cardiometabolic health development over time in relation to parity. This longitudinal study comprised a large, well-phenotyped cohort with uniform assessment of all measurements during a median follow-up of 6 years. The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects. However, several limitations need to be discussed. The mean age of women para > 2 was significantly higher than women who were nulliparous, para 1 or para 2. In addition, women para > 2 less often used oral contraceptives and more often used antihypertensive medication. This might result in a slightly different metabolic profile although correction of the GEE-analysis for age and oral contraceptive use did not significantly change the results. Despite extensive phenotyping, age at first delivery, inter-pregnancy interval and lactation have not been assessed in the PREVEND study and therefore, adjustment of the analyses for these factors was not possible. Since parity itself was not assessed in the PREVEND, we used the number of children as a proxy for the number of childbirths. This might lead to inaccurate estimates of parity numbers, as the possibility of surrogacy or twin pregnancies is not taken into account. Additionally, no information was available regarding subfertility and several pregnancy complications, which leads to a lower number of children in these women and might reflect influence the cardiometabolic profile in later life as well. Lastly, pre-pregnancy BMI and gestational weight gain have not been assessed in the PREVEND study either, although their role on postpartum weight retention seemed limited in a recent publication.[9,18]

The PREVEND study was enriched with subjects with an elevated albumin excretion, which mostly results in an unfavorable cardiovascular risk profile compared to the general population. However,

albuminuria did not significantly differ among the groups within our analyses. Although our findings suggest an effect of parity itself on metabolic parameters, it should be noted that causality cannot be determined in our study. Therefore, one could argue that the relationship is reversed, e.g. women with higher BMI or lower HDL cholesterol are more fertile and therefore have more children. Prospective research assessing pre-pregnancy determinants of cardiometabolic health are warranted to further assess the possible causal effect of pregnancy itself.

Conclusion

In this longitudinal cohort study, higher parity is associated with higher BMI and lower HDL cholesterol. This difference among parity groups is constant over time. Furthermore, higher parity is associated with a higher prevalence of cardiovascular risk factors among the parity groups over time. These findings warrant for prospective research assessing determinants of cardiometabolic health at earlier age to understand the role of pregnancy in the development of cardiovascular disease in women.

References

1. Bartels a., O'Donoghue K. Cholesterol in pregnancy: a review of knowns and unknowns. *Obstet Med*. 2011;4(4):147-151. doi:10.1258/om.2011.110003.
2. Tan EK, Tan EL. Alterations in physiology and anatomy during pregnancy. *Best Pract Res Clin Obstet Gynaecol*. 2013;27(6):791-802. doi:10.1016/j.bpobgyn.2013.08.0011.
3. Jebeile H, Mijatovic J, Louie JCY, Prvan T, Brand-Miller JC. A systematic review and metaanalysis of energy intake and weight gain in pregnancy. *Am J Obstet Gynecol*. 2016;214(4):465-483. doi:10.1016/j.ajog.2015.12.049.
4. de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J, Spaanderman ME. Physiologic adaptation of plasma volume during pregnancy: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2016;d(November 2016):177-187. doi:10.1002/uog.17360.
5. Berge L, Armesen E, Forsdahl A. Pregnancy related changes in some cardiovascular risk factors. *Acta Obstet Gynecol Scand*. 1996;75(5):439-442.
6. Humphries KH, Westendorp ICD, Bots ML, et al. Parity and Carotid Artery Atherosclerosis in Elderly Women: The Rotterdam Study. *Stroke*. 2001;32:2259-2264.
7. Lawlor D a., Emberson JR, Ebrahim S, et al. Is the association between parity and coronary heart disease due to biological effects of pregnancy or adverse lifestyle risk factors associated with child-rearing? Findings from the British

women's heart and health study and the British regional heart st. *Circulation*. 2003;107(9):1260-1264. doi:10.1161/01.CIR.0000053441.43495.1A.

8. Vladutiu CJ, Siega-Riz AM, Sotres-Alvarez D, et al. Parity and components of the metabolic syndrome among US hispanic/latina women: Results from the hispanic community health study/study of latinos. *Circ Cardiovasc Qual Outcomes*. 2016;9(2_suppl_1):S62-S69. doi:10.1161/CIRCOUTCOMES.115.002464.

9. Rong K, Yu K, Han X, et al. Pre-pregnancy BMI, gestational weight gain and postpartum weight retention: a meta-analysis of observational studies. *Public Health Nutr*. 2015;18(12):2172-2182. doi:10.1017/S1368980014002523.

10. Amorim AR, Rössner S, Neovius M, Lourenço PM, Linné Y. Does excess pregnancy weight gain constitute a major risk for increasing long-term BMI? *Obesity (Silver Spring)*. 2007;15(5):1278-1286. doi:10.1038/oby.2007.149.

11. Al Mamun a. Associations of excess weight gain during pregnancy with long-term maternal obesity, hypertension and diabetes: Evidence from 21 years postpartum follow-up. *Obes Rev*. 2010;11:51. doi:http://dx.doi.org/10.1111/j.1467-789X.2010.00763-4.x.

12. Fraser a, Tilling K, Macdonald C, Hughes R, Sattar N. Associations of gestational weight gain with maternal body mass index, waist circumference, and blood pressure measured 16 y after pregnancy: the Avon Longitudinal Study of Parents and Children. *Am J Clin Nutr*. 2011;93(6):1-4. doi:10.3945/ajcn.110.008326.1.

13. Steenland K, Lally C, Thun Mi. Parity and COronary Heart Disease among Women in the American Cancer Society CPS II Population. *Epidemiology*. 1996;7:641-643.

14. de Kleijn MJ, van der Schouw YT, van der Graaf Y. Reproductive history and cardiovascular disease risk in postmenopausal women: a review of the literature. *Maturitas*. 1999;33(1):7-36.

15. Chung E, Kim Y, Usen O. Associations Between Parity, Obesity, and Cardiovascular Risk Factors Among Middle-Aged Women. *J Women's Heal*. 2016;25(8):818-825. doi:10.1089/jwh.2015.5581.

16. Robinson WR, Cheng MM, Hoggatt KJ, Stürmer T, Siega-Riz AM. Childbearing is not associated with young women's long-term obesity risk. *Obesity*. 2014;22(4):1126-1132. doi:10.1002/oby.20593.

17. Schmitt NM, Nicholson WK, Schmitt J. The association of pregnancy and the development of obesity - results of a systematic review and meta-analysis on the natural history of postpartum weight retention. *Int J Obes (Lond)*. 2007;31(11):1642-1651. doi:10.1038/sj.ijo.0803655.

18. Nehring I, Schmoll S, Beyerlein A, Hauner H, Von Kries R. Gestational weight gain and long-term postpartum weight retention: A meta-analysis. *Am J Clin Nutr*. 2011;94(5):1225-1231. doi:10.3945/ajcn.111.015289.

19. Cohen A, Pieper CF, Brown AJ, Bastian L a. Number of children and risk of metabolic syndrome in women. *J Womens Health (Larchmt)*. 2006;15(6):763-773. doi:10.1089/jwh.2006.15.763.

20. Ness RB, Harris T, Cobb J, et al. Number of pregnancies and the subsequent risk of cardiovascular disease. *N Engl J Med*. 1993;328:1528-1533.

21. Pinto-Sietsma S-J, Mulder J, Janssen WMT, Hillege HL, Zeeuw D De, De Jong PE. Smoking Is Related to Albuminuria and Abnormal Renal Function in Nondiabetic Persons. *Ann Intern Med*. 2000;(133):585-591.

22. Hillege HL, Janssen WMT, Bak a. a a, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med*. 2001;249(6):519-526. doi:10.1046/j.1365-2796.2001.00833.x.

23. Joosten MM, Gansevoort RT, Mukamal KJ, Harst P Van Der, Geleijnse JM, Feskens EJM. Urinary and plasma magnesium and risk of ischemic heart disease 1 – 3. *October*. 2013;97(13):1299-1306.

doi:10.3945/ajcn.112.054114.INTRODUCTION.

24. Abbasi A, Corpeleijn E, Gansevoort RT, et al. Role of HDL cholesterol and estimates of HDL particle composition in future development of type 2 diabetes in the general population: The PREVEND study. *J Clin Endocrinol Metab*. 2013;98(8):1352-1359. doi:10.1210/jc.2013-1680.

25. Lamon-Fava S, Wilson PWF, Schaefer EJ. Impact of Body Mass Index on Coronary Heart Disease Risk Factors in Men and Women: The Framingham Offspring Study. *Arterioscler Thromb Vasc Biol*. 1996;16:1509-15151.

26. Wilson PWF, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch Intern Med*. 2002;162(16):1867-1872. doi:10.1001/archinte.162.16.1867.

27. Bogers R, Bemelmans W, Hoogenveen R, et al. Association of overweight with increased risk of coronary heart disease partly independent of blood pressure and cholesterol levels. *Arch Intern Med*. 2007;167(16):1720-1728. doi:10.1001/archinte.167.16.1720.

28. Mongraw-Chaffin ML, Peters S a E, Huxley RR, Woodward M. The sex-specific relationship between body mass index and coronary heart disease: A systematic review and meta-analysis of 95 cohorts with 1.2 million participants. *Lancet Diabetes Endocrinol*. 2015;3(6):437-449. doi:10.1002/aur.1474.Replication.

29. Hardy R, Lawlor D a., Black S, Wadsworth MEJ, Kuh D. Number of children and coronary heart disease risk factors in men and women from a British birth cohort. *BJOG An Int J Obstet Gynaecol*. 2007;114(6):721-730. doi:10.1111/j.1471-0528.2007.01324.x.

30. Gunderson EP, Jacobs DR, Chiang V, et al. Childbearing is associated with higher incidence of the metabolic syndrome among women of reproductive age controlling for measurements before pregnancy: the CARDIA study. *Am J Obstet Gynecol*. 2009;201(2):177.e1-177.e9. doi:10.1016/j.ajog.2009.03.031.

31. McClure CK, Catov JM, Ness RB, Schwarz EB. Lactation and maternal subclinical cardiovascular disease among premenopausal women. *Am J Obstet Gynecol*. 2012;207(1):1-15. doi:10.1016/j.ajog.2012.04.030.

32. Natland Fagerhaug T, Forsmo S, Jacobsen GW, Midthjell K, Andersen LF, Ivar Lund Nilsen T. A prospective population-based cohort study of lactation and cardiovascular disease mortality: the HUNT study. *BMC Public Health*. 2013;13(1):1070. doi:10.1186/1471-2458-13-1070.

33. Baker JL, Gamborg M, Heitmann BL, Lissner L, Sørensen TI a, Rasmussen KM. Breastfeeding reduces postpartum weight retention. *Am J Clin Nutr*. 2008;88(6):1543-1551. doi:10.3945/ajcn.2008.26379.

34. Jarlenski MP, Bennett WL, Bleich SN, Barry CL, Stuart E a. Effects of breastfeeding on postpartum weight loss among U.S. women. *Prev Med*. 14AD;69:146-150. doi:10.1016/j.ypmed.2014.09.018.Effects.

35. Britz SE, McDermott KC, Pierce CB, Blomquist JL, Handa VL. Changes in maternal weight 5-10 years after a first delivery. *Womens Health (Lond Engl)*. 2012;8(5):513-519. doi:10.2217/whe.12.35.

36. He X, Zhu M, Hu C, et al. Breast-feeding and postpartum weight retention: a systematic review and meta-analysis. *Public Health Nutr*. 2015;18(18):3308-3316. doi:10.1017/S1368980015000828.

37. Sherwood OD. Relaxin's physiological roles and other diverse actions. *Endocr Rev*. 2004;25(2):205-234. doi:10.1210/er.2003-0013.

38. Bani D. Relaxin as a natural agent for vascular health. *Vasc Health Risk Manag*. 2008;4(3):515-524.

39. Szepietowska B, Gorska M, Szelachowska M. Plasma relaxin concentration is related to beta-cell function and insulin sensitivity in women with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2008;79(3):2007-2009. doi:10.1016/j.diabres.2007.10.017.

Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work; no relationships with companies that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

Author's contributions

GAZ, NDP, KJG, RTG, HG and ATL were involved in conception and design of the study. GAZ, KJG and ATL drafted the manuscript. All authors edited the manuscript; all authors read and approved the final manuscript.

Data sharing statement

Data sharing: patient level data and full dataset and technical appendix and statistical code are available from the corresponding author (gzoet@umcutrecht.nl). Informed consent was not obtained but the presented data are anonymized and the risk of identification is low.

Figure 1: Flowchart

Table 1: at entry table PREVEND stratified for parity

	No children (N=464) ‡	One child (N=277) ‡	Two children (N=1021) ‡	More than two children (N=697) ‡	*P-value*
General characteristics					
Age (years)	52.2 (10.1)	52.2 (9.0)	52.3 (8.4)	56.9 (9.6)	**<0.001**
Follow-up time (years)	11 (11-12)	11 (11-12)	11 (11-12)	11 (11-12)	0.71
Caucasian (n (%))	445 (96.9%)	264 (96.4%)	995 (98.0%)	663 (95.7%)	**0.04**
Job (n (%))	315 (68.3%)	127 (47.4%)	467 (46.5%)	264 (38.6%)	**<0.001**
Cardiovascular risk profile					
BMI (kg/m²)	24.6 (22.5-27.7)	25.3 (22.5-28.6)	25.8 (23.4-28.8)	26.9 (24.0-30.2)	**<0.001**
SBP (mmHg)	120 (109-138)	121 (110-140)	122 (111-138)	130 (115-146)	**<0.001**
DBP (mmHg)	71 (65-78)	71 (67-77)	71 (66-78)	73 (67-79)	**0.004**
Current smoker (n (%))	139 (30.0%)	105 (37.9%)	326 (31.9%)	195 (28.0%)	**0.02**
Alcohol use (n (%))	203 (44.0%)	137 (49.6%)	503 (49.4%)	378 (54.5%)	**0.007**
Cardiovascular comorbidity (n (%))	11 (2.4%)	11 (4.1%)	24 (2.4%)	24 (3.6%)	0.30
Renal disease requiring dialysis (n (%))	1 (0.2%)	0 (0%)	3 (0.3%)	1 (0.1%)	0.78
Laboratory results					
Glucose (mmol/L)	4.6 (4.3-5.0)	4.7 (4.3-5.1)	4.7 (4.3-5.0)	4.8 (4.4-5.2)	**0.002**
Insulin (mmol/L)	7.0 (4.9-10.7)	7.6 (5.1-11.5)	7.8 (5.6-11.2)	8.5 (6.0-13.4)	**<0.001**
HOMAir	1.4 (1.0-2.4)	1.6 (1.0-2.5)	1.6 (1.1-2.4)	1.8 (1.2-3.0)	**<0.001**
Total Cholesterol (mmol/L)	5.7 (5.0-6.4)	5.7 (5.1-6.5)	5.8 (5.0-6.7)	6.0 (5.3-6.7)	**<0.001**
HDL Cholesterol (mmol/L)	1.5 (1.2-1.8)	1.5 (1.2-1.8)	1.4 (1.2-1.7)	1.4 (1.2-1.7)	**<0.001**
Triglycerides (mmol/L)	1.0 (0.8-1.5)	1.1 (0.9-1.7)	1.1 (0.9-1.6)	1.2 (0.9-1.6)	**<0.001**
Medication use					
Total blood pressure lowering (n (%))	65 (16.4%)	39 (16.5%)	140 (15.6%)	151 (24.4%)	**<0.001**
ACEi/ARB (n (%))	19 (4.7%)	8 (3.3%)	41 (4.5%)	44 (7.0%)	0.07
Glucose lowering (n (%))	7 (1.7%)	2 (0.8%)	12 (1.3%)	12 (1.9%)	0.63
Lipid lowering (n (%))	19 (4.7%)	15 (6.3%)	32 (3.5%)	37 (5.9%)	0.11
Oral contraceptive use (n (%))	79 (18.2%)	50 (19.5%)	198 (21.1%)	90 (14.6%)	**0.01**

Data are presented as median (25^{th} – 75^{th} percentile) unless otherwise stated. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; ACEi/ARB: angiotensin converting enzyme inhibitor and/or angiotensin receptor blocker; HOMAir: homeostatic model assessment index.

‡ Total number that participated at visit 1 of the study, not all variables were available for each participant at baseline

Figure 2: Development over time of BMI (A), HDL cholesterol (B) and MAP (C), stratified for parity

Figure 3: Development of CVD risk factors over time

Legend: Hypertension = RR ≥140/90 mm/Hg and/or use of antihypertensive medication;
Obesity = BMI ≥30kg/m2; Low HDL cholesterol = HDL cholesterol <1.29 mmol/L;
Diabetes = fasting plasma glucose ≥7.0 mmol/L, self-report of a physician
diagnosis and/or use of glucose-lowering medication.
□ = first visit; ■ = follow-up visit

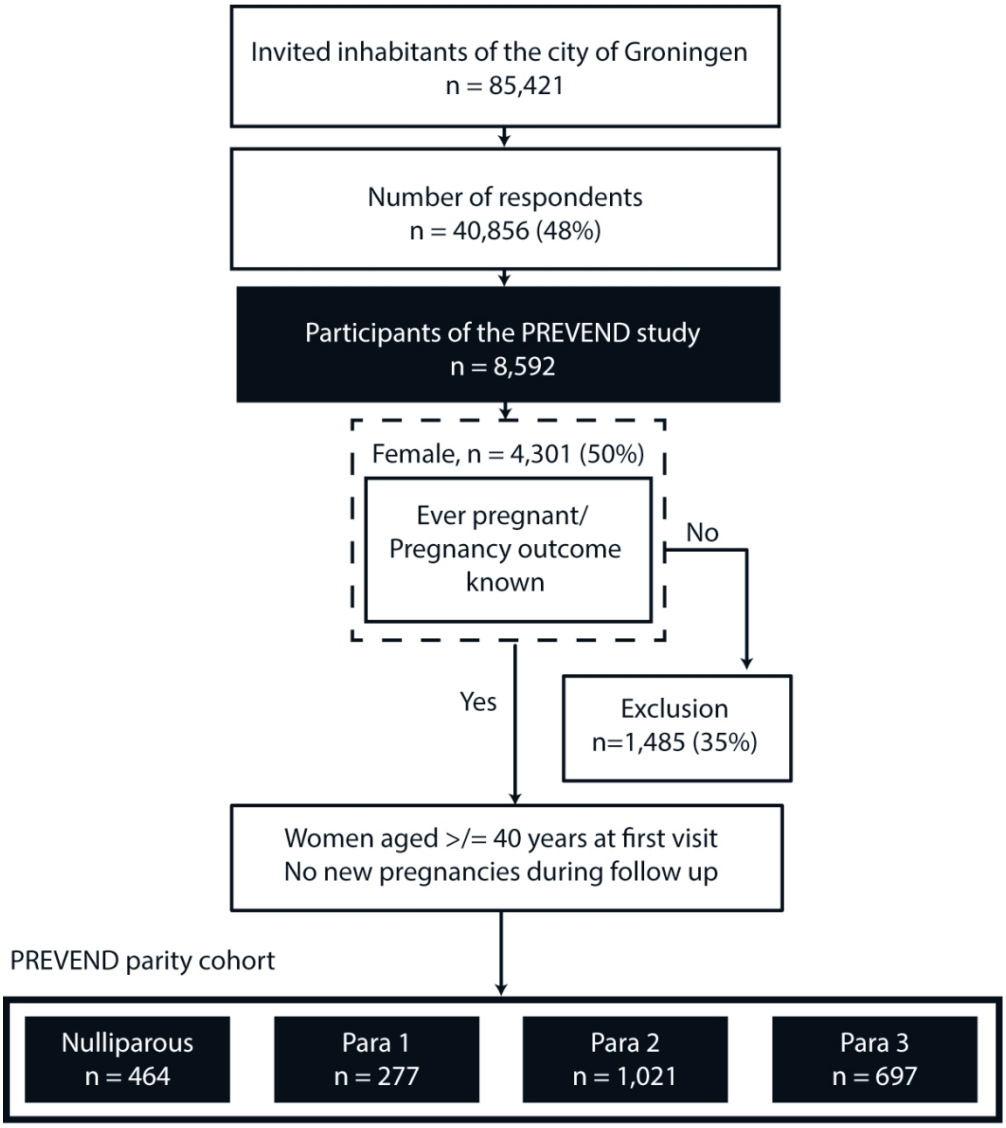


Figure 1: Flowchart

101x117mm (300 x 300 DPI)

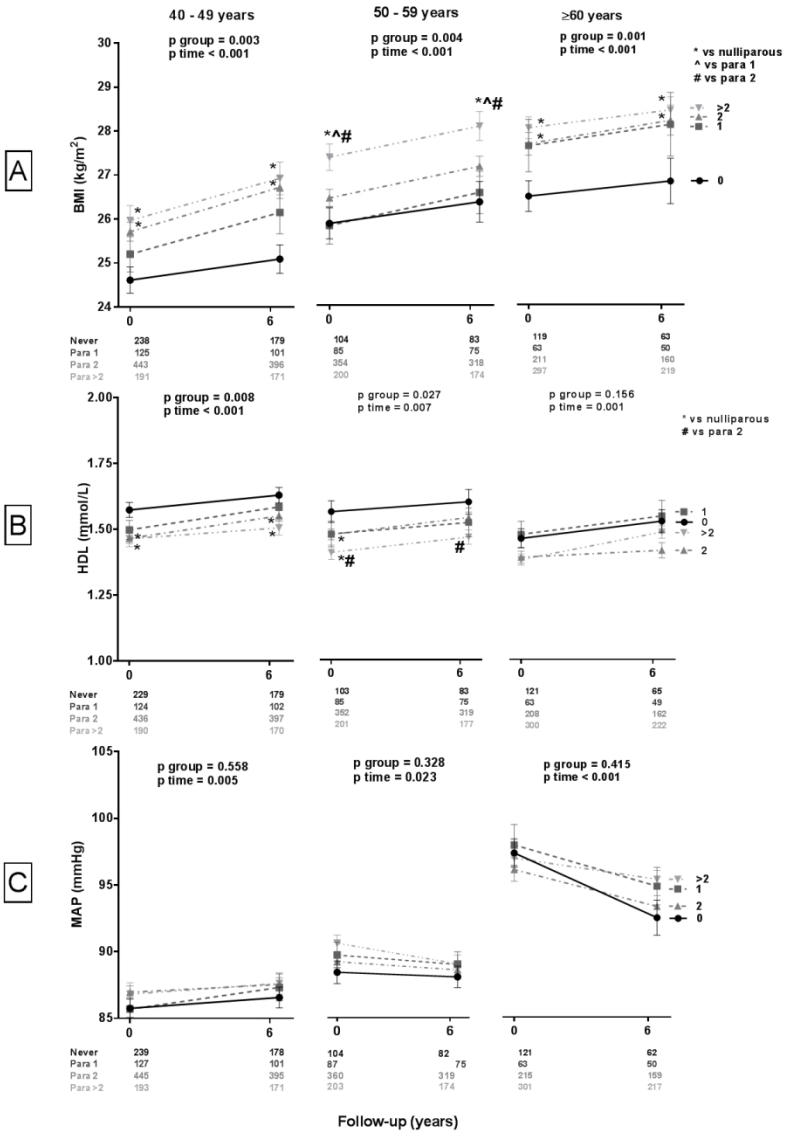


Figure 2: Development over time of BMI (A), HDL cholesterol (B) and MAP (C), stratified for parity

114x160mm (300 x 300 DPI)

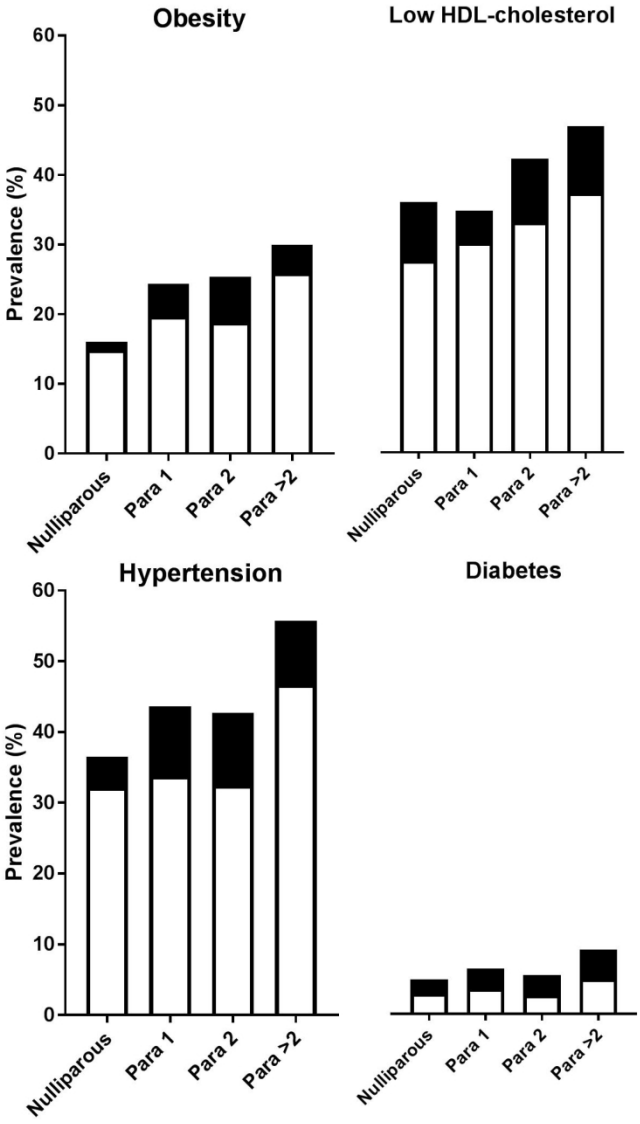


Figure 3: Development of CVD risk factors over time

111x187mm (300 x 300 DPI)

Supplemental table 1: stepwise correction for GEE-analysis

Table 1A: Correction models for GEE-analysis, stratified at age 40 – 50 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & education**			**Adjusted for age & OCC**			**Adjusted for age & education & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.003	<0.001	0.667	0.011	0.938	0.694	0.323	0.552	0.685	0.010	0.910	0.8	0.342	0.64	0.628
HDL-cholesterol	0.008	<0.001	0.530	0.003	0.737	0.520	0.047	0.472	0.519	0.006	0.666	0.5	0.080	0.438	0.565
MAP	0.558	0.005	0.815	0.866	<0.001	0.779	0.871	<0.001	0.777	0.874	<0.001	0.8	0.904	<0.001	0.871

Abbreviation: OCC, oral contraceptives

Table 1B: Correction models for GEE-analysis, stratified at age 50 – 60 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & education**			**Adjusted for age & OCC**			**Adjusted for age & education & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.004	<0.001	0.947	0.006	0.197	0.945	0.009	0.031	0.920	0.011	0.269	0.849	0.017	0.048	0.812
HDL-cholesterol	0.027	0.007	0.184	0.031	0.040	0.171	0.051	0.152	0.151	0.085	0.035	0.055	0.118	0.126	0.045
MAP	0.328	0.023	0.494	0.411	<0.001	0.478	0.522	0.005	0.457	0.637	0.003	0.802	0.669	0.025	0.786

Abbreviation: OCC, oral contraceptives

Table 1C: Correction models for GEE-analysis, stratified at age > 60 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & education**			**Adjusted for age & OCC**			**Adjusted for age & education & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.001	<0.001	0.662	0.001	0.116	0.662	0.006	0.087	0.663	0.002	0.011	0.625	0.006	0.009	0.626
HDL-cholesterol	0.156	0.001	0.163	0.155	0.114	0.191	0.314	0.142	0.201	0.162	0.102	0.358	0.299	0.132	0.384
MAP	0.415	<0.001	0.348	0.646	<0.001	0.407	0.649	<0.001	0.407	0.667	<0.001	0.508	0.678	<0.001	0.507

Abbreviation: OCC, oral contraceptives

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohort reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	5, 6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	5

	#6b	For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6
Bias	#9	Describe any efforts to address potential sources of bias	5, 6, 7
Study size	#10	Explain how the study size was arrived at	5
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	5, 7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6, 7
	#12b	Describe any methods used to examine subgroups and interactions	6, 7
	#12c	Explain how missing data were addressed	6, 7
	#12d	If applicable, explain how loss to follow-up was addressed	6, 7
	#12e	Describe any sensitivity analyses	na
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	5
	#13b	Give reasons for non-participation at each stage	5
	#13c	Consider use of a flow diagram	figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential	7, table 1

		confounders. Give information separately for exposed and unexposed groups if applicable.	
	#14b	Indicate number of participants with missing data for each variable of interest	Figure 2
	#14c	Summarise follow-up time (eg, average and total amount)	6, 8, 9
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	7, 8, 9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7, 8, 9
	#16b	Report category boundaries when continuous variables were categorized	7, 8, 9
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	na
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	na
Key results	#18	Summarise key results with reference to study objectives	9, 10
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11, 12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	See note 1
Generalisability	#21	Discuss the generalisability (external validity) of the study results	See note 2
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

Author notes

1. 10, 11, 12, 13

2. 10, 11, 12, 13

 This checklist was completed on 18. May 2018 using http://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

Association between parity and persistent weight gain at age 40-60 years: a longitudinal prospective cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-024279.R2
Article Type:	Research
Date Submitted by the Author:	17-Feb-2019
Complete List of Authors:	Zoet, Gerbrand; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Paauw, Nina; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Groenhof, Katrien; Universitair Medisch Centrum Utrecht, Julius Center for Health Sciences and Primary Care Franx, Arie; University Medical Center Utrecht, Obstetrics & Gynaecology Gansevoort, Ron T.; University Medical Center Groningen, Division of Nephrology Groen, Henk; University Medical Centre Groningen, Department of Epidemiology Van Rijn, Bas; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology Lely, Titia; Universitair Medisch Centrum Utrecht, Obstetrics & Gynaecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Cardiovascular medicine, Epidemiology
Keywords:	Pregnancy, Parity, Cardiovascular risk factors, BMI, HDL cholesterol, Hypertension < CARDIOLOGY

Association between parity and persistent weight gain at age 40-60 years: a longitudinal prospective cohort study

Gerbrand A. Zoet, MD[a]; Nina D. Paauw, MD[a]; T. Katrien J. Groenhof, MD[b], Arie Franx, MD PhD[a]; Ron T. Gansevoort, MD, PhD[c]; Henk Groen[d], MD, PhD; Bas B. van Rijn*, MD, PhD[a,e,f], A.Titia Lely*, MD, PhD[a].

* Both authors contributed equally

[a] Wilhelmina Children's Hospital Birth Center, University Medical Center Utrecht, Lundlaan 6, 3508 AB, Utrecht, the Netherlands

[b] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands

[c] Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands

[d] Department of Epidemiology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, Groningen, the Netherlands

[e] Academic Unit of Human Development and Health, University of Southampton, Princess Anne Hospital, Coxford Road, Southampton SO16 5YA, United Kingdom

[f] Department of Obstetrics and Prenatal Medicine, Erasmus University Medical Center, Wytemaweg 80, 3015CN, Rotterdam, the Netherlands

Corresponding author at: G.A. Zoet, University Medical Center Utrecht, Room KE 02.510.2, Lundlaan 6, PO Box 85090, 3508 AB, Utrecht, The Netherlands. Tel.: +31 88 7557526, e-mail: gzoet@umcutrecht.nl

Short title: Parity and persistent weight gain

Total word count: 3080 (excluding title page, abstract, references, figures and tables)

Abstract

Objectives: Physiological metabolic adaptations occur in the pregnant woman. These may persist postpartum and thereby contribute to an unfavorable cardiovascular disease (CVD) risk profile in parous women. The aim of the current study is to assess time-dependent changes of cardiometabolic health in parous women compared to nulliparous women.

Design and setting: We studied data of 2459 women who participated in the PREVEND study, a population-based prospective longitudinal cohort for assessment of CVD and renal disease in the general population.

Participants: We selected women ≥40 years at the first visit, who reported no new pregnancies during the 4 follow-up visits. All women were categorized in parity groups, and stratified for age.

Outcome measures: We compared BMI, high-density lipoprotein (HDL) cholesterol, blood pressure as continuous measurements and as clinical relevant CVD risk factors among parity groups over the course of six years using generalized estimating equation (GEE) models adjusted for age.

Results: The BMI was significantly higher in women para 2 or more in all age categories: per child, the BMI was 0.6 kg/m^2 higher. corresponding with 1.5–2.0 kg weight gain per child. HDL cholesterol was significantly lower in women para 2 or more aged 40-49 and 50-59 years: per child, the HDL cholesterol was up to 0.09 mmol/L lower. Blood pressure did not differ among parity groups in any of the age categories.

Conclusions: Higher parity is associated with higher BMI, lower HDL cholesterol and a higher prevalence of cardiovascular risk factors, which is constant over time. These findings warrant for prospective research assessing determinants of cardiometabolic health at earlier age to understand the role of pregnancy in the development of cardiovascular disease in women.

Keywords: Pregnancy; parity; cardiovascular risk factors; BMI; HDL cholesterol; hypertension.

Study summary: Strengths and limitations of this study

- This longitudinal cohort comprised a large, well-phenotyped cohort with uniform assessment of all measurements during a median follow-up of 6 years.
- The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects.
- Since parity itself was not assessed in this cohort, we used the number of children as a proxy for the number of childbirths.
- Women para > 2 were older, less often used oral contraceptives and more often used antihypertensive medication which might have resulted in a slightly different metabolic profile.
- Age at first delivery, inter-pregnancy interval and lactation have not been assessed in this cohort and therefore, adjustment of the analyses for these factors was not possible.

Funding: The PREVEND study was supported by Dutch Kidney Foundation (Grant E.033), The Groningen University Medical Center (Beleidsruimte) and Dade Behring, Ausam, Roche, Abbott and Gentian who financed laboratory equipment and reagents for various laboratory determinations. Gerbrand Zoet is supported by the Dutch Heart Foundation (grant number 2013T083). Titia Lely is supported by the ZonMW Clinical Fellowship (40-000703-97-12463).

Introduction

Pregnancy is associated with major alterations to the cardiovascular system and metabolic profile.[1–4] Increases in weight, lipid levels, blood plasma volume and cardiac output are needed to maintain a healthy, physiological pregnancy. Postpartum, this maternal adaptation reverses to its pre-pregnancy state, although several changes, (e.g. increased body weight and hypercholesterolemia) may persist for several months or longer.[5] Possibly, these persisting changes contribute to an increased prevalence of metabolic syndrome (MetS) and an unfavorable cardiometabolic profile in parous women.[6–8] The amount of gestational weight gain (GWG) affects postpartum weight retention.[9] At long-term follow up, excessive gestational weight gain is associated with an increased BMI, up to a 3–4 kg/m^2 21 years after pregnancy.[10–12]

Previous studies assessing the relation between parity and cardiometabolic health showed conflicting results and even the association between parity and obesity is questioned in some studies.[13–16] Long-term effects have only scarcely been investigated and most studies had a follow-up of only 1 to 3 years postpartum.[17,18] A recent large cross-sectional study among Hispanic women in the US demonstrated that multiparity (i.e. more than 4 or more than 6 children) was associated with obesity, low HDL cholesterol and elevated fasting glucose, also after adjustment for sociodemographic and lifestyle factors.[8] In addition, results from the cross-sectional Rotterdam study showed an lower HDL cholesterol and higher total cholesterol and glucose/insulin ratios with higher parity in Caucasian women at 70 years of age.[6]

Studies on the development of cardiovascular risk factors over time and the quantification of this effect per childbirth are conflicting. Some studies suggested a linear association between number of children and an unfavorable cardiometabolic profile, while others stated that only multiparity is associated with an increased cardiovascular disease risk.[6–8,19,20]

Some studies even showed a 'J-shaped' association in which women with two children had the lowest prevalence of coronary heart disease.[6–8]

The aim of the current study was to assess time-dependent changes of cardiometabolic health in parous women, stratified for number of children, as compared with nulliparous controls. This study was performed in a well-defined longitudinal prospective cohort study that primarily assessed development of CVD, albuminuria and renal disease.[21]

Methods

Participants

The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a longitudinal cohort follow-up study for assessment of cardiovascular and renal disease in the general population. Details of this study have previously been published elsewhere.[22,23] In summary, all inhabitants of the city of Groningen, the Netherlands, from 1997 to 1998 at age 28–75 years (n = 85,421) were invited to participate. All that applied, filled out a questionnaire and collected a first-morning void urine sample. Pregnant women and subjects with type 1 diabetes mellitus were excluded. The urinary albumin concentration was assessed in a total of 40,856 (47.8%) responders. A total of 6,000 participants were enrolled out of 7,768 subjects with a urinary albumin concentration ≥ 10mg/L. In addition, 2,592 participants were enrolled out of 3,394 subjects with a urinary albumin concentration < 10mg/L. Thereby, the PREVEND study was enriched by subjects with an elevated albumin excretion.

In total, 4,301 women were enrolled in the PREVEND study (**Figure 1**). For the current analysis, only women aged 40 years or older at the first visit and who reported no new pregnancies during the follow-up visits were included. Women who reported no children were categorized as nulliparous (n = 464; 18.9%). Women who reported one child, two children or more than two children, were categorized as para 1 (n = 277; 11.3%), para 2 (n = 1021; 41.5%) and para > 2 (n = 697; 28.3%). The PREVEND study has

been approved by the medical ethics committee of the University Medical Centre Groningen. Written informed consent was obtained from all participants.

Patient and Public Involvement

No participants were involved with setting out the research question, developing the outcome measures or planning the study design. The results of study results will be disseminated by the newsletter and the study website.

Measurements and visits

Between 1997 and 2012, the screening visits took place every 2-4 years. Participants completed questionnaires and underwent physical examinations. Blood samples and 24-hour urine samples were taken. The questionnaires included questions regarding parity. Participants reported their number of children, which was used as a proxy for the number of childbirths. In addition, education level, current alcohol use and current smoking were assessed in these questionnaires. Details of clinical and laboratory measurements have previously been described elsewhere.[22] Prescription data from pharmacies was used to assess the use of antihypertensive and lipid lowering medication. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg or the use of blood pressure lowering medication. Type 2 diabetes mellitus (T2DM) was defined as a fasting plasma glucose ≥ 7.0 mmol/L, random sample plasma glucose ≥ 11.1 mmol/L, self-reported physician diagnosis of type 2 diabetes mellitus, and/or the use of glucose-lowering medication.[24] Obesity was defined as BMI ≥ 30 kg/m^2.

Data selection for analyses was based on a fixed time interval of six years between the visits.

Statistical analysis

Data was arranged per patient per visit. Continuous variables with a normal distribution are presented as mean ± standard deviation (SD) and analyzed using Student *t*-test or One-Way ANOVA followed by Tukey post-hoc analysis. Continuous variables with a skewed distribution were expressed as median with 25^{th}–75^{th} percentile and analyzed using Mann-Whitney U test or Kruskall Wallis. Categorical variables were analyzed using Pearson Chi square test or Fisher's exact test, where appropriate.

For longitudinal assessment (time factor) of the outcome measures among the different parity groups (group factor), a generalized estimating equations (GEE) analysis was performed, including the interaction term group*visit (interaction factor). All analyses were performed using an autoregressive correlation matrix structure. This assumes a variable correlation between measurements depending on the time between measurements, as was expected in the current analysis. For GEE analyses of continuous dependent variables (BMI, HDL cholesterol and MAP), participants were further stratified in three age categories (40 – 49 years old, 50 – 59 years old, and ≥ 60 years old). In addition, we performed a stepwise correction strategy, correcting for age (model 1), age and education (model 2), age and oral contraceptive use (model 3) and age, education and oral contraceptive use (model 4).

Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad Software Inc. San Diego, CA, USA). In all analyses, a p-value <0.05 was considered statistically significant.

Results

Study population

Table 1 provides an overview of the baseline characteristics of women who were nulliparous, para 1, para 2 and para > 2 at the first PREVEND visit. The mean age was significantly higher in women who were para > 2. The majority of all women were Caucasian. The median follow-up time was 6 years in all groups. The cardiovascular risk profile among the parity groups differed significantly on BMI, blood pressure, smoking and use of alcohol. Unfavorable blood glucose measurements and cholesterol profiles

were related to higher parity. The use of blood pressure lowering medication was higher in women who were para > 2 compared to the other groups, but the use of glucose and lipid lowering medication did not differ among the groups. Women who were para > 2 less often used oral contraceptives compared to women who were nulliparous, para 1 or para 2.

Cardiometabolic profile in relation to parity and age

During the 6-year study period, there was a constant, significant difference in BMI among the parity groups at all age categories (**Figure 2A**). The BMI was higher with every increase of parity at all age categories: per child, the BMI was 0.6kg/m2 higher, equal to 1.5-2.0 kg. In women para > 2, the BMI was 0.7–2.0 kg/m^2 higher compared to the other parity groups. Over time, BMI increased significantly in all age groups (p_{time}<0.001), the change in BMI over time was similar among all parity groups ($p_{interaction}$=0.662–0.947). In a stepwise correction model, correction for age alone and correction for age and oral contraceptive use did not influence the differences in BMI among parity groups at all age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use, differences among parity groups were statistically significant at age 50-59 and >60 years only.

The prevalence of obesity increased with increasing parity at entry ($p_{for\ trend}$<0.001) and at 6 year follow up ($p_{for\ trend}$<0.001; **Figure 3**). At visit one, 15% of the nulliparous women was obese, compared to 26% of the women para >2. After the course of six years, this was increased to 16% of the nulliparous women compared to 30% of the para >2. The increase in prevalence over time was similar among the groups (p=0.450). In a stepwise correction model, correction for age alone and correction for age and oral contraceptive use did not influence the differences in HDL cholesterol among parity groups at all age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use, differences among parity groups were not statistically significant anymore at all age groups.

HDL cholesterol differed among the groups, except for participants aged ≥ 60 years (**Figure 2B**). The HDL cholesterol was lower with every increase of parity, except for participants older than 60 years: per child, the HDL decreased with 0.05 mmol/L. Women para 2 and women para > 2 had significant lower HDL cholesterol at both measurements compared to nulliparous women aged 40–49 years (0.08–0.12 mmol/L) and 50–59 years (0.10–0.17 mmol/L). Over time, HDL cholesterol increased significantly in all age groups (p_{time}=0.001–0.007) and the change in HDL cholesterol over time was similar among all parity groups ($p_{interaction}$=0.163–0.530).

Low HDL cholesterol (<1.29 mmol/L) prevalence differed among the groups at visit one ($p_{for\ trend}$<0.001) and at follow up visit ($p_{for\ trend}$=0.006); low HDL cholesterol was more common when parity increased (**Figure 3**). Low HDL cholesterol prevalence inclined similar in all groups over time (p=0.160).

There were no differences among the parity groups over time in MAP at all ages, although MAP increased in all groups at age 40–49 years and 50–59 years and decreased in all groups at age ≥ 60 years (**Figure 2C**). The change in MAP over time was similar among all parity groups ($p_{interaction}$=0.348–0.815). Prevalence of hypertension increased with parity both at entry visit ($p_{for\ trend}$<0.001) and at follow up ($p_{for\ trend}$<0.001). Hypertension prevalence increased similar in all groups over time by 4–10% (p=0.761; **Figure 3**).

Occurrence of T2DM did not differ among the groups at entry ($p_{for\ trend}$=0.094), although a positive association was found between T2DM prevalence and parity after six years ($p_{for\ trend}$=0.018). The increase in T2DM over time was comparable at all groups (p=0.336). T2DM prevalence was < 10% at all groups at both visits (**Figure 3**).

Discussion

In this longitudinal cohort study, having 2 children or more was associated with a higher BMI in all age categories and with a lower HDL cholesterol, but not with changes in blood pressure. A higher parity was associated with higher prevalence of obesity, low HDL cholesterol and a higher prevalence of hypertension. These associations were constant over time. As analyses were stratified and/or adjusted for age, these results suggest a direct association of parity itself with BMI, HDL cholesterol levels and cardiovascular risk factor prevalence. Other factors attributing to parity, i.e. education and oral contraceptive use, might have contributed to these differences and therefore, our results should be interpreted with caution.

Since BMI appears to be one of the most important cardiometabolic risk factors, the influence of parity on BMI is of great interest. This strong effect of BMI is not only due to the direct effect on cardiovascular disease onset, but also due to its adverse effect on lipid profile and blood pressure.[25–28] Results from a population-based cohort study among 4699 women suggested that weight or weight chances might be an important mediator in the effect of parity on metabolic syndrome, thus confirming the importance of BMI in regard to cardiometabolic health.[19] In our study, roughly each extra child is associated with 1.5–2.0 kg weight gain. Similar, each extra child is associated with 0.05 mmol/L decrease in HDL cholesterol, which might be the result of the increased BMI.

Specific BMI measures, HDL cholesterol levels and MAP measures differed among the three age groups. Because women from all different ages were seen throughout all screening visits, we expect this to be an effect of age itself, thereby reflecting the growing influence of age on cardiometabolic health with increasing age.

Parallel to these metabolic differences in continuous measurements among the groups, occurrence of several clinical relevant cardiovascular risk factors, such as obesity and hypertension, differed among the groups as well. This is in line with cross-sectional studies assessing metabolic profile in relation to the number of children.[6–8,19] However, some studies could not confirm the relation between parity and metabolic health, especially after adjustment for covariates such as lifestyle.[14,15,19,29] In the stepwise correction, we found no or minimal influence of age, age and education level or age and oral contraceptive use on our results. Only after full correction for age, education level and oral contraceptive use, the statistical significance among parity groups diminished. Consequently, our findings should be interpreted with caution, as these factors and others, such as lifestyle changes following childbirth, might influence cardiovascular health next to parity itself.[29] Moreover, lifestyle effects of family life and the protective effect of lactation could explain the influence of parity on cardiometabolic health.[30–32]

Another possible explanation behind the mechanism of this relationship between parity and cardiovascular risk factors could be found in the effect of disturbances by pregnancy itself that continue to last postpartum. For example, breastfeeding is associated with a lower risk of cardiovascular risk factors, i.e. T2DM, although the role of breastfeeding remains controversial and a recent meta-analysis showed no significant effect of breastfeeding on postpartum weight retention.[33–36] Other factors involved in the relationship between parity and cardiovascular risk factors might be found in circulation markers. An example might be found in the ovarian peptide hormone relaxin, produced by the corpus luteum or placental throughout pregnancy, has emerged as cardiovascular modulator involved in vasodilatation and inducing angiogenesis.[37,38] Moreover, relaxin was positively associated with insulin sensitivity and lipid profile in women with type 2 diabetes mellitus as well.[39]

Previous cohort studies showed a 'J-shaped' association between parity and coronary heart disease, with lowest prevalence in women with two children, or stated that only multiparity (i.e. more than four or five children) was associated with increased cardiovascular disease risk.[6–8] However, our results indicate a stepwise effect of parity on cardiometabolic health and having two children or more than two children already affects BMI, HDL cholesterol levels and cardiovascular risk factor prevalence.

Our paper is the first study providing detailed assessment of cardiometabolic health development over time in relation to parity. This longitudinal study comprised a large, well-phenotyped cohort with uniform assessment of all measurements during a median follow-up of 6 years. The GEE analysis which was performed, allowed us to assess differences among groups over time, focusing on group effects. However, several limitations need to be discussed. The mean age of women para > 2 was significantly higher than women who were nulliparous, para 1 or para 2. In addition, women para > 2 less often used oral contraceptives and more often used antihypertensive medication. This might result in a slightly different metabolic profile although correction of the GEE-analysis for age and oral contraceptive use did not significantly change the results. Despite extensive phenotyping, age at first delivery, inter-pregnancy interval and lactation have not been assessed in the PREVEND study and therefore, adjustment of the analyses for these factors was not possible. Since parity itself was not assessed in the PREVEND, we used the number of children as a proxy for the number of childbirths. This might lead to inaccurate estimates of parity numbers, as the possibility of surrogacy or twin pregnancies is not taken into account. Additionally, no information was available regarding subfertility and several pregnancy complications, which leads to a lower number of children in these women and might reflect influence the cardiometabolic profile in later life as well. More extensive information regarding socio-economic status was not measured as well, thereby it was only possible to correct for education but not for other socio-economic factors. Lastly, pre-pregnancy BMI and gestational weight gain have not been assessed

in the PREVEND study either, although their role on postpartum weight retention seemed limited in a recent publication.[9,18]

The PREVEND study was enriched with subjects with an elevated albumin excretion, which mostly results in an unfavorable cardiovascular risk profile compared to the general population. However, albuminuria did not significantly differ among the groups within our analyses. Although our findings suggest an effect of parity itself on metabolic parameters, it should be noted that causality cannot be determined in our study. Therefore, one could argue that the relationship is reversed, e.g. women with higher BMI or lower HDL cholesterol are more fertile and therefore have more children. Prospective research assessing pre-pregnancy determinants of cardiometabolic health are warranted to further assess the possible causal effect of pregnancy itself.

Conclusion

In this longitudinal cohort study, higher parity is associated with higher BMI and lower HDL cholesterol. This difference among parity groups is constant over time. Furthermore, higher parity is associated with a higher prevalence of cardiovascular risk factors among the parity groups over time. These findings warrant for prospective research assessing determinants of cardiometabolic health at earlier age to understand the role of pregnancy and the influence of lifestyle factors in the development of cardiovascular disease in women.

References

1. Bartels a., O'Donoghue K. Cholesterol in pregnancy: a review of knowns and unknowns. *Obstet Med*. 2011;4(4):147-151. doi:10.1258/om.2011.110003.
2. Tan EK, Tan EL. Alterations in physiology and anatomy during pregnancy. *Best Pract Res Clin Obstet Gynaecol*. 2013;27(6):791-802. doi:10.1016/j.bpobgyn.2013.08.0011.
3. Jebeile H, Mijatovic J, Louie JCY, Prvan T, Brand-Miller JC. A systematic review and metaanalysis of energy intake and

weight gain in pregnancy. *Am J Obstet Gynecol*. 2016;214(4):465-483. doi:10.1016/j.ajog.2015.12.049.

4. de Haas S, Ghossein-Doha C, van Kuijk SM, van Drongelen J, Spaanderman ME. Physiologic adaptation of plasma volume during pregnancy: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2016;d(November 2016):177-187. doi:10.1002/uog.17360.

5. Berge L, Armesen E, Forsdahl A. Pregnancy related changes in some cardiovascular risk factors. *Acta Obstet Gynecol Scand*. 1996;75(5):439-442.

6. Humphries KH, Westendorp ICD, Bots ML, et al. Parity and Carotid Artery Atherosclerosis in Elderly Women: The Rotterdam Study. *Stroke*. 2001;32:2259-2264.

7. Lawlor D a., Emberson JR, Ebrahim S, et al. Is the association between parity and coronary heart disease due to biological effects of pregnancy or adverse lifestyle risk factors associated with child-rearing? Findings from the British women's heart and health study and the British regional heart st. *Circulation*. 2003;107(9):1260-1264. doi:10.1161/01.CIR.0000053441.43495.1A.

8. Vladutiu CJ, Siega-Riz AM, Sotres-Alvarez D, et al. Parity and components of the metabolic syndrome among US hispanic/latina women: Results from the hispanic community health study/study of latinos. *Circ Cardiovasc Qual Outcomes*. 2016;9(2_suppl_1):S62-S69. doi:10.1161/CIRCOUTCOMES.115.002464.

9. Rong K, Yu K, Han X, et al. Pre-pregnancy BMI, gestational weight gain and postpartum weight retention: a meta-analysis of observational studies. *Public Health Nutr*. 2015;18(12):2172-2182. doi:10.1017/S1368980014002523.

10. Amorim AR, Rössner S, Neovius M, Lourenço PM, Linné Y. Does excess pregnancy weight gain constitute a major risk for increasing long-term BMI? *Obesity (Silver Spring)*. 2007;15(5):1278-1286. doi:10.1038/oby.2007.149.

11. Al Mamun a. Associations of excess weight gain during pregnancy with long-term maternal obesity, hypertension and diabetes: Evidence from 21 years postpartum follow-up. *Obes Rev*. 2010;11:51. doi:http://dx.doi.org/10.1111/j.1467-789X.2010.00763-4.x.

12. Fraser a, Tilling K, Macdonald C, Hughes R, Sattar N. Associations of gestational weight gain with maternal body mass index, waist circumference, and blood pressure measured 16 y after pregnancy: the Avon Longitudinal Study of Parents and Children. *Am J Clin Nutr*. 2011;93(6):1-4. doi:10.3945/ajcn.110.008326.1.

13. Steenland K, Lally C, Thun Mi. Parity and COronary Heart Disease among Women in the American Cancer Society CPS II Population. *Epidemiology*. 1996;7:641-643.

14. de Kleijn MJ, van der Schouw YT, van der Graaf Y. Reproductive history and cardiovascular disease risk in postmenopausal women: a review of the literature. *Maturitas*. 1999;33(1):7-36.

15. Chung E, Kim Y, Usen O. Associations Between Parity, Obesity, and Cardiovascular Risk Factors Among Middle-Aged Women. *J Women's Heal*. 2016;25(8):818-825. doi:10.1089/jwh.2015.5581.

16. Robinson WR, Cheng MM, Hoggatt KJ, Stürmer T, Siega-Riz AM. Childbearing is not associated with young women's long-term obesity risk. *Obesity*. 2014;22(4):1126-1132. doi:10.1002/oby.20593.

17. Schmitt NM, Nicholson WK, Schmitt J. The association of pregnancy and the development of obesity - results of a systematic review and meta-analysis on the natural history of postpartum weight retention. *Int J Obes (Lond)*. 2007;31(11):1642-1651. doi:10.1038/sj.ijo.0803655.

18. Nehring I, Schmoll S, Beyerlein A, Hauner H, Von Kries R. Gestational weight gain and long-term postpartum weight retention: A meta-analysis. *Am J Clin Nutr*. 2011;94(5):1225-1231. doi:10.3945/ajcn.111.015289.

19. Cohen A, Pieper CF, Brown AJ, Bastian L a. Number of children and risk of metabolic syndrome in women. *J Womens*

Health (Larchmt). 2006;15(6):763-773. doi:10.1089/jwh.2006.15.763.

20. Ness RB, Harris T, Cobb J, et al. Number of pregnancies and the subsequent risk of cardiovascular disease. *N Engl J Med*. 1993;328:1528-1533.
21. Pinto-Sietsma S-J, Mulder J, Janssen WMT, Hillege HL, Zeeuw D De, De Jong PE. Smoking Is Related to Albuminuria and Abnormal Renal Function in Nondiabetic Persons. *Ann Intern Med*. 2000;(133):585-591.
22. Hillege HL, Janssen WMT, Bak a. a a, et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med*. 2001;249(6):519-526. doi:10.1046/j.1365-2796.2001.00833.x.
23. Joosten MM, Gansevoort RT, Mukamal KJ, Harst P Van Der, Geleijnse JM, Feskens EJM. Urinary and plasma magnesium and risk of ischemic heart disease 1 – 3. *October*. 2013;97(13):1299-1306. doi:10.3945/ajcn.112.054114.INTRODUCTION.
24. Abbasi A, Corpeleijn E, Gansevoort RT, et al. Role of HDL cholesterol and estimates of HDL particle composition in future development of type 2 diabetes in the general population: The PREVEND study. *J Clin Endocrinol Metab*. 2013;98(8):1352-1359. doi:10.1210/jc.2013-1680.
25. Lamon-Fava S, Wilson PWF, Schaefer EJ. Impact of Body Mass Index on Coronary Heart Disease Risk Factors in Men and Women: The Framingham Offspring Study. *Arterioscler Thromb Vasc Biol*. 1996;16:1509-15151.
26. Wilson PWF, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Arch Intern Med*. 2002;162(16):1867-1872. doi:10.1001/archinte.162.16.1867.
27. Bogers R, Bemelmans W, Hoogenveen R, et al. Association of overweight with increased risk of coronary heart disease partly independent of blood pressure and cholesterol levels. *Arch Intern Med*. 2007;167(16):1720-1728. doi:10.1001/archinte.167.16.1720.
28. Mongraw-Chaffin ML, Peters S a E, Huxley RR, Woodward M. The sex-specific relationship between body mass index and coronary heart disease: A systematic review and meta-analysis of 95 cohorts with 1.2 million participants. *Lancet Diabetes Endocrinol*. 2015;3(6):437-449. doi:10.1002/aur.1474.Replication.
29. Hardy R, Lawlor D a., Black S, Wadsworth MEJ, Kuh D. Number of children and coronary heart disease risk factors in men and women from a British birth cohort. *BJOG An Int J Obstet Gynaecol*. 2007;114(6):721-730. doi:10.1111/j.1471-0528.2007.01324.x.
30. Gunderson EP, Jacobs DR, Chiang V, et al. Childbearing is associated with higher incidence of the metabolic syndrome among women of reproductive age controlling for measurements before pregnancy: the CARDIA study. *Am J Obstet Gynecol*. 2009;201(2):177.e1-177.e9. doi:10.1016/j.ajog.2009.03.031.
31. McClure CK, Catov JM, Ness RB, Schwarz EB. Lactation and maternal subclinical cardiovascular disease among premenopausal women. *Am J Obstet Gynecol*. 2012;207(1):1-15. doi:10.1016/j.ajog.2012.04.030.
32. Natland Fagerhaug T, Forsmo S, Jacobsen GW, Midthjell K, Andersen LF, Ivar Lund Nilsen T. A prospective population-based cohort study of lactation and cardiovascular disease mortality: the HUNT study. *BMC Public Health*. 2013;13(1):1070. doi:10.1186/1471-2458-13-1070.
33. Baker JL, Gamborg M, Heitmann BL, Lissner L, Sørensen TI a, Rasmussen KM. Breastfeeding reduces postpartum weight retention. *Am J Clin Nutr*. 2008;88(6):1543-1551. doi:10.3945/ajcn.2008.26379.
34. Jarlenski MP, Bennett WL, Bleich SN, Barry CL, Stuart E a. Effects of breastfeeding on postpartum weight loss among U.S. women. *Prev Med*. 14AD;69:146-150. doi:10.1016/j.ypmed.2014.09.018.Effects.

35. Britz SE, McDermott KC, Pierce CB, Blomquist JL, Handa VL. Changes in maternal weight 5-10 years after a first delivery. *Womens Health (Lond Engl)*. 2012;8(5):513-519. doi:10.2217/whe.12.35.

36. He X, Zhu M, Hu C, et al. Breast-feeding and postpartum weight retention: a systematic review and meta-analysis. *Public Health Nutr*. 2015;18(18):3308-3316. doi:10.1017/S1368980015000828.

37. Sherwood OD. Relaxin's physiological roles and other diverse actions. *Endocr Rev*. 2004;25(2):205-234. doi:10.1210/er.2003-0013.

38. Bani D. Relaxin as a natural agent for vascular health. *Vasc Health Risk Manag*. 2008;4(3):515-524.

39. Szepietowska B, Gorska M, Szelachowska M. Plasma relaxin concentration is related to beta-cell function and insulin sensitivity in women with type 2 diabetes mellitus. *Diabetes Res Clin Pract*. 2008;79(3):2007-2009. doi:10.1016/j.diabres.2007.10.017.

Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work; no relationships with companies that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

Author's contributions

Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof, Ron T. Gansevoort, Henk Groen and A.Titia Lely were involved in conception and design of the study. Data analyses was performed by Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof and Henk Groen. Interpretation of the results was performed by Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof, Ron T. Gansevoort, Henk Groen, Arie Franx, Bas B. van Rijn and A.Titia Lely. Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof and A.Titia Lely drafted the manuscript. Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof, Ron T. Gansevoort, Henk Groen, Arie Franx, Bas B. van Rijn and A.Titia Lely edited the manuscript. All authors read and approved the final manuscript.

Data sharing statement

Data sharing: patient level data and full dataset and technical appendix and statistical code are available from the corresponding author (g.zoet@umcutrecht.nl). Informed consent was not obtained but the presented data are anonymized and the risk of identification is low.

Figure 1: Flowchart

Table 1: at entry table PREVEND stratified for parity

	No children (N=464) ‡	One child (N=277) ‡	Two children (N=1021) ‡	More than two children (N=697) ‡	*P-value*
General characteristics					
Age (years)	52.2 (10.1)	52.2 (9.0)	52.3 (8.4)	56.9 (9.6)	**<0.001**
Follow-up time (years)	11 (11-12)	11 (11-12)	11 (11-12)	11 (11-12)	0.71
Caucasian (n (%))	445 (96.9%)	264 (96.4%)	995 (98.0%)	663 (95.7%)	**0.04**
Job (n (%))	315 (68.3%)	127 (47.4%)	467 (46.5%)	264 (38.6%)	**<0.001**
Cardiovascular risk profile					
BMI (kg/m²)	24.6 (22.5-27.7)	25.3 (22.5-28.6)	25.8 (23.4-28.8)	26.9 (24.0-30.2)	**<0.001**
SBP (mmHg)	120 (109-138)	121 (110-140)	122 (111-138)	130 (115-146)	**<0.001**
DBP (mmHg)	71 (65-78)	71 (67-77)	71 (66-78)	73 (67-79)	**0.004**
Current smoker (n (%))	139 (30.0%)	105 (37.9%)	326 (31.9%)	195 (28.0%)	**0.02**
Alcohol use (n (%))	203 (44.0%)	137 (49.6%)	503 (49.4%)	378 (54.5%)	**0.007**
Cardiovascular comorbidity (n (%))	11 (2.4%)	11 (4.1%)	24 (2.4%)	24 (3.6%)	0.30
Renal disease requiring dialysis (n (%))	1 (0.2%)	0 (0%)	3 (0.3%)	1 (0.1%)	0.78
Laboratory results					
Glucose (mmol/L)	4.6 (4.3-5.0)	4.7 (4.3-5.1)	4.7 (4.3-5.0)	4.8 (4.4-5.2)	**0.002**
Insulin (mmol/L)	7.0 (4.9-10.7)	7.6 (5.1-11.5)	7.8 (5.6-11.2)	9.5 (6.0-13.4)	**<0.001**
HOMAir	1.4 (1.0-2.4)	1.6 (1.0-2.5)	1.6 (1.1-2.4)	1.8 (1.2-3.0)	**<0.001**
Total Cholesterol (mmol/L)	5.7 (5.0-6.4)	5.7 (5.1-6.5)	5.8 (5.0-6.7)	6.0 (5.3-6.7)	**<0.001**
HDL Cholesterol (mmol/L)	1.5 (1.2-1.8)	1.5 (1.2-1.8)	1.4 (1.2-1.7)	1.4 (1.2-1.7)	**<0.001**
Triglycerides (mmol/L)	1.0 (0.8-1.5)	1.1 (0.9-1.7)	1.1 (0.9-1.6)	1.2 (0.9-1.6)	**<0.001**
Medication use					
Total blood pressure lowering (n (%))	65 (16.4%)	39 (16.5%)	140 (15.6%)	151 (24.4%)	**<0.001**
ACEi/ARB (n (%))	19 (4.7%)	8 (3.3%)	41 (4.5%)	44 (7.0%)	0.07
Glucose lowering (n (%))	7 (1.7%)	2 (0.8%)	12 (1.3%)	12 (1.9%)	0.63
Lipid lowering (n (%))	19 (4.7%)	15 (6.3%)	32 (3.5%)	37 (5.9%)	0.11
Oral contraceptive use (n (%))	79 (18.2%)	50 (19.5%)	198 (21.1%)	90 (14.6%)	**0.01**

Data are presented as median (25^{th} – 75^{th} percentile) unless otherwise stated. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; ACEi/ARB: angiotensin converting enzyme inhibitor and/or angiotensin receptor blocker; HOMAir: homeostatic model assessment index.

‡ Total number that participated at visit 1 of the study, not all variables were available for each participant at baseline.

Figure 2: Development over time of BMI (A), HDL cholesterol (B) and MAP (C), stratified for parity

Figure 3: CVD risk factors at entry

Legend: Hypertension = RR ≥140/90 mm/Hg and/or use of antihypertensive medication;
Obesity = BMI ≥30kg/m2; Low HDL cholesterol = HDL cholesterol <1.29 mmol/L;
Diabetes = fasting plasma glucose ≥7.0 mmol/L, self-report of a physician diagnosis and/or use of glucose-lowering medication.
□ = first visit; ■ = follow-up visit

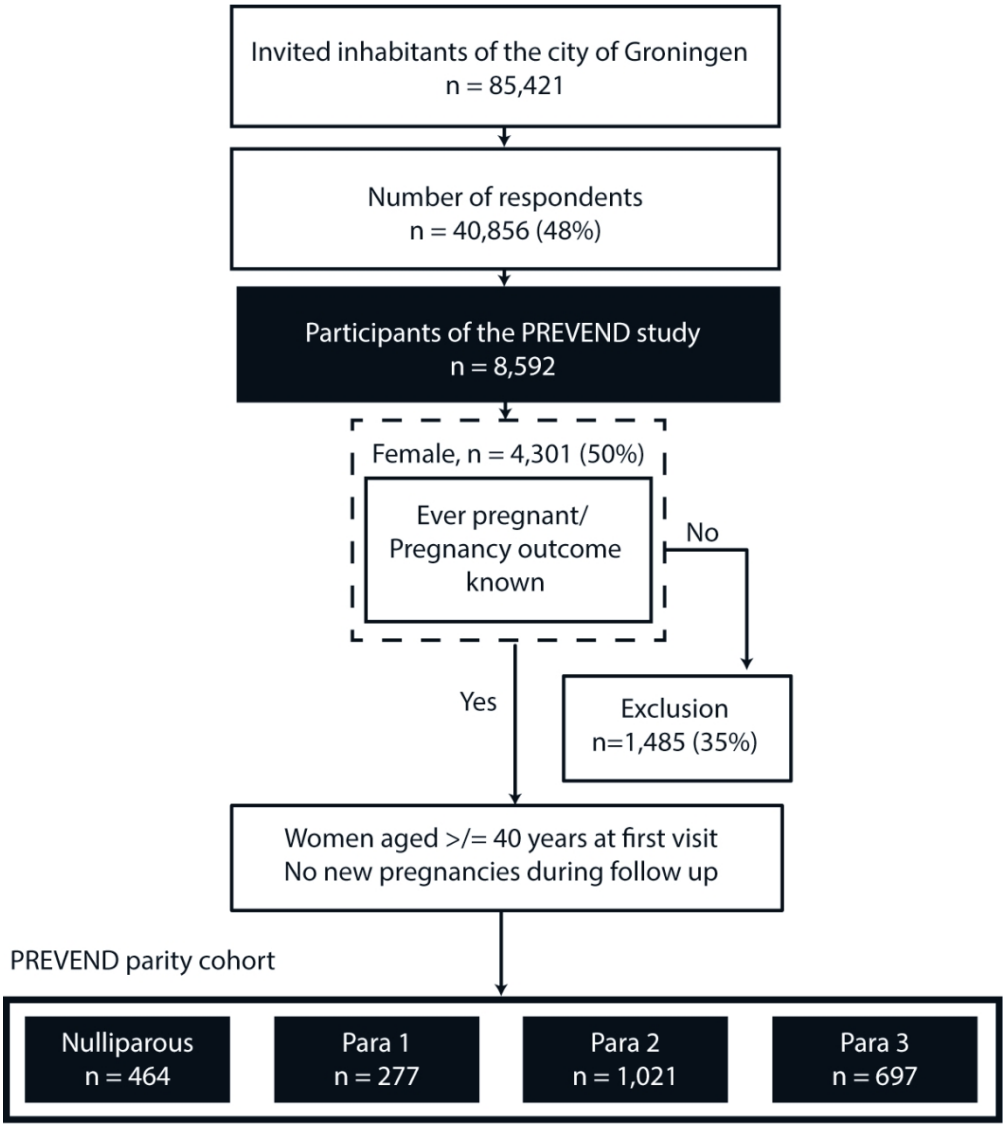


Figure 1: Flowchart

101x117mm (300 x 300 DPI)

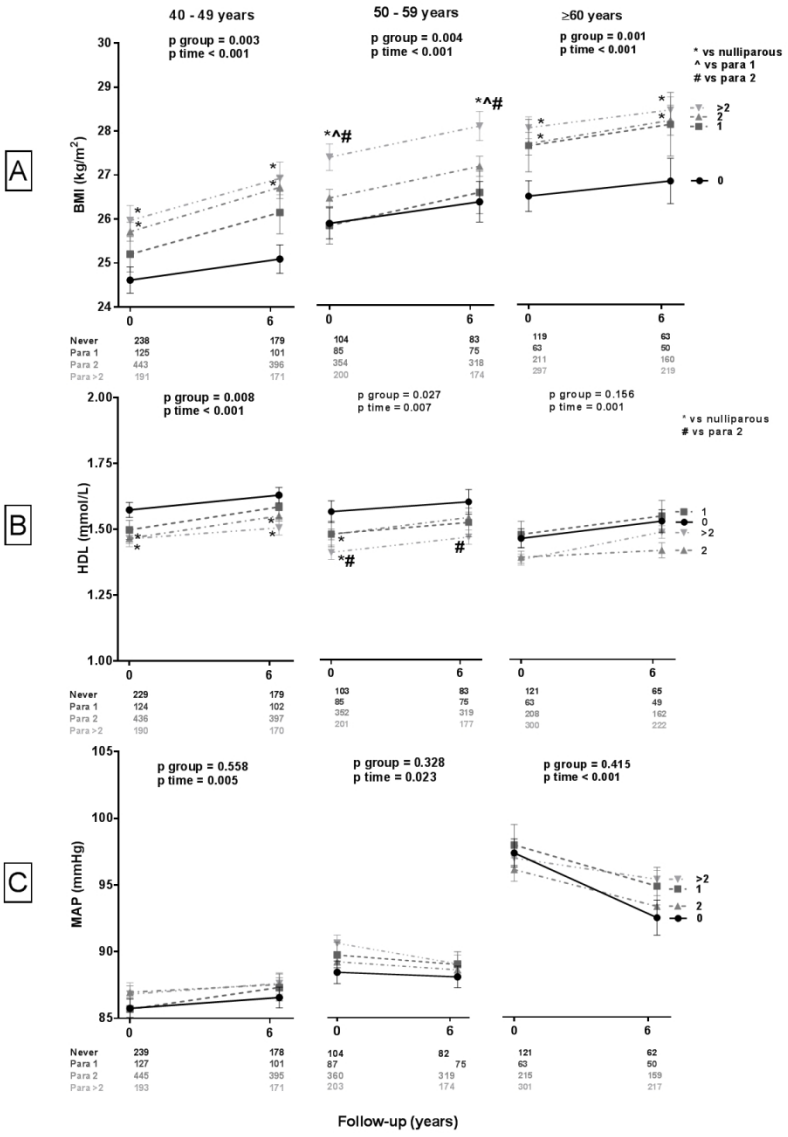


Figure 2: Development over time of BMI (A), HDL cholesterol (B) and MAP (C), stratified for parity

114x160mm (300 x 300 DPI)

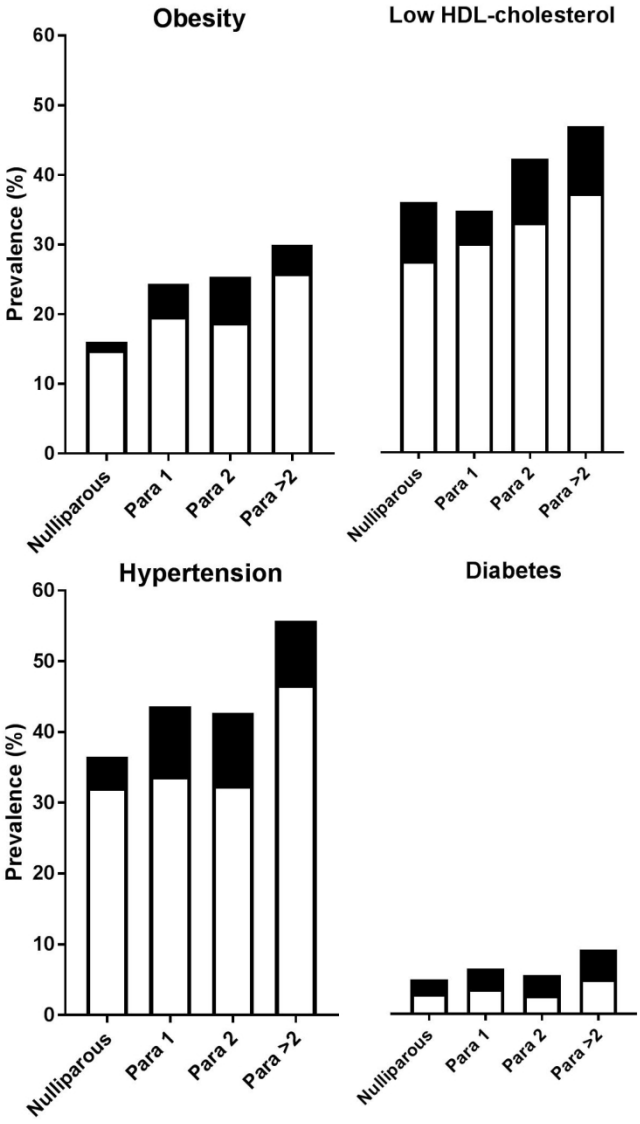


Figure 3: Development of CVD risk factors over time

111x187mm (300 x 300 DPI)

Supplemental table 1: stepwise correction for GEE-analysis

Table 1A: Correction models for GEE-analysis, stratified at age 40 – 50 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & education**			**Adjusted for age & OCC**			**Adjusted for age & education & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.003	<0.001	0.667	0.011	0.938	0.694	0.323	0.552	0.685	0.010	0.910	0.8	0.342	0.64	0.628
HDL-cholesterol	0.008	<0.001	0.530	0.003	0.737	0.520	0.047	0.472	0.519	0.006	0.666	0.5	0.080	0.438	0.565
MAP	0.558	0.005	0.815	0.866	<0.001	0.779	0.871	<0.001	0.777	0.874	<0.001	0.8	0.904	<0.001	0.871

Abbreviation: OCC, oral contraceptives

Table 1B: Correction models for GEE-analysis, stratified at age 50 – 60 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & education**			**Adjusted for age & OCC**			**Adjusted for age & education & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.004	<0.001	0.947	0.006	0.197	0.945	0.009	0.031	0.920	0.011	0.269	0.849	0.017	0.048	0.812
HDL-cholesterol	0.027	0.007	0.184	0.031	0.040	0.171	0.051	0.152	0.151	0.085	0.035	0.055	0.118	0.126	0.045
MAP	0.328	0.023	0.494	0.411	<0.001	0.478	0.522	0.005	0.457	0.637	0.003	0.802	0.669	0.025	0.786

Abbreviation: OCC, oral contraceptives

Table 1C: Correction models for GEE-analysis, stratified at age > 60 years

	Unadjusted			**Adjusted for age**			**Adjusted for age & education**			**Adjusted for age & OCC**			**Adjusted for age & education & OCC**		
	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$	P_{group}	P_{time}	$P_{interaction}$
BMI	0.001	<0.001	0.662	0.001	0.116	0.662	0.006	0.087	0.663	0.002	0.011	0.625	0.006	0.009	0.626
HDL-cholesterol	0.156	0.001	0.163	0.155	0.114	0.191	0.314	0.142	0.201	0.162	0.102	0.358	0.299	0.132	0.384
MAP	0.415	<0.001	0.348	0.646	<0.001	0.407	0.649	<0.001	0.407	0.667	<0.001	0.508	0.678	<0.001	0.507

Abbreviation: OCC, oral contraceptives

136/bmjopen-2018-024279 on 5 May 2019. Downloaded from

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohort reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	4, 5
Objectives	#3	State specific objectives, including any prespecified hypotheses	5
Study design	#4	Present key elements of study design early in the paper	5, 6
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	5

	#6b	For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	6
Bias	#9	Describe any efforts to address potential sources of bias	5, 6, 7
Study size	#10	Explain how the study size was arrived at	5
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	5, 7
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	6, 7
	#12b	Describe any methods used to examine subgroups and interactions	6, 7
	#12c	Explain how missing data were addressed	6, 7
	#12d	If applicable, explain how loss to follow-up was addressed	6, 7
	#12e	Describe any sensitivity analyses	na
Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	5
	#13b	Give reasons for non-participation at each stage	5
	#13c	Consider use of a flow diagram	figure 1
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential	7, table 1

		confounders. Give information separately for exposed and unexposed groups if applicable.	
	#14b	Indicate number of participants with missing data for each variable of interest	Figure 2
	#14c	Summarise follow-up time (eg, average and total amount)	6, 8, 9
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	7, 8, 9
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7, 8, 9
	#16b	Report category boundaries when continuous variables were categorized	7, 8, 9
	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	na
Other analyses	#17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	na
Key results	#18	Summarise key results with reference to study objectives	9, 10
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	11, 12
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	See note 1
Generalisability	#21	Discuss the generalisability (external validity) of the study results	See note 2
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

Author notes

1. 10, 11, 12, 13

2. 10, 11, 12, 13