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

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3 1 **Association between parity and persistent weight gain at age 40-60 years: a longitudinal**
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6 2 **prospective cohort study**

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39 19 **Short title:** Parity and persistent weight gain

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23 **Abstract**

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

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

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

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

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

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

45
46 **Keywords:** Pregnancy; parity; cardiovascular risk factors; BMI; HDL cholesterol; hypertension.

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3 47 **Study summary: Strengths and limitations of this study**
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- 5 48 • This longitudinal cohort comprised a large, well-phenotyped cohort with uniform assessment of all
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7 49 measurements during a median follow-up of 6 years.
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9
10 50 • The GEE analysis which was performed, allowed us to assess differences among groups over time,
11
12 51 focusing on group effects.
13
14 52 • Since parity itself was not assessed in this cohort, we used the number of children as a proxy for the
15
16 53 number of childbirths.
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19 54 • Women para > 2 were older, less often used oral contraceptives and more often used
20
21 55 antihypertensive medication which might have resulted in a slightly different metabolic profile.
22
23 56 • Age at first delivery, inter-pregnancy interval and lactation have not been assessed in this cohort
24
25 57 and therefore, adjustment of the analyses for these factors was not possible.
26
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29

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31
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33
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35
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38
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66 Introduction

67 Pregnancy is associated with major alterations to the cardiovascular system and metabolic profile.¹⁻⁴

68 Increases in weight, lipid levels, blood plasma volume and cardiac output are needed to maintain a
69 healthy, physiological pregnancy. Postpartum, this maternal adaptation reverses to its pre-pregnancy
70 state, although several changes, (e.g. increased body weight and hypercholesterolemia) may persist for
71 several months or longer.⁵ Possibly, these persisting changes contribute to an increased prevalence of
72 metabolic syndrome (MetS) and an unfavorable cardiometabolic profile in parous women.⁶⁻⁸ The
73 amount of gestational weight gain (GWG) affects postpartum weight retention.⁹ At long-term follow up,
74 excessive gestational weight gain is associated with an increased BMI, up to a 3-4 kg/m² 21 years after
75 pregnancy.¹⁰⁻¹²

76
77 Previous studies assessing the relation between parity and cardiometabolic health showed conflicting
78 results and even the association between parity and obesity is questioned in some studies.¹³⁻¹⁶ Long-
79 term effects have only scarcely been investigated and most studies had a follow-up of only 1 to 3 years
80 postpartum.^{17,18} A recent large cross-sectional study among Hispanic women in the US demonstrated
81 that multiparity (i.e. more than 4 or more than 6 children) was associated with obesity, low HDL
82 cholesterol and elevated fasting glucose, also after adjustment for sociodemographic and lifestyle
83 factors.⁸ In addition, results from the cross-sectional Rotterdam study showed a lower HDL cholesterol
84 and higher total cholesterol and glucose/insulin ratios with higher parity in Caucasian women at 70
85 years of age.⁶

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

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3 90 Some studies even showed a 'J-shaped' association in which women with two children had the lowest
4
5 91 prevalence of coronary heart disease.⁶⁻⁸
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7 92 The aim of the current study was to assess time-dependent changes of cardiometabolic health in parous
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9 93 women, stratified for number of children, as compared with nulliparous controls. This study was
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11 94 performed in a well-defined longitudinal prospective cohort study that primarily assessed development
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13 95 of CVD, albuminuria and renal disease.²¹
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19 97 **Methods**

20 21 98 **Participants**

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23 99 The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a longitudinal cohort follow-
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25 100 up study for assessment of cardiovascular and renal disease in the general population. Details of this
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27 101 study have previously been published elsewhere.^{22,23} In summary, all inhabitants of the city of
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29 102 Groningen, the Netherlands, from 1997 to 1998 at age 28–75 years (n = 85,421) were invited to
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31 103 participate. All that applied, filled out a questionnaire and collected a first-morning void urine sample.
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33 104 Pregnant women and subjects with type 1 diabetes mellitus were excluded. The urinary albumin
34
35 105 concentration was assessed in a total of 40,856 (47.8%) responders. A total of 6,000 participants were
36
37 106 enrolled out of 7,768 subjects with a urinary albumin concentration $\geq 10\text{mg/L}$. In addition, 2,592
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39 107 participants were enrolled out of 3,394 subjects with a urinary albumin concentration $< 10\text{mg/L}$.
40
41 108 Thereby, the PREVEND study was enriched by subjects with an elevated albumin excretion.
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43
44 109 In total, 4,301 women were enrolled in the PREVEND study (**Figure 1**). For the current analysis, only
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46 110 women aged 40 years or older at the first visit and who reported no new pregnancies during the follow-
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48 111 up visits were included. Women who reported no children were categorized as nulliparous (n = 464;
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50 112 18.9%). Women who reported one child, two children or more than two children, were categorized as
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54 113 para 1 (n = 277; 11.3%), para 2 (n = 1021; 41.5%) and para > 2 (n = 697; 28.3%). The PREVEND study has

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3 114 been approved by the medical ethics committee of the University Medical Centre Groningen. Written
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5 115 informed consent was obtained from all participants.
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10 117 **Measurements and visits**

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12 118 Between 1997 and 2012, the screening visits took place every 2-4 years. Participants completed
13
14 119 questionnaires and underwent physical examinations. Blood samples and 24-hour urine samples were
15
16 120 taken. The questionnaires included questions regarding parity. Participants reported their number of
17
18 121 children, which was used as a proxy for the number of childbirths. Details of clinical and laboratory
19
20 122 measurements have previously been described elsewhere.²² Prescription data from pharmacies was
21
22 123 used to assess the use of antihypertensive and lipid lowering medication. Hypertension was defined as a
23
24 124 systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg or the use of blood
25
26 125 pressure lowering medication. Type 2 diabetes mellitus (T2DM) was defined as a fasting plasma glucose
27
28 126 ≥ 7.0 mmol/L, random sample plasma glucose ≥ 11.1 mmol/L, self-reported physician diagnosis of type 2
29
30 127 diabetes mellitus, and/or the use of glucose-lowering medication.²⁴ Obesity was defined as BMI ≥ 30
31
32 128 kg/m².

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36 129 Data selection for analyses was based on a fixed median time interval of six years between the visits.
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40 41 131 **Statistical analysis**

42
43 132 Data was arranged per patient per visit. Continuous variables with a normal distribution are presented
44
45 133 as mean \pm standard deviation (SD) and analyzed using Student *t*-test or One-Way ANOVA followed by
46
47 134 Tukey post-hoc analysis. Continuous variables with a skewed distribution were expressed as median
48
49 135 with 25th–75th percentile and analyzed using Mann-Whitney U test or Kruskal Wallis. Categorical
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51 136 variables were analyzed using Pearson Chi square test or Fisher's exact test, where appropriate.
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3 137 For longitudinal assessment (time factor) of the outcome measures among the different parity groups
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5 138 (group factor), a generalized estimating equations (GEE) analysis was performed, including the
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7 139 interaction term group*visit (interaction factor). All analyses were performed using an autoregressive
8
9 140 correlation matrix structure. This assumes a variable correlation between measurements depending on
10
11 141 the time between measurements, as was expected in the current analysis. For GEE analyses of
12
13 142 continuous dependent variables (BMI, HDL cholesterol and MAP), participants were further stratified in
14
15 143 three age categories (40 – 49 years old, 50 – 59 years old, and ≥ 60 years old). In addition, we performed
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17 144 a stepwise correction strategy, correcting for age (model 1), age and education (model 2), age and oral
18
19 145 contraceptive use (model 3) and age, education and oral contraceptive use (model 4).
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23 146 Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad
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25 147 Software Inc. San Diego, CA, USA). In all analyses, a p-value <0.05 was considered statistically significant.
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30 149 **Results**

31 150 **Study population**

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34 151 **Table 1** provides an overview of the baseline characteristics of women who were nulliparous, para 1,
35
36 152 para 2 and para > 2 at the first PREVEND visit. The mean age was significantly higher in women who
37
38 153 were para > 2 . The majority of all women were Caucasian. The median follow-up time was 6 years in all
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40 154 groups. The cardiovascular risk profile among the parity groups differed significantly on BMI, blood
41
42 155 pressure, smoking and use of alcohol. Unfavorable blood glucose measurements and cholesterol profiles
43
44 156 were related to higher parity. The use of blood pressure lowering medication was higher in women who
45
46 157 were para > 2 compared to the other groups, but the use of glucose and lipid lowering medication did
47
48 158 not differ among the groups. Women who were para > 2 less often used oral contraceptives compared
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50 159 to women who were nulliparous, para 1 or para 2.
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161 **Cardiometabolic profile in relation to parity and age**

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

171
172 The prevalence of obesity increased with increasing parity at entry ($p_{\text{for trend}} < 0.001$) and at 6 year follow
173 up ($p_{\text{for trend}} < 0.001$; **Figure 3**). At visit one, 15% of the nulliparous women was obese, compared to 26% of
174 the women para >2. After the course of six years, this was increased to 16% of the nulliparous women
175 compared to 30% of the para >2. The increase in prevalence over time was similar among the groups
176 ($p = 0.450$). In a stepwise correction model, correction for age alone and correction for age and oral
177 contraceptive use did not influence the differences in HDL cholesterol among parity groups at all age
178 categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use,
179 differences among parity groups were not statistically significant anymore at all age groups.

180
181 HDL cholesterol differed among the groups, except for participants aged ≥ 60 years (**Figure 2B**). The
182 HDL cholesterol was lower with every increase of parity, except for participants older than 60
183 years: per child, the HDL decreased with 0.05 mmol/L. Women para 2 and women para > 2 had

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3 184 significant lower HDL cholesterol at both measurements compared to nulliparous women aged 40–49
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5 185 years (0.08–0.12 mmol/L) and 50–59 years (0.10–0.17 mmol/L). Over time, HDL cholesterol increased
6
7 186 significantly in all age groups ($p_{\text{time}}=0.001\text{--}0.007$) and the change in HDL cholesterol over time was
8
9 187 similar among all parity groups ($p_{\text{interaction}}=0.163\text{--}0.530$).

10 188 Low HDL cholesterol (<1.29 mmol/L) prevalence differed among the groups at visit one ($p_{\text{for trend}}<0.001$)
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12 189 and at follow up visit ($p_{\text{for trend}}=0.006$); low HDL cholesterol was more common when parity increased
13
14 190 **(Figure 3)**. Low HDL cholesterol prevalence inclined similar in all groups over time ($p=0.160$).
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21 192 There were no differences among the parity groups over time in MAP at all ages, although MAP
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23 193 increased in all groups at age 40–49 years and 50–59 years and decreased in all groups at age ≥ 60 years
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25 194 **(Figure 2C)**. The change in MAP over time was similar among all parity groups ($p_{\text{interaction}}=0.348\text{--}0.815$).

26
27 195 Prevalence of hypertension increased with parity both at entry visit ($p_{\text{for trend}}<0.001$) and at follow up
28
29 196 ($p_{\text{for trend}}<0.001$). Hypertension prevalence increased similar in all groups over time by 4–10% ($p=0.761$;
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31 197 **Figure 3**).

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36 199 Occurrence of T2DM did not differ among the groups at entry ($p_{\text{for trend}}=0.094$), although a positive
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38 200 association was found between T2DM prevalence and parity after six years ($p_{\text{for trend}}=0.018$). The increase
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40 201 in T2DM over time was comparable at all groups ($p=0.336$). T2DM prevalence was < 10% at all groups at
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42 202 both visits **(Figure 3)**.

43 44 203 45 46 204 **Discussion**

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48 205 In this longitudinal cohort study, having 2 children or more was associated with a higher BMI in all age
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50 206 categories and with a lower HDL cholesterol, but not with changes in blood pressure. A higher parity was
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52 207 associated with higher prevalence of obesity, low HDL cholesterol and a higher prevalence of
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3 208 hypertension. These associations were constant over time. As analyses were stratified and/or
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5 209 adjusted for age, these results suggest a direct association of parity itself with BMI, HDL cholesterol
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7 210 levels and cardiovascular risk factor prevalence. Other factors attributing to parity, i.e. socio-economic
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9 211 status and oral contraceptive use, might have contributed to these differences.
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14 213 Especially the effect of parity on BMI is of great interest, since BMI appears to be one of the most
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16 214 important cardiometabolic risk factors. This is not only due to the direct effect on cardiovascular disease
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18 215 onset, but also due to its adverse effect on lipid profile and blood pressure.^{25–28} Results from a
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20 216 population-based cohort study among 4699 women suggested that weight or weight changes might be
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22 217 an important mediator in the effect of parity on metabolic syndrome, thus confirming the importance of
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24 218 BMI in regard to cardiometabolic health.¹⁹ In our study, roughly each extra child is associated with 1.5–
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26 219 2.0 kg weight gain. Similar, each extra child is associated with 0.05 mmol/L decrease in HDL cholesterol,
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28 220 which might be the result of the increased BMI.
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33 222 Parallel to these metabolic differences in continuous measurements among the groups, occurrence of
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35 223 several clinical relevant cardiovascular risk factors, such as obesity and hypertension, differed among
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37 224 the groups as well. This is in line with cross-sectional studies assessing metabolic profile in relation to
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39 225 the number of children.^{6–8,19} However, some studies could not confirm the relation between parity and
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41 226 metabolic health, especially after adjustment for covariates such as lifestyle.^{14,15,19,29} In the stepwise
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43 227 correction, we found no or minimal influence of age, age and education level or age and oral
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45 228 contraceptive use on our results. Only after full correction for age, education level and oral
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47 229 contraceptive use, the statistical significance among parity groups diminished. Consequently, our
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49 230 findings should be interpreted with caution, as these factors and others, such as lifestyle changes
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51 231 following childbirth, might influence cardiovascular health next to parity itself.²⁹ Moreover, lifestyle
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3 232 effects of family life and the protective effect of lactation could explain the influence of parity on
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5 233 cardiometabolic health.^{30–32}
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10 235 Another possible explanation behind the mechanism of this relationship between parity and
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12 236 cardiovascular risk factors could be found in the effect of disturbances by pregnancy itself that continue
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14 237 to last postpartum. For example, breastfeeding is associated with a lower risk of cardiovascular risk
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16 238 factors, i.e. T2DM, although the role of breastfeeding remains controversial and a recent meta-analysis
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18 239 showed no significant effect of breastfeeding on postpartum weight retention.^{33–36} Other factors
19
20 240 involved in the relationship between parity and cardiovascular risk factors might be found in circulation
21
22 241 markers. An example might be found in the ovarian peptide hormone relaxin, produced by the corpus
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24 242 luteum or placental throughout pregnancy, has emerged as cardiovascular modulator involved in
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26 243 vasodilatation and inducing angiogenesis.^{37,38} Moreover, relaxin was positively associated with insulin
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28 244 sensitivity and lipid profile in women with type 2 diabetes mellitus as well.³⁹
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30 245 Previous cohort studies showed a ‘J-shaped’ association between parity and coronary heart disease,
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32 246 with lowest prevalence in women with two children, or stated that only multiparity (i.e. more than four
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34 247 or five children) was associated with increased cardiovascular disease risk.^{6–8} However, our results
35
36 248 indicate a stepwise effect of parity on cardiometabolic health and having two children or more than two
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38 249 children already affects BMI, HDL cholesterol levels and cardiovascular risk factor prevalence.
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42
43 251 Our paper is the first study providing detailed assessment of cardiometabolic health development over
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45 252 time in relation to parity. This longitudinal study comprised a large, well-phenotyped cohort with
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47 253 uniform assessment of all measurements during a median follow-up of 6 years. The GEE analysis which
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49 254 was performed, allowed us to assess differences among groups over time, focusing on group effects.
50
51 255 However, several limitations need to be discussed. The mean age of women para > 2 was significantly
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3 256 higher than women who were nulliparous, para 1 or para 2. In addition, women para > 2 less often used
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5 257 oral contraceptives and more often used antihypertensive medication. This might result in a slightly
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7 258 different metabolic profile although correction of the GEE-analysis for age and oral contraceptive use
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9
10 259 did not significantly change the results. Despite extensive phenotyping, age at first delivery, inter-
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12 260 pregnancy interval and lactation have not been assessed in the PREVEND study and therefore,
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14 261 adjustment of the analyses for these factors was not possible. Since parity itself was not assessed in the
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16 262 PREVEND, we used the number of children as a proxy for the number of childbirths. This might lead to
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18 263 inaccurate estimates of parity numbers, as the possibility of surrogacy or twin pregnancies is not taken
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21 264 into account. Additionally, no information was available regarding subfertility and several pregnancy
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23 265 complications, which leads to a lower number of children in these women and might reflect influence
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25 266 the cardiometabolic profile in later life as well. Lastly, pre-pregnancy BMI and gestational weight gain
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27 267 have not been assessed in the PREVEND study either, although their role on postpartum weight
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29
30 268 retention seemed limited in a recent publication.^{9,18}

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34 270 The PREVEND study was enriched with subjects with an elevated albumin excretion, which mostly
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36 271 results in an unfavorable cardiovascular risk profile compared to the general population. However,
37
38 272 albuminuria did not significantly differ among the groups within our analyses. In addition, adjustment
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40 273 for albuminuria did not change the results (data not shown). Although our findings suggest an effect of
41
42 274 parity itself on metabolic parameters, it should be noted that causality cannot be determined in our
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44 275 study. Therefore, one could argue that the relationship is reversed, e.g. women with higher BMI or
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46 276 lower HDL cholesterol are more fertile and therefore have more children. Prospective research assessing
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48 277 pre-pregnancy determinants of cardiometabolic health are warranted to further assess the possible
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50 278 causal effect of pregnancy itself.

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280 Conclusion

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

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382 **Competing interests**

42 383 All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf
43
44 384 (available on request from the corresponding author) and declare no support from any organization for
45
46 385 the submitted work; no relationships with companies that might have an interest in the submitted work
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48 386 in the previous 3 years; no other relationships or activities that could appear to have influenced the
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51 387 submitted work.
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3 389 **Author's contributions**
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5 390 GAZ, NDP, KJG, RTG, HG and ATL were involved in conception and design of the study. GAZ, KJG
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8 391 and ATL drafted the manuscript. All authors edited the manuscript; all authors read and
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10 392 approved the final manuscript.
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15 394 **Data sharing statement**
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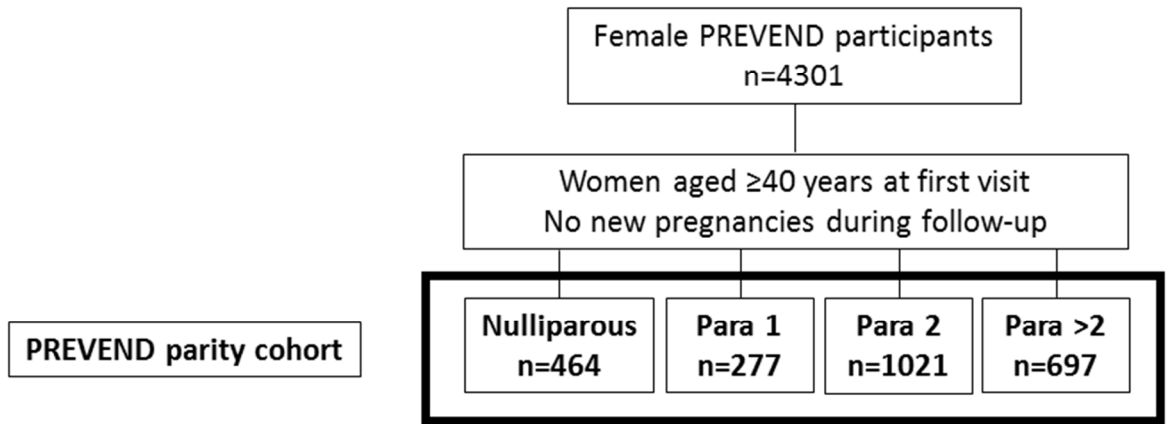
17 395 Data sharing: patient level data and full dataset and technical appendix and statistical code are available
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19 396 from the corresponding author (g.zoet@umcutrecht.nl). Informed consent was not obtained but the
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21 397 presented data are anonymized and the risk of identification is low.
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399 **Figure 1: Flowchart**

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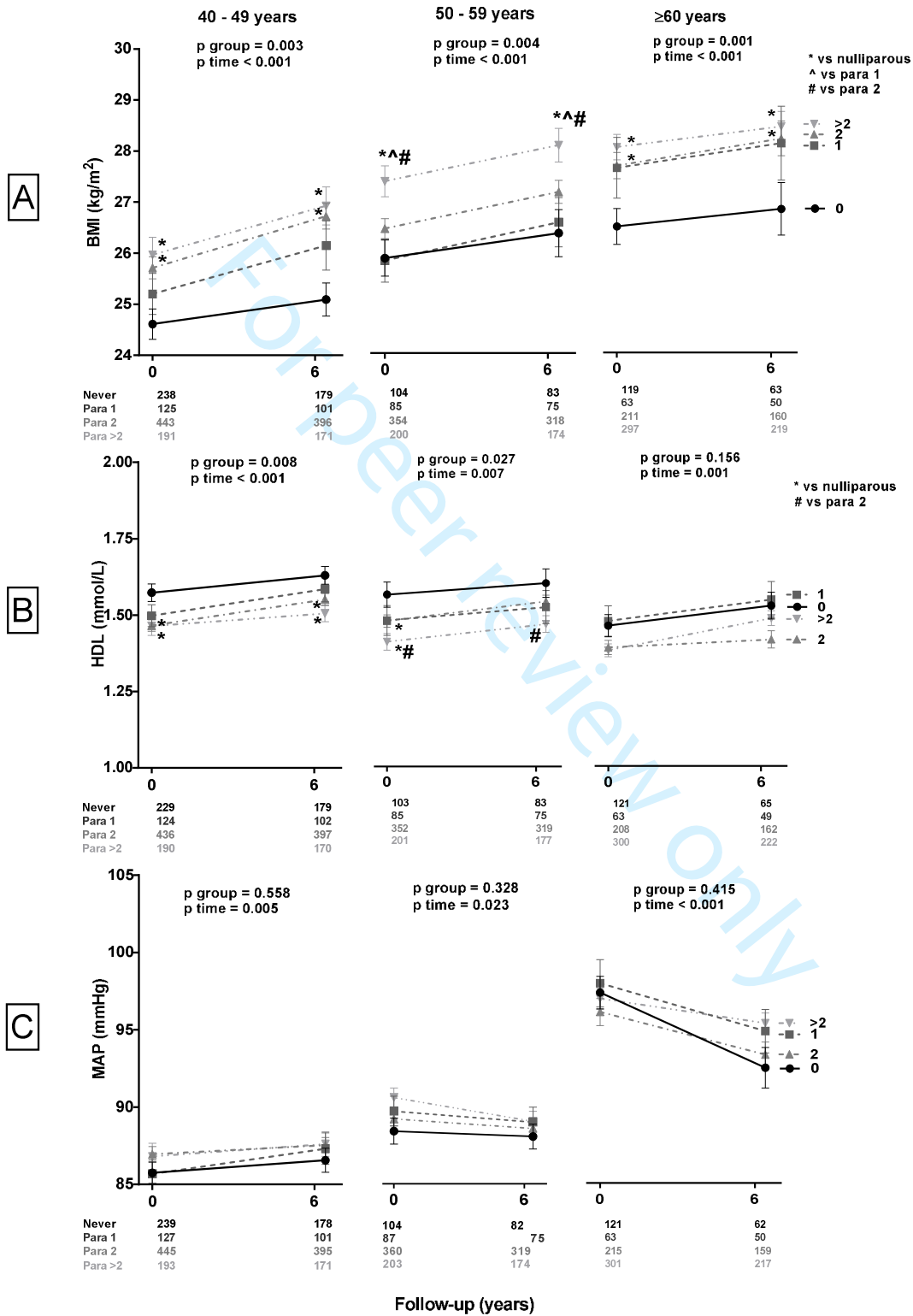
403 **Table 1: at entry table PREVEND stratified for parity**

	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/m ²)	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
HOMA _{1c}	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

405 Data are presented as median (25th – 75th 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; HOMA_{1c}: homeostatic model
407 assessment index.

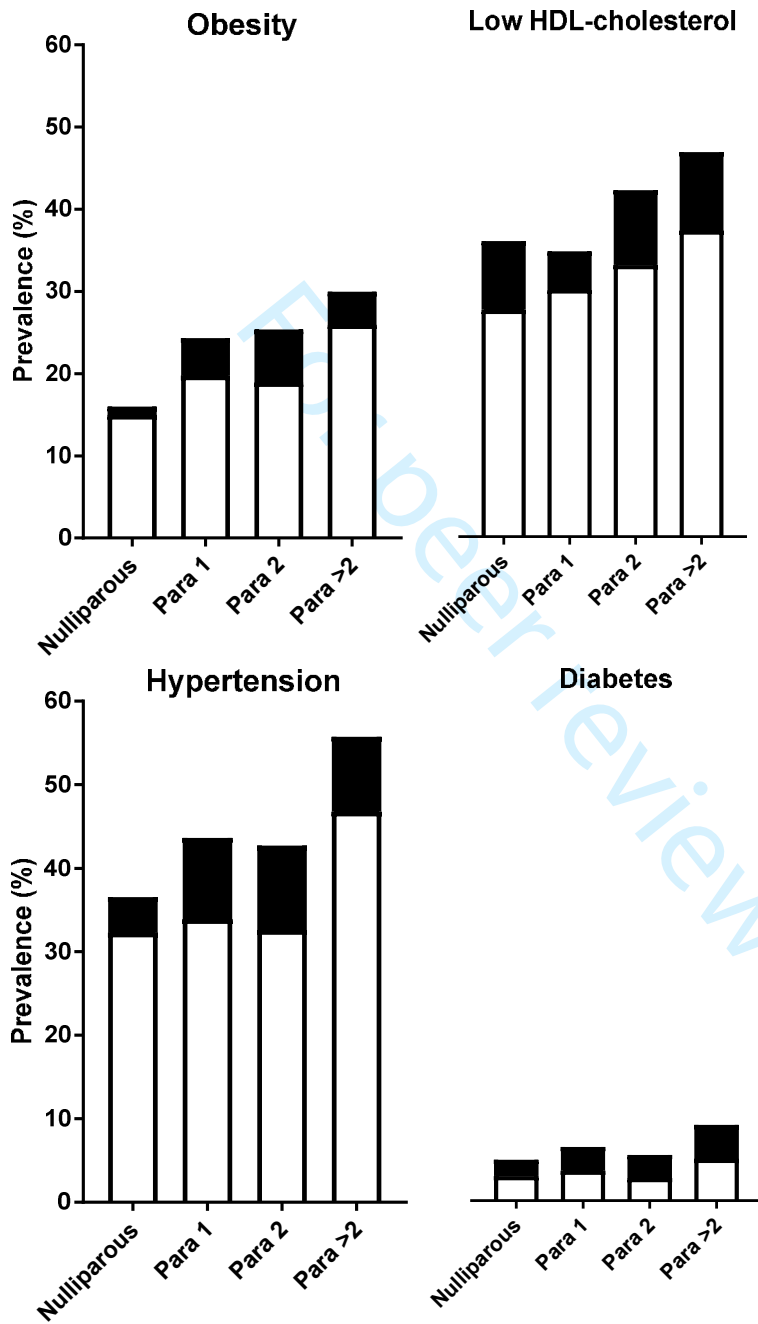
408 ‡ Total number that participated at visit 1 of the study, not all variables were available for each participant at baseline

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



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411 **Figure 3: CVD risk factors at entry**



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 413 Legend: Hypertension = RR \geq 140/90 mm/Hg and/or use of antihypertensive medication;
 414 Obesity = BMI \geq 30kg/m²; Low HDL cholesterol = HDL cholesterol <1.29 mmol/L;
 415 Diabetes = fasting plasma glucose \geq 7.0 mmol/L, self-report of a physician
 416 diagnosis and/or use of glucose-lowering medication.
 417 □ = 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, Gøtzsche 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

1		#6b	For matched studies, give matching criteria and number of	na
2			exposed and unexposed	
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5	Variables	#7	Clearly define all outcomes, exposures, predictors, potential	6
6			confounders, and effect modifiers. Give diagnostic criteria, if	
7			applicable	
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10	Data sources /	#8	For each variable of interest give sources of data and details of	6
11	measurement		methods of assessment (measurement). Describe	
12			comparability of assessment methods if there is more than one	
13			group. Give information separately for for exposed and	
14			unexposed groups if applicable.	
15				
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18	Bias	#9	Describe any efforts to address potential sources of bias	5, 6, 7
19				
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21	Study size	#10	Explain how the study size was arrived at	5
22				
23	Quantitative	#11	Explain how quantitative variables were handled in the	5, 7
24	variables		analyses. If applicable, describe which groupings were chosen,	
25			and why	
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28	Statistical	#12a	Describe all statistical methods, including those used to control	6, 7
29	methods		for confounding	
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31				
32		#12b	Describe any methods used to examine subgroups and	6, 7
33			interactions	
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35				
36		#12c	Explain how missing data were addressed	6, 7
37				
38		#12d	If applicable, explain how loss to follow-up was addressed	6, 7
39				
40				
41		#12e	Describe any sensitivity analyses	na
42				
43	Participants	#13a	Report numbers of individuals at each stage of study—eg	5
44			numbers potentially eligible, examined for eligibility, confirmed	
45			eligible, included in the study, completing follow-up, and	
46			analysed. Give information separately for for exposed and	
47			unexposed groups if applicable.	
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51		#13b	Give reasons for non-participation at each stage	5
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54		#13c	Consider use of a flow diagram	figure 1
55				
56	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	7, table
57			clinical, social) and information on exposures and potential	1
58				
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1		confounders. Give information separately for exposed and	
2		unexposed groups if applicable.	
3			
4		#14b Indicate number of participants with missing data for each	Figure 2
5		variable of interest	
6			
7		#14c Summarise follow-up time (eg, average and total amount)	6, 8, 9
8			
9			
10	Outcome data	#15 Report numbers of outcome events or summary measures	7, 8, 9
11		over time. Give information separately for exposed and	
12		unexposed groups if applicable.	
13			
14			
15	Main results	#16a Give unadjusted estimates and, if applicable, confounder-	7, 8, 9
16		adjusted estimates and their precision (eg, 95% confidence	
17		interval). Make clear which confounders were adjusted for and	
18		why they were included	
19			
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21			
22		#16b Report category boundaries when continuous variables were	7, 8, 9
23		categorized	
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26		#16c If relevant, consider translating estimates of relative risk into	na
27		absolute risk for a meaningful time period	
28			
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30	Other analyses	#17 Report other analyses done—e.g., analyses of subgroups and	na
31		interactions, and sensitivity analyses	
32			
33			
34	Key results	#18 Summarise key results with reference to study objectives	9, 10
35			
36	Limitations	#19 Discuss limitations of the study, taking into account sources of	11, 12
37		potential bias or imprecision. Discuss both direction and	
38		magnitude of any potential bias.	
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41	Interpretation	#20 Give a cautious overall interpretation considering objectives,	See note
42		limitations, multiplicity of analyses, results from similar studies,	1
43		and other relevant evidence.	
44			
45			
46	Generalisability	#21 Discuss the generalisability (external validity) of the study	See note
47		results	2
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49			
50	Funding	#22 Give the source of funding and the role of the funders for the	3
51		present study and, if applicable, for the original study on which	
52		the present article is based	
53			
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Author notes

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1 2. 10, 11, 12, 13

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3 CC-BY. This checklist was completed on 18. May 2018 using <http://www.goodreports.org/>, a tool
4 made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)
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BMJ Open

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

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Manuscripts

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3 1 **Association between parity and persistent weight gain at age 40-60 years: a longitudinal**
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6 2 **prospective cohort study**

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39 19 **Short title:** Parity and persistent weight gain

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23 **Abstract**

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

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

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

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

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

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

45
46 **Keywords:** Pregnancy; parity; cardiovascular risk factors; BMI; HDL cholesterol; hypertension.

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3 47 **Study summary: Strengths and limitations of this study**
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- 5 48 • This longitudinal cohort comprised a large, well-phenotyped cohort with uniform assessment of all
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7 49 measurements during a median follow-up of 6 years.
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10 50 • The GEE analysis which was performed, allowed us to assess differences among groups over time,
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12 51 focusing on group effects.
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14 52 • Since parity itself was not assessed in this cohort, we used the number of children as a proxy for the
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16 53 number of childbirths.
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19 54 • Women para > 2 were older, less often used oral contraceptives and more often used
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21 55 antihypertensive medication which might have resulted in a slightly different metabolic profile.
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23 56 • Age at first delivery, inter-pregnancy interval and lactation have not been assessed in this cohort
24
25 57 and therefore, adjustment of the analyses for these factors was not possible.
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29
30 59 **Funding:** The PREVEND study was supported by Dutch Kidney Foundation (Grant E.033), The Groningen
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37
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66 Introduction

67 Pregnancy is associated with major alterations to the cardiovascular system and metabolic profile.¹⁻⁴

68 Increases in weight, lipid levels, blood plasma volume and cardiac output are needed to maintain a
69 healthy, physiological pregnancy. Postpartum, this maternal adaptation reverses to its pre-pregnancy
70 state, although several changes, (e.g. increased body weight and hypercholesterolemia) may persist for
71 several months or longer.⁵ Possibly, these persisting changes contribute to an increased prevalence of
72 metabolic syndrome (MetS) and an unfavorable cardiometabolic profile in parous women.⁶⁻⁸ The
73 amount of gestational weight gain (GWG) affects postpartum weight retention.⁹ At long-term follow up,
74 excessive gestational weight gain is associated with an increased BMI, up to a 3-4 kg/m² 21 years after
75 pregnancy.¹⁰⁻¹²

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

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

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3 90 Some studies even showed a 'J-shaped' association in which women with two children had the lowest
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5 91 prevalence of coronary heart disease.⁶⁻⁸
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7 92 The aim of the current study was to assess time-dependent changes of cardiometabolic health in parous
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9 93 women, stratified for number of children, as compared with nulliparous controls. This study was
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11 94 performed in a well-defined longitudinal prospective cohort study that primarily assessed development
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13 95 of CVD, albuminuria and renal disease.²¹
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19 97 **Methods**

20 21 98 **Participants**

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23 99 The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a longitudinal cohort follow-
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25 100 up study for assessment of cardiovascular and renal disease in the general population. Details of this
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27 101 study have previously been published elsewhere.^{22,23} In summary, all inhabitants of the city of
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29 102 Groningen, the Netherlands, from 1997 to 1998 at age 28–75 years (n = 85,421) were invited to
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31 103 participate. All that applied, filled out a questionnaire and collected a first-morning void urine sample.
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33 104 Pregnant women and subjects with type 1 diabetes mellitus were excluded. The urinary albumin
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35 105 concentration was assessed in a total of 40,856 (47.8%) responders. A total of 6,000 participants were
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37 106 enrolled out of 7,768 subjects with a urinary albumin concentration $\geq 10\text{mg/L}$. In addition, 2,592
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39 107 participants were enrolled out of 3,394 subjects with a urinary albumin concentration $< 10\text{mg/L}$.
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41 108 Thereby, the PREVEND study was enriched by subjects with an elevated albumin excretion.
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44 109 In total, 4,301 women were enrolled in the PREVEND study (**Figure 1**). For the current analysis, only
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46 110 women aged 40 years or older at the first visit and who reported no new pregnancies during the follow-
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48 111 up visits were included. Women who reported no children were categorized as nulliparous (n = 464;
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50 112 18.9%). Women who reported one child, two children or more than two children, were categorized as
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52 113 para 1 (n = 277; 11.3%), para 2 (n = 1021; 41.5%) and para > 2 (n = 697; 28.3%). The PREVEND study has
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3 114 been approved by the medical ethics committee of the University Medical Centre Groningen. Written
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5 115 informed consent was obtained from all participants.
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10 117 **Patient and Public Involvement**

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12 118 No participants were involved with setting out the research question, developing the outcome measures
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14 119 or planning the study design. The results of study results will be disseminated by the newsletter and the
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16 120 study website.
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20 21 22 **Measurements and visits**

23 123 Between 1997 and 2012, the screening visits took place every 2-4 years. Participants completed
24
25 124 questionnaires and underwent physical examinations. Blood samples and 24-hour urine samples were
26
27 125 taken. The questionnaires included questions regarding parity. Participants reported their number of
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29 126 children, which was used as a proxy for the number of childbirths. In addition, education level, current
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31 127 alcohol use and current smoking were assessed in these questionnaires. Details of clinical and laboratory
32
33 128 measurements have previously been described elsewhere.²² Prescription data from pharmacies was
34
35 129 used to assess the use of antihypertensive and lipid lowering medication. Hypertension was defined as a
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37 130 systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg or the use of blood
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39 131 pressure lowering medication. Type 2 diabetes mellitus (T2DM) was defined as a fasting plasma glucose
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41 132 ≥ 7.0 mmol/L, random sample plasma glucose ≥ 11.1 mmol/L, self-reported physician diagnosis of type 2
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43 133 diabetes mellitus, and/or the use of glucose-lowering medication.²⁴ Obesity was defined as BMI ≥ 30
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46 134 kg/m².

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50 135 Data selection for analyses was based on a fixed time interval of six years between the visits.
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53 54 55 137 **Statistical analysis**

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3 138 Data was arranged per patient per visit. Continuous variables with a normal distribution are presented
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5 139 as mean \pm standard deviation (SD) and analyzed using Student *t*-test or One-Way ANOVA followed by
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7 140 Tukey post-hoc analysis. Continuous variables with a skewed distribution were expressed as median
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10 141 with 25th–75th percentile and analyzed using Mann-Whitney U test or Kruskal Wallis. Categorical
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12 142 variables were analyzed using Pearson Chi square test or Fisher's exact test, where appropriate.
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14 143 For longitudinal assessment (time factor) of the outcome measures among the different parity groups
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16 144 (group factor), a generalized estimating equations (GEE) analysis was performed, including the
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18 145 interaction term group*visit (interaction factor). All analyses were performed using an autoregressive
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20 146 correlation matrix structure. This assumes a variable correlation between measurements depending on
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22 147 the time between measurements, as was expected in the current analysis. For GEE analyses of
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24 148 continuous dependent variables (BMI, HDL cholesterol and MAP), participants were further stratified in
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26 149 three age categories (40 – 49 years old, 50 – 59 years old, and \geq 60 years old). In addition, we performed
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28 150 a stepwise correction strategy, correcting for age (model 1), age and education (model 2), age and oral
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30 151 contraceptive use (model 3) and age, education and oral contraceptive use (model 4).
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32 152 Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad
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34 153 Software Inc. San Diego, CA, USA). In all analyses, a p-value <0.05 was considered statistically significant.
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41 155 **Results**

42 156 **Study population**

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44 157 **Table 1** provides an overview of the baseline characteristics of women who were nulliparous, para 1,
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46 158 para 2 and para > 2 at the first PREVEND visit. The mean age was significantly higher in women who
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48 159 were para > 2 . The majority of all women were Caucasian. The median follow-up time was 6 years in all
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50 160 groups. The cardiovascular risk profile among the parity groups differed significantly on BMI, blood
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52 161 pressure, smoking and use of alcohol. Unfavorable blood glucose measurements and cholesterol profiles
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3 162 were related to higher parity. The use of blood pressure lowering medication was higher in women who
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5 163 were para > 2 compared to the other groups, but the use of glucose and lipid lowering medication did
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7 164 not differ among the groups. Women who were para > 2 less often used oral contraceptives compared
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10 165 to women who were nulliparous, para 1 or para 2.
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14 167 **Cardiometabolic profile in relation to parity and age**

16 168 During the 6-year study period, there was a constant, significant difference in BMI among the parity
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18 169 groups at all age categories (**Figure 2A**). The BMI was higher with every increase of parity at all age
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20 170 categories: per child, the BMI was 0.6kg/m² higher, equal to 1.5-2.0 kg. In women para > 2, the
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22 171 BMI was 0.7–2.0 kg/m² higher compared to the other parity groups. Over time, BMI increased
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24 172 significantly in all age groups ($p_{\text{time}} < 0.001$), the change in BMI over time was similar among all parity
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26 173 groups ($p_{\text{interaction}} = 0.662-0.947$). In a stepwise correction model, correction for age alone and correction
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28 174 for age and oral contraceptive use did not influence the differences in BMI among parity groups at all
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30 175 age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive
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32 176 use, differences among parity groups were not statistically significant anymore at age 50-59 years only.
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36 178 The prevalence of obesity increased with increasing parity at entry ($p_{\text{for trend}} < 0.001$) and at 6 year follow
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38 179 up ($p_{\text{for trend}} < 0.001$; **Figure 3**). At visit one, 15% of the nulliparous women was obese, compared to 26% of
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40 180 the women para >2. After the course of six years, this was increased to 16% of the nulliparous women
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42 181 compared to 30% of the para >2. The increase in prevalence over time was similar among the groups
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44 182 ($p = 0.450$). In a stepwise correction model, correction for age alone and correction for age and oral
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46 183 contraceptive use did not influence the differences in HDL cholesterol among parity groups at all age
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48 184 categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use,
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50 185 differences among parity groups were not statistically significant anymore at all age groups.
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6 187 HDL cholesterol differed among the groups, except for participants aged ≥ 60 years (**Figure 2B**). The
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8 188 HDL cholesterol was lower with every increase of parity, except for participants older than 60
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10 189 years: per child, the HDL decreased with 0.05 mmol/L. Women para 2 and women para > 2 had
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12 190 significant lower HDL cholesterol at both measurements compared to nulliparous women aged 40–49
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14 191 years (0.08–0.12 mmol/L) and 50–59 years (0.10–0.17 mmol/L). Over time, HDL cholesterol increased
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16 192 significantly in all age groups ($p_{\text{time}}=0.001\text{--}0.007$) and the change in HDL cholesterol over time was
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18 193 similar among all parity groups ($p_{\text{interaction}}=0.163\text{--}0.530$).

19 194 Low HDL cholesterol (<1.29 mmol/L) prevalence differed among the groups at visit one ($p_{\text{for trend}}<0.001$)
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21 195 and at follow up visit ($p_{\text{for trend}}=0.006$); low HDL cholesterol was more common when parity increased
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23 196 (**Figure 3**). Low HDL cholesterol prevalence inclined similar in all groups over time ($p=0.160$).

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31 198 There were no differences among the parity groups over time in MAP at all ages, although MAP
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33 199 increased in all groups at age 40–49 years and 50–59 years and decreased in all groups at age ≥ 60 years
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35 200 (**Figure 2C**). The change in MAP over time was similar among all parity groups ($p_{\text{interaction}}=0.348\text{--}0.815$).
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37 201 Prevalence of hypertension increased with parity both at entry visit ($p_{\text{for trend}}<0.001$) and at follow up
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39 202 ($p_{\text{for trend}}<0.001$). Hypertension prevalence increased similar in all groups over time by 4–10% ($p=0.761$;
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41 203 **Figure 3**).

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46 205 Occurrence of T2DM did not differ among the groups at entry ($p_{\text{for trend}}=0.094$), although a positive
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48 206 association was found between T2DM prevalence and parity after six years ($p_{\text{for trend}}=0.018$). The increase
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50 207 in T2DM over time was comparable at all groups ($p=0.336$). T2DM prevalence was $< 10\%$ at all groups at
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52 208 both visits (**Figure 3**).

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3 210 **Discussion**
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5 211 In this longitudinal cohort study, having 2 children or more was associated with a higher BMI in all age
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7 212 categories and with a lower HDL cholesterol, but not with changes in blood pressure. A higher parity was
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9 associated with higher prevalence of obesity, low HDL cholesterol and a higher prevalence of
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11 hypertension. These associations were constant over time. As analyses were stratified and/or adjusted
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13 for age, these results suggest a direct association of parity itself with BMI, HDL cholesterol levels and
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15 cardiovascular risk factor prevalence. Other factors attributing to parity, i.e. socio-economic status and
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17 oral contraceptive use, might have contributed to these differences.
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23 219 BMI appears to be one of the most important cardiometabolic risk factors because it has direct effect on
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25 220 cardiovascular disease onset, but also due to its adverse effect on lipid profile and blood pressure^{25–28}
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27 and therefore the influence of parity on BMI is of great interest. Results from a population-based cohort
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29 study among 4699 women suggested that weight or weight changes might be an important mediator in
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31 the effect of parity on metabolic syndrome, thus confirming the importance of BMI in regard to
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33 cardiometabolic health.¹⁹ In our study, roughly each extra child is associated with 1.5–2.0 kg weight gain.
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35 Similar, each extra child is associated with 0.05 mmol/L decrease in HDL cholesterol, which might be the
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37 result of the increased BMI.
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41 227 Specific BMI measures, HDL cholesterol levels and MAP measures differed among the three age groups.
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43 228 Because women from all different ages were seen throughout all screening visits, we expect this to be
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45 229 an effect of age itself, thereby reflecting the growing influence of age on cardiometabolic health with
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47 increasing age.
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52 232 Parallel to these metabolic differences in continuous measurements among the groups, occurrence of
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54 233 several clinical relevant cardiovascular risk factors, such as obesity and hypertension, differed among
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3 234 the groups as well. This is in line with cross-sectional studies assessing metabolic profile in relation to
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5 235 the number of children.^{6-8,19} However, some studies could not confirm the relation between parity and
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7 236 metabolic health, especially after adjustment for covariates such as lifestyle.^{14,15,19,29} In the stepwise
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9 237 correction, we found no or minimal influence of age, age and education level or age and oral
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11 238 contraceptive use on our results. Only after full correction for age, education level and oral
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13 239 contraceptive use, the statistical significance among parity groups diminished. Consequently, our
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15 240 findings should be interpreted with caution, as these factors and others, such as lifestyle changes
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17 241 following childbirth, might influence cardiovascular health next to parity itself.²⁹ Moreover, lifestyle
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19 242 effects of family life and the protective effect of lactation could explain the influence of parity on
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21 243 cardiometabolic health.³⁰⁻³²
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28 245 Another possible explanation behind the mechanism of this relationship between parity and
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30 246 cardiovascular risk factors could be found in the effect of disturbances by pregnancy itself that continue
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32 247 to last postpartum. For example, breastfeeding is associated with a lower risk of cardiovascular risk
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34 248 factors, i.e. T2DM, although the role of breastfeeding remains controversial and a recent meta-analysis
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36 249 showed no significant effect of breastfeeding on postpartum weight retention.³³⁻³⁶ Other factors
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38 250 involved in the relationship between parity and cardiovascular risk factors might be found in circulation
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40 251 markers. An example might be found in the ovarian peptide hormone relaxin, produced by the corpus
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42 252 luteum or placental throughout pregnancy, has emerged as cardiovascular modulator involved in
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44 253 vasodilatation and inducing angiogenesis.^{37,38} Moreover, relaxin was positively associated with insulin
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46 254 sensitivity and lipid profile in women with type 2 diabetes mellitus as well.³⁹
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48 255 Previous cohort studies showed a 'J-shaped' association between parity and coronary heart disease,
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50 256 with lowest prevalence in women with two children, or stated that only multiparity (i.e. more than four
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52 257 or five children) was associated with increased cardiovascular disease risk.⁶⁻⁸ However, our results
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3 258 indicate a stepwise effect of parity on cardiometabolic health and having two children or more than two
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5 259 children already affects BMI, HDL cholesterol levels and cardiovascular risk factor prevalence.
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10 261 Our paper is the first study providing detailed assessment of cardiometabolic health development over
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12 262 time in relation to parity. This longitudinal study comprised a large, well-phenotyped cohort with
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14 263 uniform assessment of all measurements during a median follow-up of 6 years. The GEE analysis which
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16 264 was performed, allowed us to assess differences among groups over time, focusing on group effects.
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18 265 However, several limitations need to be discussed. The mean age of women para > 2 was significantly
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20 266 higher than women who were nulliparous, para 1 or para 2. In addition, women para > 2 less often used
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22 267 oral contraceptives and more often used antihypertensive medication. This might result in a slightly
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24 268 different metabolic profile although correction of the GEE-analysis for age and oral contraceptive use
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26 269 did not significantly change the results. Despite extensive phenotyping, age at first delivery, inter-
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28 270 pregnancy interval and lactation have not been assessed in the PREVEND study and therefore,
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30 271 adjustment of the analyses for these factors was not possible. Since parity itself was not assessed in the
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32 272 PREVEND, we used the number of children as a proxy for the number of childbirths. This might lead to
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34 273 inaccurate estimates of parity numbers, as the possibility of surrogacy or twin pregnancies is not taken
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36 274 into account. Additionally, no information was available regarding subfertility and several pregnancy
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38 275 complications, which leads to a lower number of children in these women and might reflect influence
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40 276 the cardiometabolic profile in later life as well. Lastly, pre-pregnancy BMI and gestational weight gain
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42 277 have not been assessed in the PREVEND study either, although their role on postpartum weight
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44 278 retention seemed limited in a recent publication.^{9,18}
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52 280 The PREVEND study was enriched with subjects with an elevated albumin excretion, which mostly
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54 281 results in an unfavorable cardiovascular risk profile compared to the general population. However,
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3 282 albuminuria did not significantly differ among the groups within our analyses. Although our findings
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5 283 suggest an effect of parity itself on metabolic parameters, it should be noted that causality cannot be
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7 284 determined in our study. Therefore, one could argue that the relationship is reversed, e.g. women with
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10 285 higher BMI or lower HDL cholesterol are more fertile and therefore have more children. Prospective
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12 286 research assessing pre-pregnancy determinants of cardiometabolic health are warranted to further
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14 287 assess the possible causal effect of pregnancy itself.
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19 289 **Conclusion**

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21 290 In this longitudinal cohort study, higher parity is associated with higher BMI and lower HDL
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23 291 cholesterol. This difference among parity groups is constant over time. Furthermore, higher parity is
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25 292 associated with a higher prevalence of cardiovascular risk factors among the parity groups over time.
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27 293 These findings warrant for prospective research assessing determinants of cardiometabolic health at
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29 294 earlier age to understand the role of pregnancy in the development of cardiovascular disease in women.
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3 3904 391 **Competing interests**5
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7 392 All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf8
9 393 (available on request from the corresponding author) and declare no support from any organization for10
11 394 the submitted work; no relationships with companies that might have an interest in the submitted work12
13 395 in the previous 3 years; no other relationships or activities that could appear to have influenced the14
15 396 submitted work.16
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18 39719
20 398 **Author's contributions**21
22 399 GAZ, NDP, KJG, RTG, HG and ATL were involved in conception and design of the study. GAZ, KJG23
24 400 and ATL drafted the manuscript. All authors edited the manuscript; all authors read and25
26 401 approved the final manuscript.27
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29 40230
31 403 **Data sharing statement**32
33 404 Data sharing: patient level data and full dataset and technical appendix and statistical code are available34
35 405 from the corresponding author (gzoet@umcutrecht.nl). Informed consent was not obtained but the36
37 406 presented data are anonymized and the risk of identification is low.38
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408 **Figure 1: Flowchart**
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410 **Table 1: at entry table PREVENT 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/m ²)	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
HOMA _{1c}	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

411 Data are presented as median (25th – 75th percentile) unless otherwise stated. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic
412 blood pressure; ACEi/ARB: angiotensin converting enzyme inhibitor and/or angiotensin receptor blocker; HOMA_{1c}: homeostatic model
413 assessment index.
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415 ‡ Total number that participated at visit 1 of the study, not all variables were available for each participant at baseline

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

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3 **418 Figure 3: Development of CVD risk factors over time**
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8 420 Legend: Hypertension = RR \geq 140/90 mm/Hg and/or use of antihypertensive medication;

9 421 Obesity = BMI \geq 30kg/m²; Low HDL cholesterol = HDL cholesterol <1.29 mmol/L;

10 422 Diabetes = fasting plasma glucose \geq 7.0 mmol/L, self-report of a physician

11 423 diagnosis and/or use of glucose-lowering medication.

12 424 = first visit; = follow-up visit
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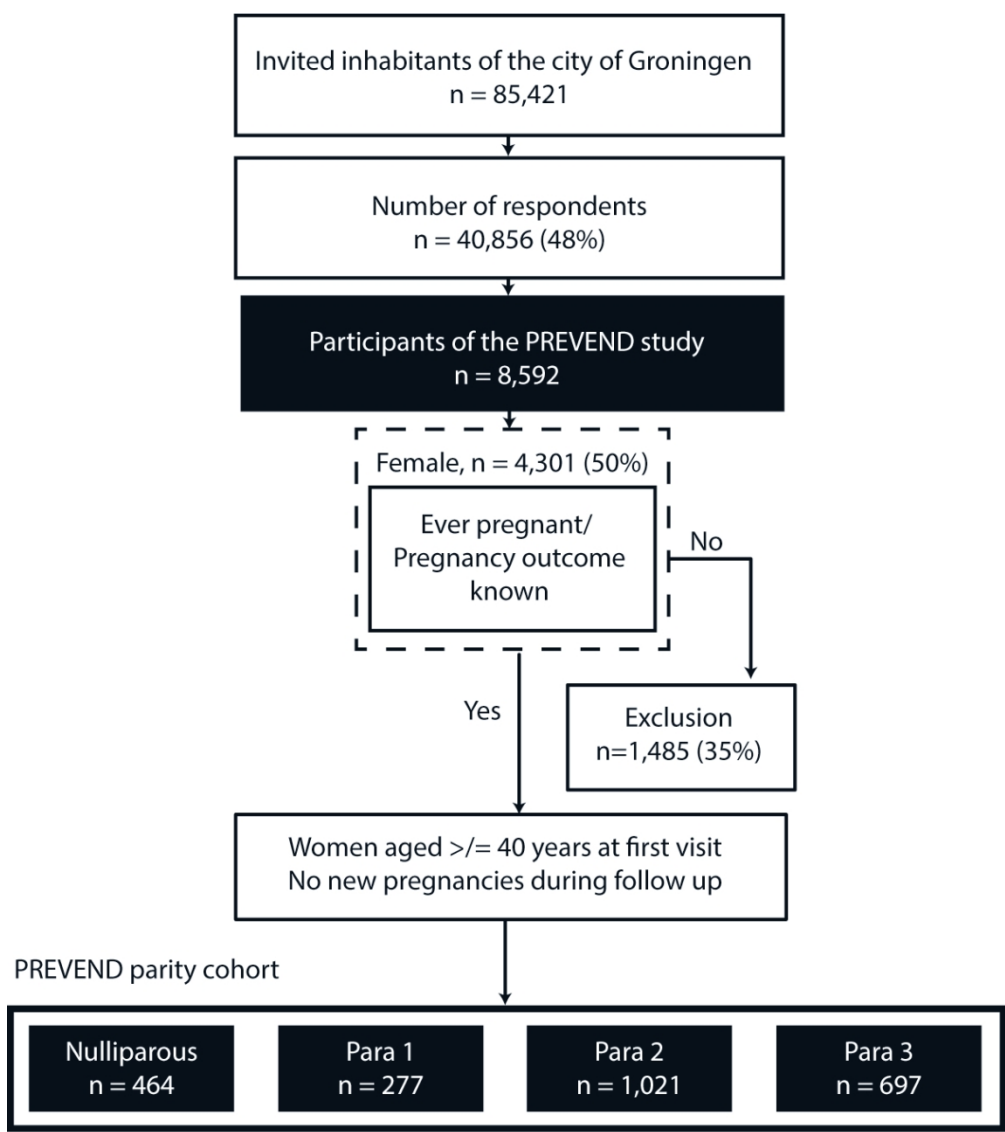


Figure 1: Flowchart

101x117mm (300 x 300 DPI)

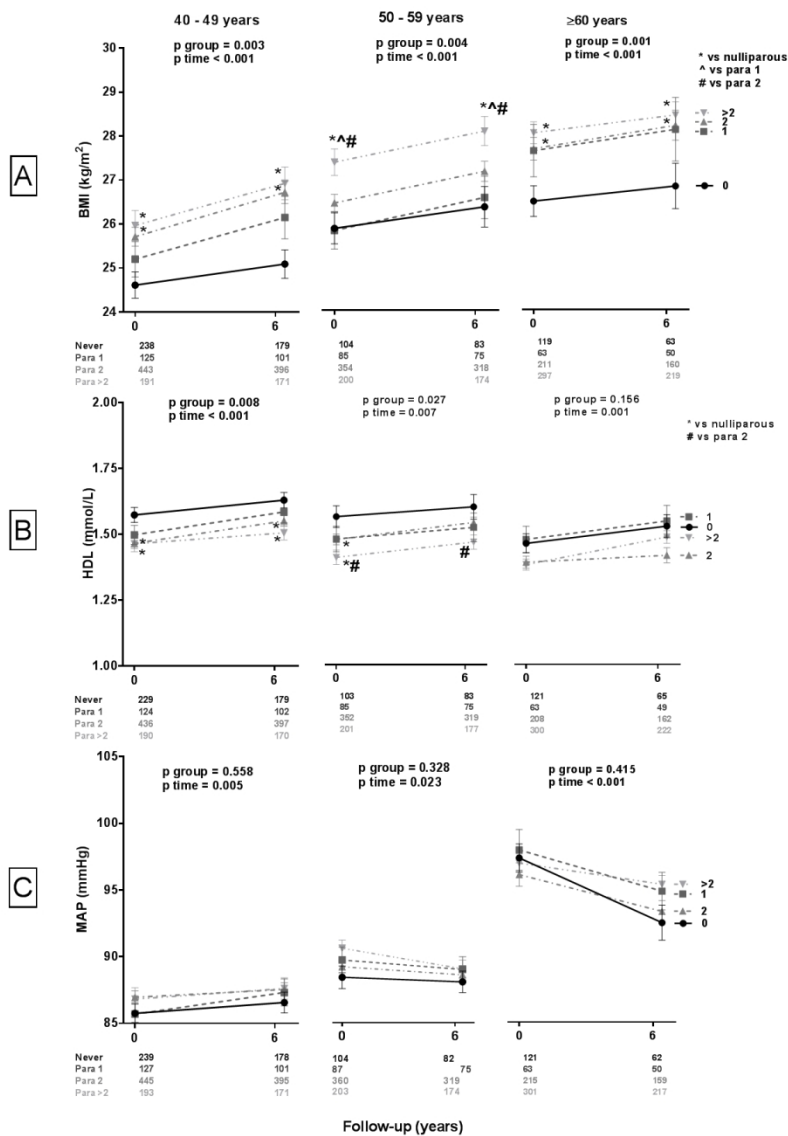


Figure 2: Development over time of BMI (A), HDL cholesterol (B) and MAP (C), stratified for parity

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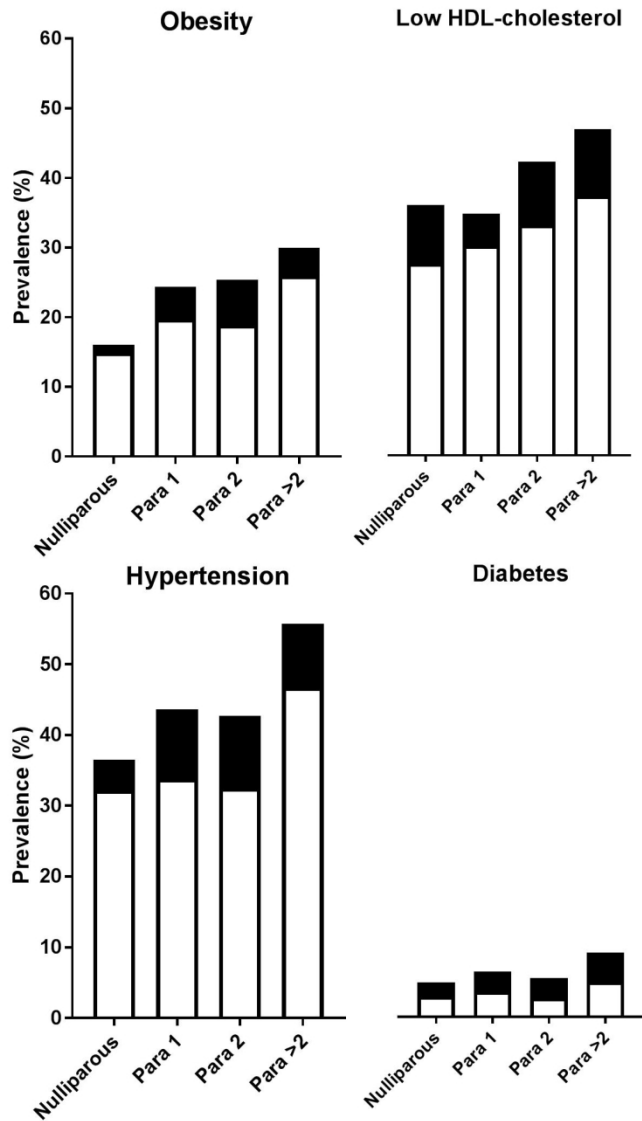


Figure 3: Development of CVD risk factors over time

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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	0.627	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

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, Gotsche 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

1		#6b	For matched studies, give matching criteria and number of	na
2			exposed and unexposed	
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5	Variables	#7	Clearly define all outcomes, exposures, predictors, potential	6
6			confounders, and effect modifiers. Give diagnostic criteria, if	
7			applicable	
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10	Data sources /	#8	For each variable of interest give sources of data and details of	6
11	measurement		methods of assessment (measurement). Describe	
12			comparability of assessment methods if there is more than one	
13			group. Give information separately for for exposed and	
14			unexposed groups if applicable.	
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18	Bias	#9	Describe any efforts to address potential sources of bias	5, 6, 7
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21	Study size	#10	Explain how the study size was arrived at	5
22				
23	Quantitative	#11	Explain how quantitative variables were handled in the	5, 7
24	variables		analyses. If applicable, describe which groupings were chosen,	
25			and why	
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28	Statistical	#12a	Describe all statistical methods, including those used to control	6, 7
29	methods		for confounding	
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32		#12b	Describe any methods used to examine subgroups and	6, 7
33			interactions	
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36		#12c	Explain how missing data were addressed	6, 7
37				
38		#12d	If applicable, explain how loss to follow-up was addressed	6, 7
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41		#12e	Describe any sensitivity analyses	na
42				
43	Participants	#13a	Report numbers of individuals at each stage of study—eg	5
44			numbers potentially eligible, examined for eligibility, confirmed	
45			eligible, included in the study, completing follow-up, and	
46			analysed. Give information separately for for exposed and	
47			unexposed groups if applicable.	
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51		#13b	Give reasons for non-participation at each stage	5
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54		#13c	Consider use of a flow diagram	figure 1
55				
56	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	7, table
57			clinical, social) and information on exposures and potential	1
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1		confounders. Give information separately for exposed and	
2		unexposed groups if applicable.	
3			
4		#14b Indicate number of participants with missing data for each	Figure 2
5		variable of interest	
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7		#14c Summarise follow-up time (eg, average and total amount)	6, 8, 9
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9			
10	Outcome data	#15 Report numbers of outcome events or summary measures	7, 8, 9
11		over time. Give information separately for exposed and	
12		unexposed groups if applicable.	
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15	Main results	#16a Give unadjusted estimates and, if applicable, confounder-	7, 8, 9
16		adjusted estimates and their precision (eg, 95% confidence	
17		interval). Make clear which confounders were adjusted for and	
18		why they were included	
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22		#16b Report category boundaries when continuous variables were	7, 8, 9
23		categorized	
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26		#16c If relevant, consider translating estimates of relative risk into	na
27		absolute risk for a meaningful time period	
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30	Other analyses	#17 Report other analyses done—e.g., analyses of subgroups and	na
31		interactions, and sensitivity analyses	
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34	Key results	#18 Summarise key results with reference to study objectives	9, 10
35			
36	Limitations	#19 Discuss limitations of the study, taking into account sources of	11, 12
37		potential bias or imprecision. Discuss both direction and	
38		magnitude of any potential bias.	
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41	Interpretation	#20 Give a cautious overall interpretation considering objectives,	See note
42		limitations, multiplicity of analyses, results from similar studies,	1
43		and other relevant evidence.	
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46	Generalisability	#21 Discuss the generalisability (external validity) of the study	See note
47		results	2
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50	Funding	#22 Give the source of funding and the role of the funders for the	3
51		present study and, if applicable, for the original study on which	
52		the present article is based	
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Author notes

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

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Manuscripts

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3 1 **Association between parity and persistent weight gain at age 40-60 years: a longitudinal**
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6 2 **prospective cohort study**

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43 21
44 22 **Short title:** Parity and persistent weight gain

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49 24 **Total word count:** 3080 (excluding title page, abstract, references, figures and tables)

26 **Abstract**

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

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

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

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

39 *Results:* The BMI was significantly higher in women para 2 or more in all age categories: per child, the
40 BMI was 0.6 kg/m² higher. corresponding with 1.5–2.0 kg weight gain per child. HDL cholesterol was
41 significantly lower in women para 2 or more aged 40-49 and 50-59 years: per child, the HDL cholesterol
42 was up to 0.09 mmol/L lower. Blood pressure did not differ among parity groups in any of the age
43 categories.

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

48
49 **Keywords:** Pregnancy; parity; cardiovascular risk factors; BMI; HDL cholesterol; hypertension.

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3 50 **Study summary: Strengths and limitations of this study**
4

- 5 51 • This longitudinal cohort comprised a large, well-phenotyped cohort with uniform assessment of all
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7 52 measurements during a median follow-up of 6 years.
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10 53 • The GEE analysis which was performed, allowed us to assess differences among groups over time,
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12 54 focusing on group effects.
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14 55 • Since parity itself was not assessed in this cohort, we used the number of children as a proxy for the
15
16 56 number of childbirths.
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19 57 • Women para > 2 were older, less often used oral contraceptives and more often used
20
21 58 antihypertensive medication which might have resulted in a slightly different metabolic profile.
22
23 59 • Age at first delivery, inter-pregnancy interval and lactation have not been assessed in this cohort
24
25 60 and therefore, adjustment of the analyses for these factors was not possible.
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28 61

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31
32 63 University Medical Center (Beleidsruimte) and Dade Behring, Ausam, Roche, Abbott and Gentian who
33
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69 Introduction

70 Pregnancy is associated with major alterations to the cardiovascular system and metabolic profile.¹⁻⁴
71 Increases in weight, lipid levels, blood plasma volume and cardiac output are needed to maintain a
72 healthy, physiological pregnancy. Postpartum, this maternal adaptation reverses to its pre-pregnancy
73 state, although several changes, (e.g. increased body weight and hypercholesterolemia) may persist for
74 several months or longer.⁵ Possibly, these persisting changes contribute to an increased prevalence of
75 metabolic syndrome (MetS) and an unfavorable cardiometabolic profile in parous women.⁶⁻⁸ The
76 amount of gestational weight gain (GWG) affects postpartum weight retention.⁹ At long-term follow up,
77 excessive gestational weight gain is associated with an increased BMI, up to a 3–4 kg/m² 21 years after
78 pregnancy.¹⁰⁻¹²

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

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

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3 93 Some studies even showed a 'J-shaped' association in which women with two children had the lowest
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5 94 prevalence of coronary heart disease.⁶⁻⁸
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7 95 The aim of the current study was to assess time-dependent changes of cardiometabolic health in parous
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9 96 women, stratified for number of children, as compared with nulliparous controls. This study was
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11 97 performed in a well-defined longitudinal prospective cohort study that primarily assessed development
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13 98 of CVD, albuminuria and renal disease.²¹
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100 **Methods**

101 **Participants**

102 The Prevention of Renal and Vascular End-stage Disease (PREVEND) study is a longitudinal cohort follow-
103 up study for assessment of cardiovascular and renal disease in the general population. Details of this
104 study have previously been published elsewhere.^{22,23} In summary, all inhabitants of the city of
105 Groningen, the Netherlands, from 1997 to 1998 at age 28–75 years (n = 85,421) were invited to
106 participate. All that applied, filled out a questionnaire and collected a first-morning void urine sample.
107 Pregnant women and subjects with type 1 diabetes mellitus were excluded. The urinary albumin
108 concentration was assessed in a total of 40,856 (47.8%) responders. A total of 6,000 participants were
109 enrolled out of 7,768 subjects with a urinary albumin concentration $\geq 10\text{mg/L}$. In addition, 2,592
110 participants were enrolled out of 3,394 subjects with a urinary albumin concentration $< 10\text{mg/L}$.
111 Thereby, the PREVEND study was enriched by subjects with an elevated albumin excretion.
112 In total, 4,301 women were enrolled in the PREVEND study (**Figure 1**). For the current analysis, only
113 women aged 40 years or older at the first visit and who reported no new pregnancies during the follow-
114 up visits were included. Women who reported no children were categorized as nulliparous (n = 464;
115 18.9%). Women who reported one child, two children or more than two children, were categorized as
116 para 1 (n = 277; 11.3%), para 2 (n = 1021; 41.5%) and para > 2 (n = 697; 28.3%). The PREVEND study has

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3 117 been approved by the medical ethics committee of the University Medical Centre Groningen. Written
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5 118 informed consent was obtained from all participants.
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10 120 **Patient and Public Involvement**

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12 121 No participants were involved with setting out the research question, developing the outcome measures
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14 122 or planning the study design. The results of study results will be disseminated by the newsletter and the
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16 123 study website.
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20 21 125 **Measurements and visits**

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23 126 Between 1997 and 2012, the screening visits took place every 2-4 years. Participants completed
24
25 127 questionnaires and underwent physical examinations. Blood samples and 24-hour urine samples were
26
27 128 taken. The questionnaires included questions regarding parity. Participants reported their number of
28
29 129 children, which was used as a proxy for the number of childbirths. In addition, education level, current
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31 130 alcohol use and current smoking were assessed in these questionnaires. Details of clinical and laboratory
32
33 131 measurements have previously been described elsewhere.²² Prescription data from pharmacies was
34
35 132 used to assess the use of antihypertensive and lipid lowering medication. Hypertension was defined as a
36
37 133 systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg or the use of blood
38
39 134 pressure lowering medication. Type 2 diabetes mellitus (T2DM) was defined as a fasting plasma glucose
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41 135 ≥ 7.0 mmol/L, random sample plasma glucose ≥ 11.1 mmol/L, self-reported physician diagnosis of type 2
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43 136 diabetes mellitus, and/or the use of glucose-lowering medication.²⁴ Obesity was defined as BMI ≥ 30
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45 137 kg/m².
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50 138 Data selection for analyses was based on a fixed time interval of six years between the visits.
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54 140 **Statistical analysis**

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3 141 Data was arranged per patient per visit. Continuous variables with a normal distribution are presented
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5 142 as mean \pm standard deviation (SD) and analyzed using Student *t*-test or One-Way ANOVA followed by
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7 143 Tukey post-hoc analysis. Continuous variables with a skewed distribution were expressed as median
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10 144 with 25th–75th percentile and analyzed using Mann-Whitney U test or Kruskal Wallis. Categorical
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12 145 variables were analyzed using Pearson Chi square test or Fisher's exact test, where appropriate.
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14 146 For longitudinal assessment (time factor) of the outcome measures among the different parity groups
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16 147 (group factor), a generalized estimating equations (GEE) analysis was performed, including the
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18 148 interaction term group*visit (interaction factor). All analyses were performed using an autoregressive
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20 149 correlation matrix structure. This assumes a variable correlation between measurements depending on
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22 150 the time between measurements, as was expected in the current analysis. For GEE analyses of
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24 151 continuous dependent variables (BMI, HDL cholesterol and MAP), participants were further stratified in
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26 152 three age categories (40 – 49 years old, 50 – 59 years old, and \geq 60 years old). In addition, we performed
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28 153 a stepwise correction strategy, correcting for age (model 1), age and education (model 2), age and oral
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30 154 contraceptive use (model 3) and age, education and oral contraceptive use (model 4).
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32 155 Data were analyzed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and GraphPad prism 5.01 (GraphPad
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34 156 Software Inc. San Diego, CA, USA). In all analyses, a p-value <0.05 was considered statistically significant.
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41 158 **Results**

42 159 **Study population**

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44 160 **Table 1** provides an overview of the baseline characteristics of women who were nulliparous, para 1,
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46 161 para 2 and para > 2 at the first PREVEND visit. The mean age was significantly higher in women who
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48 162 were para > 2 . The majority of all women were Caucasian. The median follow-up time was 6 years in all
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50 163 groups. The cardiovascular risk profile among the parity groups differed significantly on BMI, blood
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52 164 pressure, smoking and use of alcohol. Unfavorable blood glucose measurements and cholesterol profiles
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3 165 were related to higher parity. The use of blood pressure lowering medication was higher in women who
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5 166 were para > 2 compared to the other groups, but the use of glucose and lipid lowering medication did
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7 167 not differ among the groups. Women who were para > 2 less often used oral contraceptives compared
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10 168 to women who were nulliparous, para 1 or para 2.

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14 170 **Cardiometabolic profile in relation to parity and age**

16 171 During the 6-year study period, there was a constant, significant difference in BMI among the parity
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18 172 groups at all age categories (**Figure 2A**). The BMI was higher with every increase of parity at all age
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20 173 categories: per child, the BMI was 0.6kg/m² higher, equal to 1.5-2.0 kg. In women para > 2, the
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22 174 BMI was 0.7–2.0 kg/m² higher compared to the other parity groups. Over time, BMI increased
23
24 175 significantly in all age groups ($p_{\text{time}} < 0.001$), the change in BMI over time was similar among all parity
25
26 176 groups ($p_{\text{interaction}} = 0.662-0.947$). In a stepwise correction model, correction for age alone and correction
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28 177 for age and oral contraceptive use did not influence the differences in BMI among parity groups at all
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30 178 age categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive
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32 179 use, differences among parity groups were statistically significant at age 50-59 and >60 years only.

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37 181 The prevalence of obesity increased with increasing parity at entry ($p_{\text{for trend}} < 0.001$) and at 6 year follow
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39 182 up ($p_{\text{for trend}} < 0.001$; **Figure 3**). At visit one, 15% of the nulliparous women was obese, compared to 26% of
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41 183 the women para >2. After the course of six years, this was increased to 16% of the nulliparous women
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43 184 compared to 30% of the para >2. The increase in prevalence over time was similar among the groups
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45 185 ($p = 0.450$). In a stepwise correction model, correction for age alone and correction for age and oral
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47 186 contraceptive use did not influence the differences in HDL cholesterol among parity groups at all age
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49 187 categories (**Supplemental table 1**). After correction for age, education level and oral contraceptive use,
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51 188 differences among parity groups were not statistically significant anymore at all age groups.

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6 190 HDL cholesterol differed among the groups, except for participants aged ≥ 60 years (**Figure 2B**). The
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8 191 HDL cholesterol was lower with every increase of parity, except for participants older than 60
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10 192 years: per child, the HDL decreased with 0.05 mmol/L. Women para 2 and women para > 2 had
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12 193 significant lower HDL cholesterol at both measurements compared to nulliparous women aged 40–49
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14 194 years (0.08–0.12 mmol/L) and 50–59 years (0.10–0.17 mmol/L). Over time, HDL cholesterol increased
15
16 195 significantly in all age groups ($p_{\text{time}}=0.001\text{--}0.007$) and the change in HDL cholesterol over time was
17
18 196 similar among all parity groups ($p_{\text{interaction}}=0.163\text{--}0.530$).

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21 197 Low HDL cholesterol (<1.29 mmol/L) prevalence differed among the groups at visit one ($p_{\text{for trend}}<0.001$)
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23 198 and at follow up visit ($p_{\text{for trend}}=0.006$); low HDL cholesterol was more common when parity increased
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25 199 (**Figure 3**). Low HDL cholesterol prevalence inclined similar in all groups over time ($p=0.160$).

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31 201 There were no differences among the parity groups over time in MAP at all ages, although MAP
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33 202 increased in all groups at age 40–49 years and 50–59 years and decreased in all groups at age ≥ 60 years
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35 203 (**Figure 2C**). The change in MAP over time was similar among all parity groups ($p_{\text{interaction}}=0.348\text{--}0.815$).
36
37 204 Prevalence of hypertension increased with parity both at entry visit ($p_{\text{for trend}}<0.001$) and at follow up
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39 205 ($p_{\text{for trend}}<0.001$). Hypertension prevalence increased similar in all groups over time by 4–10% ($p=0.761$;
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41 206 **Figure 3**).

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46 208 Occurrence of T2DM did not differ among the groups at entry ($p_{\text{for trend}}=0.094$), although a positive
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48 209 association was found between T2DM prevalence and parity after six years ($p_{\text{for trend}}=0.018$). The increase
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50 210 in T2DM over time was comparable at all groups ($p=0.336$). T2DM prevalence was $< 10\%$ at all groups at
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52 211 both visits (**Figure 3**).

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213 Discussion

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

222
223 Since BMI appears to be one of the most important cardiometabolic risk factors, the influence of parity
224 on BMI is of great interest. This strong effect of BMI is not only due to the direct effect on cardiovascular
225 disease onset, but also due to its adverse effect on lipid profile and blood pressure.^{25–28} Results from a
226 population-based cohort study among 4699 women suggested that weight or weight changes might be
227 an important mediator in the effect of parity on metabolic syndrome, thus confirming the importance of
228 BMI in regard to cardiometabolic health.¹⁹ In our study, roughly each extra child is associated with 1.5–
229 2.0 kg weight gain. Similar, each extra child is associated with 0.05 mmol/L decrease in HDL cholesterol,
230 which might be the result of the increased BMI.

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

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3 236 Parallel to these metabolic differences in continuous measurements among the groups, occurrence of
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5 237 several clinical relevant cardiovascular risk factors, such as obesity and hypertension, differed among
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7 238 the groups as well. This is in line with cross-sectional studies assessing metabolic profile in relation to
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10 239 the number of children.^{6-8,19} However, some studies could not confirm the relation between parity and
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12 240 metabolic health, especially after adjustment for covariates such as lifestyle.^{14,15,19,29} In the stepwise
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14 241 correction, we found no or minimal influence of age, age and education level or age and oral
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16 242 contraceptive use on our results. Only after full correction for age, education level and oral
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18 243 contraceptive use, the statistical significance among parity groups diminished. Consequently, our
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21 244 findings should be interpreted with caution, as these factors and others, such as lifestyle changes
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23 245 following childbirth, might influence cardiovascular health next to parity itself.²⁹ Moreover, lifestyle
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25 246 effects of family life and the protective effect of lactation could explain the influence of parity on
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28 247 cardiometabolic health.³⁰⁻³²
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32 249 Another possible explanation behind the mechanism of this relationship between parity and
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34 250 cardiovascular risk factors could be found in the effect of disturbances by pregnancy itself that continue
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37 251 to last postpartum. For example, breastfeeding is associated with a lower risk of cardiovascular risk
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39 252 factors, i.e. T2DM, although the role of breastfeeding remains controversial and a recent meta-analysis
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41 253 showed no significant effect of breastfeeding on postpartum weight retention.³³⁻³⁶ Other factors
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43 254 involved in the relationship between parity and cardiovascular risk factors might be found in circulation
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46 255 markers. An example might be found in the ovarian peptide hormone relaxin, produced by the corpus
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48 256 luteum or placental throughout pregnancy, has emerged as cardiovascular modulator involved in
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50 257 vasodilatation and inducing angiogenesis.^{37,38} Moreover, relaxin was positively associated with insulin
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52 258 sensitivity and lipid profile in women with type 2 diabetes mellitus as well.³⁹
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3 259 Previous cohort studies showed a 'J-shaped' association between parity and coronary heart disease,
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5 260 with lowest prevalence in women with two children, or stated that only multiparity (i.e. more than four
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7 261 or five children) was associated with increased cardiovascular disease risk.⁶⁻⁸ However, our results
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9
10 262 indicate a stepwise effect of parity on cardiometabolic health and having two children or more than two
11
12 263 children already affects BMI, HDL cholesterol levels and cardiovascular risk factor prevalence.
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15
16 265 Our paper is the first study providing detailed assessment of cardiometabolic health development over
17
18 266 time in relation to parity. This longitudinal study comprised a large, well-phenotyped cohort with
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20 267 uniform assessment of all measurements during a median follow-up of 6 years. The GEE analysis which
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22 268 was performed, allowed us to assess differences among groups over time, focusing on group effects.
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24
25 269 However, several limitations need to be discussed. The mean age of women para > 2 was significantly
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27 270 higher than women who were nulliparous, para 1 or para 2. In addition, women para > 2 less often used
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29 271 oral contraceptives and more often used antihypertensive medication. This might result in a slightly
30
31 272 different metabolic profile although correction of the GEE-analysis for age and oral contraceptive use
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33 273 did not significantly change the results. Despite extensive phenotyping, age at first delivery, inter-
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35 274 pregnancy interval and lactation have not been assessed in the PREVEND study and therefore,
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37 275 adjustment of the analyses for these factors was not possible. Since parity itself was not assessed in the
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39 276 PREVEND, we used the number of children as a proxy for the number of childbirths. This might lead to
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41 277 inaccurate estimates of parity numbers, as the possibility of surrogacy or twin pregnancies is not taken
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43 278 into account. Additionally, no information was available regarding subfertility and several pregnancy
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45 279 complications, which leads to a lower number of children in these women and might reflect influence
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47 280 the cardiometabolic profile in later life as well. More extensive information regarding socio-economic
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49 281 status was not measured as well, thereby it was only possible to correct for education but not for other
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51 282 socio-economic factors. Lastly, pre-pregnancy BMI and gestational weight gain have not been assessed
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3 283 in the PREVEND study either, although their role on postpartum weight retention seemed limited in a
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5 284 recent publication.^{9,18}
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10 286 The PREVEND study was enriched with subjects with an elevated albumin excretion, which mostly
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12 287 results in an unfavorable cardiovascular risk profile compared to the general population. However,
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14 288 albuminuria did not significantly differ among the groups within our analyses. Although our findings
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16 289 suggest an effect of parity itself on metabolic parameters, it should be noted that causality cannot be
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18 290 determined in our study. Therefore, one could argue that the relationship is reversed, e.g. women with
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20 291 higher BMI or lower HDL cholesterol are more fertile and therefore have more children. Prospective
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22 292 research assessing pre-pregnancy determinants of cardiometabolic health are warranted to further
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24 293 assess the possible causal effect of pregnancy itself.
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30 295 **Conclusion**

31
32 296 In this longitudinal cohort study, higher parity is associated with higher BMI and lower HDL
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34 297 cholesterol. This difference among parity groups is constant over time. Furthermore, higher parity is
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36 298 associated with a higher prevalence of cardiovascular risk factors among the parity groups over time.
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38 299 These findings warrant for prospective research assessing determinants of cardiometabolic health at
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40 300 earlier age to understand the role of pregnancy and the influence of lifestyle factors in the development
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42 301 of cardiovascular disease in women.
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18 398 **Competing interests**

19
20 399 All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf
21
22 (available on request from the corresponding author) and declare no support from any organization for
23 400
24 the submitted work; no relationships with companies that might have an interest in the submitted work
25 401
26 in the previous 3 years; no other relationships or activities that could appear to have influenced the
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28 submitted work.
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32 404 **Author's contributions**

33
34 405
35 406 Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof, Ron T. Gansevoort, Henk Groen
36
37 and A.Titia Lely were involved in conception and design of the study. Data analyses was performed by
38 407
39 Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof and Henk Groen. Interpretation of the results
40 408
41 was performed by Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J. Groenhof, Ron T. Gansevoort, Henk
42 409
43 Groen, Arie Franx, Bas B. van Rijn and A.Titia Lely. Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J.
44 410
45 Groenhof and A.Titia Lely drafted the manuscript. Gerbrand A. Zoet, Nina D. Paauw, T. Katrien J.
46 411
47 Groenhof, Ron T. Gansevoort, Henk Groen, Arie Franx, Bas B. van Rijn and A.Titia Lely edited the
48 412
49 manuscript. All authors read and approved the final manuscript.
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3 415 **Data sharing statement**
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5 416 Data sharing: patient level data and full dataset and technical appendix and statistical code are available
6
7 417 from the corresponding author (g.zoet@umcutrecht.nl). Informed consent was not obtained but the
8
9 418 presented data are anonymized and the risk of identification is low.
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3 420 **Figure 1: Flowchart**

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422 **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/m ²)	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
HOMA _{1c}	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

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424 Data are presented as median (25th – 75th percentile) unless otherwise stated. BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic
425 blood pressure; ACEi/ARB: angiotensin converting enzyme inhibitor and/or angiotensin receptor blocker; HOMA_{1c}: homeostatic model
426 assessment index.

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

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

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3 **430 Figure 3: CVD risk factors at entry**
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8 432 Legend: Hypertension = RR \geq 140/90 mm/Hg and/or use of antihypertensive medication;

9 433 Obesity = BMI \geq 30kg/m²; Low HDL cholesterol = HDL cholesterol <1.29 mmol/L;

10 434 Diabetes = fasting plasma glucose \geq 7.0 mmol/L, self-report of a physician

11 435 diagnosis and/or use of glucose-lowering medication.

12 436 = first visit; = follow-up visit
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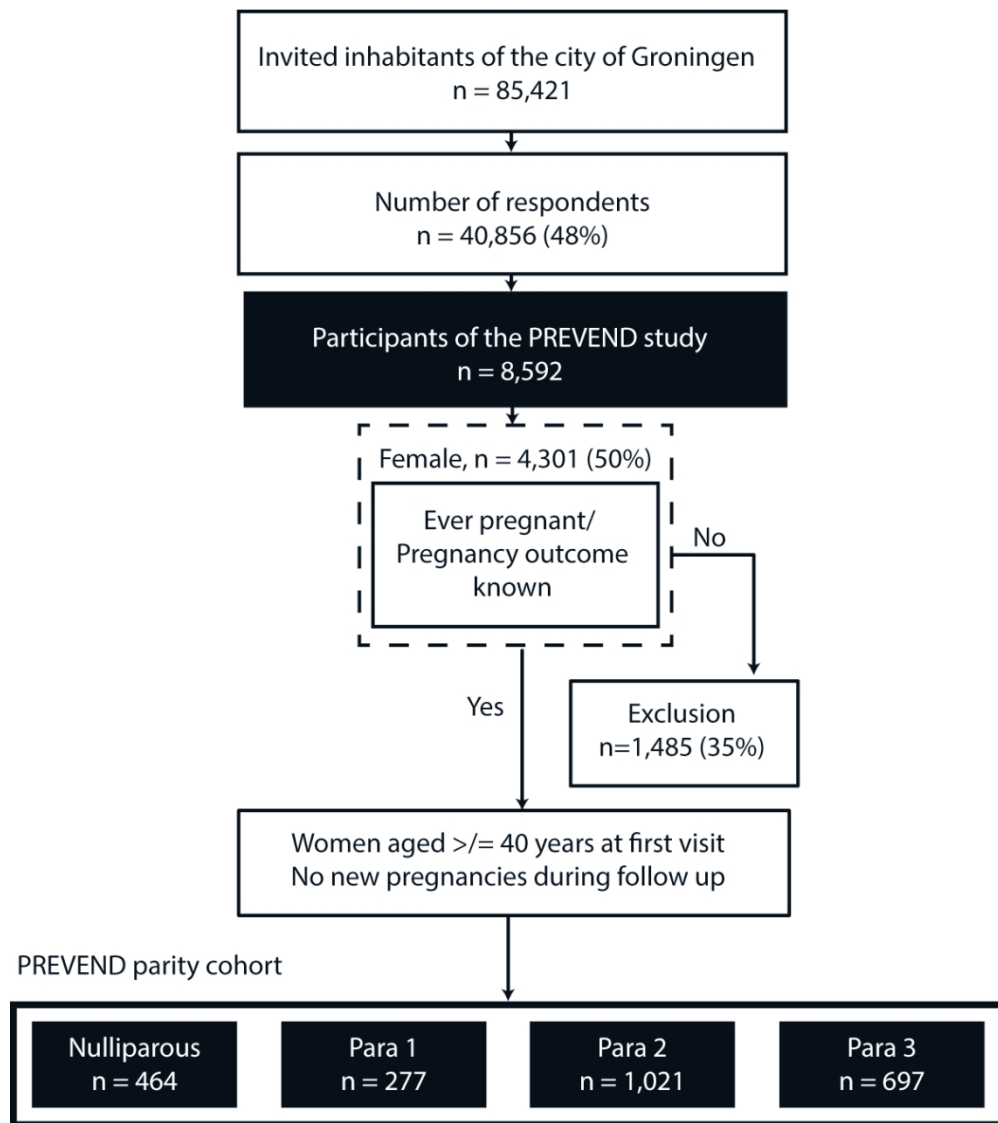


Figure 1: Flowchart

101x117mm (300 x 300 DPI)

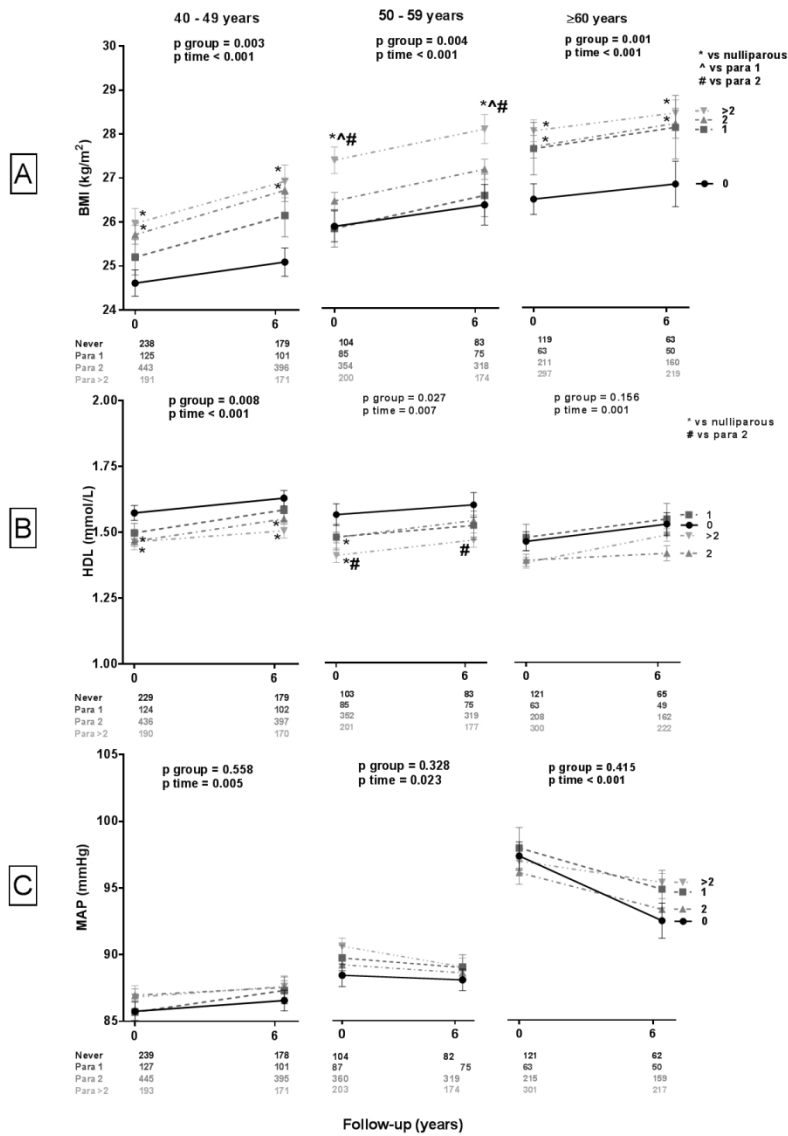


Figure 2: Development over time of BMI (A), HDL cholesterol (B) and MAP (C), stratified for parity

114x160mm (300 x 300 DPI)

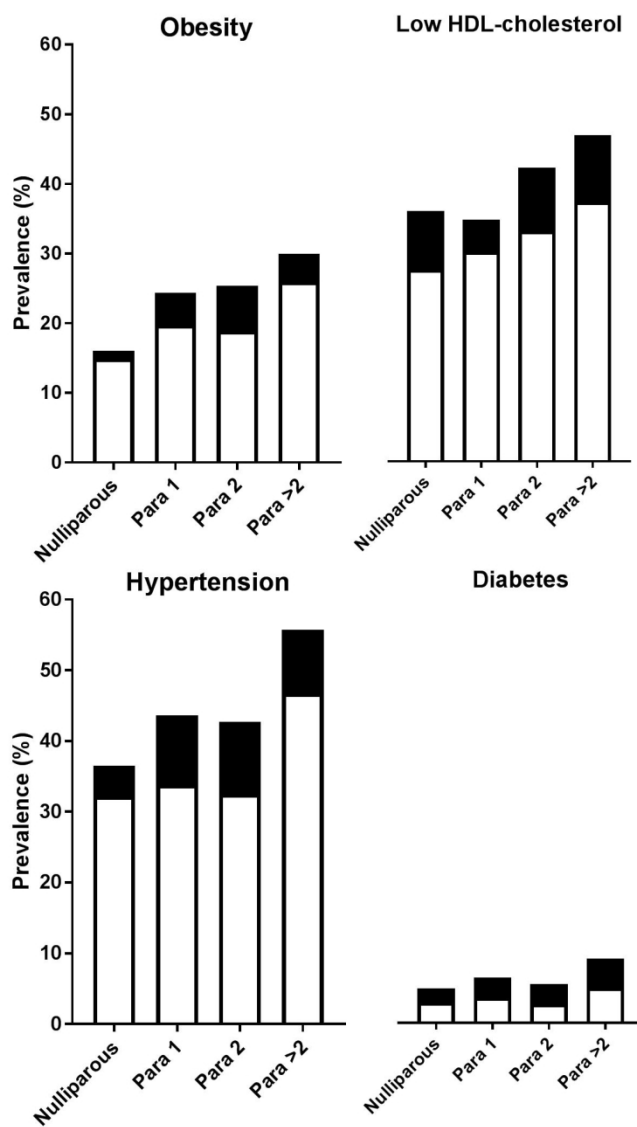


Figure 3: Development of CVD risk factors over time

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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	0.627	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

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.

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

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		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

1		#6b	For matched studies, give matching criteria and number of	na
2			exposed and unexposed	
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5	Variables	#7	Clearly define all outcomes, exposures, predictors, potential	6
6			confounders, and effect modifiers. Give diagnostic criteria, if	
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10	Data sources /	#8	For each variable of interest give sources of data and details of	6
11	measurement		methods of assessment (measurement). Describe	
12			comparability of assessment methods if there is more than one	
13			group. Give information separately for for exposed and	
14			unexposed groups if applicable.	
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18	Bias	#9	Describe any efforts to address potential sources of bias	5, 6, 7
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20				
21	Study size	#10	Explain how the study size was arrived at	5
22				
23	Quantitative	#11	Explain how quantitative variables were handled in the	5, 7
24	variables		analyses. If applicable, describe which groupings were chosen,	
25			and why	
26				
27				
28	Statistical	#12a	Describe all statistical methods, including those used to control	6, 7
29	methods		for confounding	
30				
31				
32		#12b	Describe any methods used to examine subgroups and	6, 7
33			interactions	
34				
35				
36		#12c	Explain how missing data were addressed	6, 7
37				
38		#12d	If applicable, explain how loss to follow-up was addressed	6, 7
39				
40				
41		#12e	Describe any sensitivity analyses	na
42				
43	Participants	#13a	Report numbers of individuals at each stage of study—eg	5
44			numbers potentially eligible, examined for eligibility, confirmed	
45			eligible, included in the study, completing follow-up, and	
46			analysed. Give information separately for for exposed and	
47			unexposed groups if applicable.	
48				
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51		#13b	Give reasons for non-participation at each stage	5
52				
53				
54		#13c	Consider use of a flow diagram	figure 1
55				
56	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	7, table
57			clinical, social) and information on exposures and potential	1
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1		confounders. Give information separately for exposed and	
2		unexposed groups if applicable.	
3			
4		#14b Indicate number of participants with missing data for each	Figure 2
5		variable of interest	
6			
7		#14c Summarise follow-up time (eg, average and total amount)	6, 8, 9
8			
9			
10	Outcome data	#15 Report numbers of outcome events or summary measures	7, 8, 9
11		over time. Give information separately for exposed and	
12		unexposed groups if applicable.	
13			
14			
15	Main results	#16a Give unadjusted estimates and, if applicable, confounder-	7, 8, 9
16		adjusted estimates and their precision (eg, 95% confidence	
17		interval). Make clear which confounders were adjusted for and	
18		why they were included	
19			
20			
21			
22		#16b Report category boundaries when continuous variables were	7, 8, 9
23		categorized	
24			
25			
26		#16c If relevant, consider translating estimates of relative risk into	na
27		absolute risk for a meaningful time period	
28			
29			
30	Other analyses	#17 Report other analyses done—e.g., analyses of subgroups and	na
31		interactions, and sensitivity analyses	
32			
33			
34	Key results	#18 Summarise key results with reference to study objectives	9, 10
35			
36	Limitations	#19 Discuss limitations of the study, taking into account sources of	11, 12
37		potential bias or imprecision. Discuss both direction and	
38		magnitude of any potential bias.	
39			
40			
41	Interpretation	#20 Give a cautious overall interpretation considering objectives,	See note
42		limitations, multiplicity of analyses, results from similar studies,	1
43		and other relevant evidence.	
44			
45			
46	Generalisability	#21 Discuss the generalisability (external validity) of the study	See note
47		results	2
48			
49			
50	Funding	#22 Give the source of funding and the role of the funders for the	3
51		present study and, if applicable, for the original study on which	
52		the present article is based	
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Author notes

1. 10, 11, 12, 13

1 2. 10, 11, 12, 13

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