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Pre-pregnant lipid levels and number of children: a prospective population-based cohort study

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Abstract

Objective: To study pre-pregnant serum lipid levels and association with number of children.

Design: Prospective population-based cohort.

Setting: Linked data from Cohort of Norway and The Medical Birth Registry of Norway.

Participants: 2 645 women giving birth to their first child during 1994 - 2003 (488 one-child mothers and 2157 women with ≥ 2 births) and 1 677 nulliparous women.

Main outcome measures: Odds ratios (ORs) for no and one lifetime pregnancy (relative to ≥ 2 pregnancies) obtained by multinomial logistic regression; adjusted for age at examination, education, body mass index (BMI), smoking, time since last meal and oral contraceptive use.

Results: Assessed in quintiles, higher pre-pregnant triglyceride (TG) and triglyceride to high density lipoprotein (TG/HDL-c) ratio levels were associated with increased risk of one lifetime pregnancy compared to having ≥ 2 children. Compared to the highest quintile, women in the lowest quintile of HDL cholesterol levels had an increased risk of one lifetime pregnancy (OR 1.7 95% CI 1.2-2.4), as were women with the highest low density lipoprotein (LDL) cholesterol, TG and TG/HDL-c ratio quintiles (compared to the lowest) (OR 1.2 95% CI 0.8-1.7; OR 2.2 95% CI 1.5-3.2; and OR 2.2 95% CI 1.5-3.2, respectively). Similar effects were found in women with BMI ≥ 25 and the highest LDL and total cholesterol levels in risk of lifetime nulliparity.

Conclusion: Women with unfavorable pre-pregnant lipid profile had higher risk of having no or only one child. These findings substantiate an association between pre-pregnant serum lipid levels and number of children. Previously observed associations between low parity and

increased cardiovascular mortality may in part be due to preexisting cardiovascular disease lipid risk factors.

Key words: Pre-pregnant lipid levels, TG/HDL ratio, maternal health, parity, female fertility.

Strengths and limitations of this study

- A large population-based health study with pre-pregnant health data.
- Linkage with the Medical Birth Registry of Norway provides complete registration of total reproduction.
- The study lack data on family planning, dietary intake and duration of oral contraceptive use, therefore the possibility of unmeasured confounding by those factors cannot be ruled out.
- Non-fasting lipid measurements were used in the study, however, adjustments in our analyses for time since last meal did not change the results.

Introduction

Cardiovascular disease (CVD) is an important public health problem and remains as the number one cause of death in women.(1) Reproductive history is important in evaluating health risks in women, as pregnancy may unmask a woman's predisposition for CVD.(1) Several studies have reported increased CVD mortality among women with no or only one lifetime pregnancy.(2, 3, 4) Efforts to elucidate the association between number of children and the risk of female CVD have been inconclusive.(1, 3) Proposed explanations are lifestyle risk factors associated with childrearing (5), sex hormone fluctuations, protective effect of future pregnancies,(3) lifestyle factors prior to conception such as elevated blood pressure and obesity (6) as well as metabolic irregularities triggered by gestation.(1) Detection of high density lipoprotein (HDL) cholesterol and apolipoprotein B (ApoB) in follicular fluid from oocytes (7, 8) suggests a relation between lipids and female reproductive function. More recent studies have reported associations between lipids and fertility in both sexes.(9) Low parity (as a feature of subfecundity) and cardiovascular events may share common pathophysiological mechanisms.(10)

While the role of serum lipids in cardio metabolic health is well established, showing low HDL and high triglycerides (TGs) to be strong predictors of CVD,(11) their role in reproduction is uncertain. It is also uncertain whether women with no or one lifetime pregnancy have a higher CVD risk to begin with, or whether future pregnancies may reduce the CVD risk.

We pursued this question by exploring the extent to which pre-pregnant serum lipid levels of total, HDL and low density lipoprotein (LDL) cholesterol, TG and TG/HDL-c ratio are associated with having no and one lifetime pregnancy.

Materials and Methods

Study Design and Population

We used linked data from Cohort of Norway (CONOR) and the Medical Birth Registry of Norway (MBRN). CONOR is a population-based collection of health data and blood samples provided by participators older than 20 years of age residing in several different regions in Norway during 1994 to 2003.(12) Our subset included women with no children at the time of examination with standardized measurements of height, weight, and non-fasting lipids levels. Lifestyle factors were obtained through an extensive questionnaire that collected self-reported information on smoking, oral contraceptive (OC) use, self-reported status on receipt of social security benefits, attained level of education and various life style factors.(12) Education in Norway consists of primary school (7 years), lower secondary school (3 years), upper secondary school (3 years) and higher education. The first 10 years are obligatory.

The MBRN has since 1967 recorded data on all deliveries in the country after 16th week of gestation.(13) Based on mandatory notification, midwives and doctors report information using standard forms throughout pregnancy and at the time of delivery. The registry includes demographic information, mother's health prior and during pregnancy, complications in pregnancy and perinatal outcome. Using the unique national identification number given to all Norwegian citizens, each woman was linked to all her subsequent births (if any) after participating in CONOR. Women reporting no children in CONOR at the time of examination and with no valid records in the MBRN were considered having no pregnancies.

Women with baseline assessment of lifestyle factors in CONOR were linked to the MBRN. We defined one-child mothers as women being 6 years out from their first pregnancy and with no additional births registered in the MBRN.

Preconception measurements

Non-fasting blood samples were analyzed on a Hitachi 911 Autoanalyzer (Hitachi, Mito, Japan).(12) Applied reagents were from Boehringer Mannheim (Manheim, Germany). Serum concentrations of total cholesterol, HDL cholesterol and TG were analyzed subsequent to sampling. The total cholesterol, HDL cholesterol levels and TGs were measured by an enzymatic method. The day-to-day coefficients of variation were 2.4% and 0.7-1.3% for total cholesterol, HDL cholesterol and TG, respectively. To calculate LDL, we used the Friedewald formula (14): Total serum cholesterol minus HDL cholesterol minus one fifth of the triglyceride concentration. LDL cholesterol levels were calculated only for participants with TG concentrations below 4.5mmol/l.(6, 14) Accordingly, TG/HDL-c ratio was expressed as mmol/l.

Trained personnel measured height and weight with the participants wearing light clothes and no shoes; measurements were taken as follows: - height to the nearest 1.0 cm and weight to the nearest 0.5 kg. Body mass index (BMI) was calculated as weight in kilogram/(height in meters)^2.

Statistical analyses

Characteristics of the analyzed women were presented as means with standard deviations for continuous data and as number with percentages for categorical data. Differences between

nulliparous women, one-child mothers and mothers with two or more children, as well as pre-pregnant health status were analyzed by Chi-square tests and T tests where appropriate. Linear associations across pre-pregnant lipid levels (in quintiles) for no and one lifetime pregnancy were assessed by p-values for trend. Odds ratios (OR) of no and one lifetime pregnancy by lipid levels and TG/HDL-c ratio, when compared to women with two or more pregnancies were calculated using multinomial logistic regression and adjusted for mother's age at examination, level of education (categorized in: <11 years and >11 years of education), smoking (current smoker: yes, no), time since last meal, OC use (now, previously, never) and BMI (linear term). To extend each woman's likelihood of completing her birth record, we separately examined women who were 7 years out from their first pregnancy. About 95% of Norwegian women will complete their second pregnancy within 7 years.(4) To test the effect of (pre-pregnant) BMI, we stratified main analyses by BMI (<25 and ≥ 25). To avoid influence from age at first delivery on number of children, we excluded women older than 34 years at the time of first delivery in a sub analysis. Additionally, we performed sensitivity analyses including only mothers who were 22-30 years old at the time of first delivery. Using presence of a partner (ever) as a proxy for exposure to pregnancy among nulliparous women, we performed logistic regression in a sub-analysis (nulliparous vs. women with ≥ 2 births) including only women with a reported partner (ever). All analyses were performed using The Statistical Package for Social Sciences (SPSS, version 22.0 and 23.0, Chicago, Illinois).

Results

There were 4 743 women with baseline assessment of lifestyle factors in CONOR (1994-2003) that were linked to the MBRN. We excluded 421 women with pregnancy at the time of examination (n =139), unsure pregnancy status (n =157) and missing lipid assessments (n =125). Thus, 4 322 women were included in the analyses (1 677 nulliparous, 488 one-child mothers and 2 157 women with ≥ 2 births, see Figure 1). Sub-analyses included only women with reported partners (228 nulliparous and 216 mothers with ≥ 2 births).

Characteristics of the included women are given in Table 1. Nulliparous women were older at the time of examination, had higher BMI and were more frequent smokers compared to women with two or more births. A higher proportion of nulliparous women had >11 years of education. One-child mothers had higher mean age both at examination and at delivery (29.5 vs. 26.7 and 32.3 vs. 29.9, respectively), were more often smokers and had lower education than mothers with ≥ 2 births. The mean BMI prior to pregnancy was higher in one-child mothers (24.2 vs. 23.5), whereas mean years from examination to first delivery were similar for the two groups. Women with no and one child were less frequent users of OCs at the time of examination compared to mothers with ≥ 2 births (27.4%, 34.6% and 48.9%, respectively).

The proportion of diabetes at first delivery in one-child mothers was higher than in women with two or more births (1.4% versus 0.9%, p =0.30). Polycystic Ovary Syndrome (PCOS) was rare and we only had three cases in our material. A significantly higher number of one-child mothers had in-vitro fertilization (IVF) in their first pregnancy (7.2% versus 2.6% in women with ≥ 2 births, p< 0.001) (data not shown). This latter finding remained after excluding mothers older than 34 years at first delivery.

OR's with 95% CI's for no and one lifetime pregnancy (vs. ≥ 2 lifetime pregnancies) by lipid levels (in quintiles) are presented in Table 2 and Figure 2. Significant trends in ORs for one lifetime pregnancy across TG and TG/HDL-c ratio quintiles were observed (p trend = 0.01). OR for having one lifetime pregnancy for women with the highest TG quintile compared to the lowest quintile was 2.2 (95% CI 1.5-3.2). ORs for having one lifetime pregnancy for women with TG/HDL-c ratio levels in the two highest quintiles were 1.7 (95% CI 1.2-2.5) and 2.2 (95% CI 1.5-3.2), respectively, compared to the lowest quintile. There were no significant trends for LDL cholesterol, total cholesterol or HDL cholesterol, although ORs of one lifetime pregnancy for the lowest HDL quintile were 1.7 (95% CI 1.2-2.4) and for the highest LDL quintile 1.2 (95% CI 0.8-1.7). We found no increased risk of being nulliparous by serum lipid levels except for the highest LDL and total cholesterol levels and these estimates were not persuasive (ORs 1.2 [95% CI 0.9-1.6] and 1.2 [95% CI 0.9-1.5], respectively). Truncation of data to extend the time for each woman to complete her birth record (to 7 years) did not appreciably alter the results, neither did exclusion of women older than 34 years at the time of first delivery nor the additional restriction of our analyses to mothers aged 22-30 years at first pregnancy. The similar effects of pre-pregnant lipids as in one child mothers were observed when sub analysis (nulliparous vs. ≥ 2 births) were performed on women who had a partner (as a proxy for ever being exposed to pregnancy). For women with partner, the risk of having no children was increased among the women in the highest quintiles of TG and TH/HDL-c ratio (compared to the lowest quintiles) and also for those in the lowest HDL quintile (compared to the highest) (OR 1.9, 95% CI 0.9-4.2; OR 2.0, 95% CI 1.0-4.1; and OR 1.6, 95% CI 0.7-3.6, respectively).

Stratified analyses by BMI are presented in Table 3. In women with BMI ≥ 25 there were significant trends in ORs of having no children or one child across increasing levels of pre-

pregnant total cholesterol, TG and TG/HDL-c ratio quintiles (p trend= 0.04 and < 0.001, respectively). The adjusted ORs of one lifetime pregnancy for women with BMI ≥ 25 and TG levels in the two highest quintiles were 2.1 (95% CI 0.9-4.8) and 3.5 (95% CI 1.6-7.4) and for the two highest TG/HDL-c ratio quintiles 3.1 (95% CI 1.3-7.4) and 4.3 (95% CI 1.9-10.0) compared to women in the lowest respective quintile. The risk of one lifetime pregnancy was also significantly increased for women with BMI ≥ 25 and the highest LDL and total cholesterol as well as the lowest HDL quintiles (ORs 1.8 [95% CI 0.8-3.8]; 1.2 [95% CI 0.6-2.4] and 2.6 [95% CI 1.3-5.3], respectively). Similarly, ORs of having no pregnancy (in women with BMI ≥ 25) were 1.7 (95% CI 1.0-3.0), 2.8 (95% CI 1.7-4.7) and 3.6 (95% CI 2.1-6.1) for women with the highest LDL, TG and TG/HDL-c ratio quintiles, respectively, compared to women with the lowest quintile. Increased risk of having no children was also found for the overweight and obese women with the lowest HDL quintile (OR 1.9, 95%CI 1.2-3.0). Unlike in one-child mothers, risk of having no pregnancy among overweight and obese women with higher total cholesterol levels only slightly changed from the main results. In women with pre-pregnant BMI < 25, there were significant trends in risk of having one lifetime pregnancy across increasing levels of pre-pregnant TG (p trend=0.04), TG/HDL-c ratio (p trend=0.04) and HDL quintiles (p trend=0.05). There were increased risks of one lifetime pregnancy in the highest TG quintile (OR 1.9, 95% CI 1.2-3.0) and the two highest TG/HDL-c ratio quintiles (OR 1.6, 95% CI 1.0-2.4 and 1.8, 95% CI 1.2-2.8, respectively), as well as the lowest HDL quintile (OR: 1.7, 95% CI: 1.1-2.6). Risks of no and one lifetime pregnancy with higher LDL and total cholesterol levels only slightly changed compared to our main results.

Discussion

Pre-pregnant lipid levels were associated with having one lifetime pregnancy. Women with high levels of LDL, TG and TG/HDL-c ratio as well as low HDL levels, measured years before conception, were at increased risk of having only one lifetime pregnancy. High levels of LDL and total cholesterol were associated with having no children, while in overweight and obese women this was true for all the lipids examined.

These findings provide a possible biological underpinning for a joint mechanistic pathway for reduced fertility and cardiovascular conditions.(10) Our study suggests that the previously observed association between low parity and increased CVD risk may be confounded by preexisting adverse lipid levels. This does not support the hypothesis that having additional pregnancies reduces CVD risk.(3) Rather, unfavorable lipid profiles may be related to both subfertility and later cardiovascular disease.

There is a lack of studies evaluating the relation between preconception lipid levels and fertility in women. The LIFE study found concentrations of free cholesterol to be associated with fecundity in both sexes.(9) In contrast to our study, TGs and total cholesterol were not found to be significant in individual and couple-based adjusted models (as well as two other measured lipid components: phospholipids and total lipids), however, authors used different study design and lipid measurement methods. In accordance with our findings is the Framingham Heart Study, which detected a trend towards TG elevation and lower HDL serum levels among women with self-reported infertility (as not achieving pregnancy for ≥ 1

year).(15) The presence of HDL cholesterol and ApoB in follicular fluid from human oocytes, suggests that these lipids play a direct role in reproduction.(7, 8, 16) Previous animal studies have reported association between dyslipidemia and infertility.(17) Posed explanations have been that abnormalities in HDL metabolism including change in structure, concentration or function compromise female fertility.(7, 8, 16) It has been suggested that genetic polymorphisms that alter function in proteins engaged in cholesterol metabolism may affect human fertility.(18, 19) A possible mechanism could be the mediating role of HDL on Paraoxonase 1 activity and related oxidative stress, a factor known to be associated with adverse cardiovascular and fertility outcomes.(19)

Recent insights suggest TG/HDL-c ratio to be a reliable marker of insulin resistance and atherogenicity,(20) highlighting its ability to identify insulin resistance in apparently healthy individuals.(21) Observed higher levels of TG/HDL-c ratio in our study are indicative of possible preexisting metabolic risk factors among women with one lifetime pregnancy, as well as subpopulation of nulliparous women (overweight, obese and with reported partners – as a proxy for exposed to pregnancy). This is also consistent with increasing rates of infertility in both sexes among population with metabolic syndrome.(9) The higher proportion of diabetes in this group of women further supports this notion. In agreement, the Japan Nurses Health study reported significant increase in risk of diabetes in young nulliparous women (<45 years of age) with ovarian infertility.(22) Accordingly, the Framingham Heart Study found infertile premenopausal women to have increased odds of diabetes and obesity.(15) Given the accompanying metabolic irregularities among major causes of female infertility,(15, 23) substantially higher proportion of IVF treatment among one-child mothers indirectly supports metabolic implications. The latter finding remains after exclusion of women older than 34 years at the time of first delivery.

In accordance with the literature,(23, 24) risk of having no and only one child showed strong effects in overweight and obese women (BMI ≥ 25) in stratified analyses (Table 3). Nevertheless, the higher risk of having only one child remained in normal weight women (BMI< 25) with the lowest HDL quintile and the highest TG and TG/HDL-c ratio quintiles. These findings mirror observations from the literature of metabolic irregularities among normal weight women as independent risk factor for future fertility impairments.(25, 26) The Life Study reported both female and male lipid concentrations to affect fecundity, irrespective of their BMI.(9)

Compared to women with two or more pregnancies, total cholesterol levels above clinically recommended range were associated with the risk of having no children, and this was irrespective of BMI. The Life Study reported greater percentage of women with a history of irregular menstrual cycles in the highest quartile of free cholesterol,(9) and the Japan Nurses Health Study found women with ovarian infertility to be at high risk of hypercholesterolemia.(22) In our study, total cholesterol levels were not associated with the risk of having one lifetime pregnancy, except in overweight and obese women. This could suggest that total cholesterol levels play varied roles in different subfecundity or infertility sub-types.

In our study, women with one lifetime pregnancy had poorer lifestyle factors (BMI, smoking), were older and less educated. Lower mean education among one-child mothers is in agreement with a Nordic demographic study,(27) which shows that later onset of childbearing is related to lower number of children finally born in women with low education. Given that educational level and occupation are key indicators of socioeconomic status,(28) observed

lower parity among women with low education could also reflect unfavorable socioeconomic position as a limiting factor to further pregnancies. However, a study exploring age at first birth, parity and post-reproductive mortality suggests that late childbearing in itself may be a signal of preexisting poor health of a woman.(29)

The observed risk differences between nulliparous women and one-child mothers in our main results (Figure 2, Table 2) could be explained by heterogeneity of causes for childlessness among nulliparous women in this cohort. The risk may, therefore, be diluted by low risk groups of women who are voluntary childless (30) or have not been exposed to pregnancy (ever). Given the lack of information on women's reproductive planning in our data, we tried to address this in a sub analysis including only women with reported partner (ever) as a proxy for being exposed to pregnancy. Here we found that the results for nulliparous women were similar to our main results for one-child mothers. Women with reported partner had higher risk of having no children (compared to partnered women with ≥ 2 births) if their TG and TG/HDL-c ratio levels were in the highest quintiles and HDL in the lowest quintile (OR 1.9, 0.9-4.2; OR 2.0, 95% CI 1.0-4.1; and OR 1.6, 95% CI 0.7-3.6, respectively). These findings support the role of serum lipids in lifetime nulliparity among women with partners.

Our subset of women was from a large population-based health study with pre-pregnant health data. Linked data from the MBRN provided complete registration of total reproduction. The prospective design minimized the potential for bias. A weakness is that blood sampling was performed in non-fasting state. Studies show that TG levels are sensitive to recent food intake, while cholesterol levels seem to be less affected.(31) We addressed this by adjusting our analyses for time since last meal and the main results were unchanged, suggesting that

non-fasting lipids are not likely to introduce a systematic bias. Non-fasting lipids have successfully been used in lipid and CVD research.(9, 32, 33) No assessments of duration or temporal proximity of OC use, dietary intake or stress were available, therefore unmeasured confounding by those factors cannot be ruled out. Smoking adversely influences female fertility,(34) with most of its effect attributed to HDL cholesterol decrease.(35) We accounted for this in our analyses; however, smoking status of participants was only available at enrollment in the survey. The ethnic homogeneity of the included women may reduce generalizability of our findings.

Unfavorable pre-pregnant lipid levels were associated with having no and one lifetime pregnancy. Women's metabolic homeostasis is important for reproduction and also has cardio metabolic implications.(25, 36) Preexisting poor lipid and metabolic profiles could represent one of the possible linkages between previously observed reduced fertility and later cardiovascular disease.

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Contributors

All authors had full access to the data and are responsible for the integrity of the data. AP, RS, LD and NHM designed the study. AP and NHM conducted the analyses; NHM created the

figure and AP created the tables and the flow chart. AP drafted the manuscript. NHM, RS and LD reviewed the preliminary analyses and initial draft and provided critical comments.

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Competing interest statement

None declared. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author).

Ethical approval

The study was approved by the regional ethical review board REK-Vest (Ref number 2013/118) and access to data was granted by the Steering Committee for CONOR and by the MBRN.

Data sharing statement

Due to the confidentiality requirements according to Norwegian low, data set containing personal data and other information on the individual level (CONOR, MBRN) cannot be

made public. Researchers who are interested in analyzing data from the CONOR cohort or MBRN may apply to the appropriate organizations (after having obtained the obligatory permits according to Norwegian low).

Figure Legends

Figure 1

Norwegian women examined in Cohort of Norway (CONOR) before conception of their first pregnancy and with linked data from the Medical Birth Registry of Norway (MBRN).

Figure 2

Odds ratios (OR) with 95% confidence interval (95%CI) for no and one lifetime pregnancy (reference: women with ≥ 2 pregnancies) by TG/HDL-c ratio quintiles in 4 322 women in Cohort of Norway, 1994-2003. The estimates were obtained by multinomial logistic regression and adjusted for age at examination, educational level, smoking, time since last meal, oral contraceptive use and body mass index (linear term).

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Table 1 Characteristics of 4 322 Norwegian women in Cohort of Norway, 1994-2003 with: no, one or ≥ 2 children. Values are numbers (percentages) unless stated otherwise.

Mean values	1 677 no child	488 one child	2 157 ≥2 children
Age (SD) at examination	30.5 (2.1)	29.5 (5.2)	26.7 (4.0)
Age (SD) at first delivery	-	32.3 (4.9)	29.9 (3.8)
Years (SD)-examination to first pregnancy	-	3.7 (2.1)	4.1 (2.3)
Body mass index (SD) at examination*	24.8 (5.1)	24.2(4.5)	23.5 (3.4)
Oral contraceptive use*			
now	455 (27.4)	168 (34.6)	1047 (48.9)
previously	724 (43.5)	239 (49.2)	779 (36.4)
never	484 (29.1)	79 (16.3)	317 (14.8)
Smoking at examination*			
yes	537 (32.2)	182 (37.4)	462 (21.5)
no	1 132 (67.8)	304 (62.6)	1 685 (78.5)
Education*			
<11 years	312 (18.8)	127 (26.3)	300 (14.1)
≥11 years	1 344 (81.2)	356 (73.7)	1 834 (85.9)

SD = standard deviation; *missing data on smoking: 8 nulliparous, 2 one child mothers and 10 women with ≥ 2 children,; education: 21 nulliparous, 5 one child mothers and 23 women with ≥ 2 children; BMI: 10 nulliparous; OC use: 4 nulliparous, 2 one child mothers and 14 women with ≥2 children.

Table 2 Odds ratios (OR) with 95% confidence interval (95%CI) for no and one lifetime pregnancy (reference: women with ≥ 2 pregnancies) by pre-pregnant lipid quintiles in 4 322 women in Cohort of Norway, 1994-2003. The estimates were obtained by multinomial logistic regression and adjusted for age at examination, educational level, smoking, time since last meal, oral contraceptive use and body mass index (linear term).

Lipid quintiles** in mmol/l	n (%)	n[a] (%)	n[b] (%)	N	Nulliparous OR (95%CI)	One child mothers OR (95%CI)	P for trend
LDL cholesterol*							
<2.42	449 (56.9)	261 (33.0)	80 (10.1)	790	1.0 reference	1.0 reference	
2.43-2.84	433 (52,3)	309 (37.4)	85 (10.3)	827	1.1 (0.9-1.4)	1.0 (0.7-1.4)	
2.85-3.24	454 (52.2)	325 (37.4)	90 (10.4)	869	1.0 (0.8-1.3)	0.9 (0.7-1.3)	0.82
3.25-3.76	426 (48.0)	353 (39.8)	108 (12.2)	887	1.1 (0.7-1.2)	1.1 (0.8-1.6)	
>3.77	391 (41.9)	421 (45.1)	121 (13.0)	933	1.2 (0.7-1.1)	1.2 (0.8-1.7)	
Total cholesterol							
<4.19	432 (55.0)	259 (33.0)	94 (12.0)	785	1.0 reference	1.0 reference	
4.20-4.61	456 (54.6)	304 (36.4)	75 (9.0)	835	1.2 (0.9-1.5)	0.8 (0.5-1.1)	
4.62-5.0	434 (52.2)	306 (36.8)	91 (11.0)	831	1.0 (0.8-1.3)	0.9 (0.6-1.2)	
5.1-5.63	442 (45.7)	415 (43.0)	109 (11.3)	966	1.3 (1.0-1.7)	1.0 (0.7-1.4)	0.26
>5.64	393 (43.4)	393 (43.4)	119 (13.1)	905	1.2 (0.9-1.6)	1.0 (0.7-1.4)	
TG (Triglyceride)							
<0.66	429 (48.6)	372 (42.2)	81 (9.2)	882	1.0 reference	1.0 reference	
0.67-0.86	447 (49.8)	350 (39.0)	100 (11.1)	897	0.9 (0.7-1.1)	1.2 (0.8-1.7)	
0.87-1.09	455 (54.4)	294 (35.1)	88 (10.5)	837	0.9 (0.7-1.2)	1.3 (0.9-1.9)	0.01
1.10-1.45	452 (53.6)	303 (35.9)	88 (10.4)	843	1.0 (0.8-1.3)	1.4 (1.0-2.0)	
>1.46	373 (43.3)	358 (41.6)	130 (15.1)	861	1.1 (0.9-1.5)	2.2 (1.5-3.2)	
HDL cholesterol							
<1.20	326 (47.5)	263 (38.3)	97 (14.1)	686	1.0 (0.8-1.3)	1.7 (1.2-2.4)	
1.21-1.40	271 (44.7)	260 (42.9)	75 (12.4)	606	1.0 (0.8-1.2)	1.2 (0.8-1.7)	
1.41-1.60	634 (53.0)	431 (36.1)	130 (10.9)	1195	1.0 (0.7-1.3)	1.2 (0.9-1.6)	0.18
1.61-1.84	443 (49.7)	356 (40.0)	92 (10.3)	891	0.9 (0.7-1.2)	1.1 (0.8-1.5)	
>1.85	483 (51.2)	367 (38.9)	94 (10.0)	944	1.0 reference	1.0 reference	

Number of women: with ≥2 children (n, reference group), nulliparous women (n[a]), one child mothers (n[b]), total women within category (N); *missing data within lipids on 16 cases of LDL and 2 cases of TG; ** Quintiles calculated on a total sample prior to pregnancy.

Table 3 Odds ratios (OR) with 95% confidence interval (95%CI) for no and one lifetime pregnancy (reference: women with ≥ 2 pregnancies) by pre-pregnant lipid quintiles in 4 322 women in Cohort of Norway, 1994-2003. The estimates were obtained by multinomial logistic regression, presented stratified by body mass index (BMI) and adjusted for age at examination, educational level, smoking, time since last meal and oral contraceptive use.

	Women with pre-pregnant BMI < 25							Women with pre-pregnant BMI ≥ 25						
Lipid quintiles ** in mmol/l	n	n[a]	n[b]	N	Nulliparous OR (95%CI)	One child mothers OR (95%CI)	p for trend	n	n[a]	n[b]	N	Nulliparous OR (95%CI)	One child mothers OR (95%CI)	p for trend
LDL cholesterol*														
<2.42	377	219	69	665	1.0 reference	1.0 reference		72	41	11	124	1.0 reference	1.0 reference	
2.43-2.84	343	240	64	647	1.1 (0.9-1.5)	0.9 (0.6-1.4)	0.84	90	68	20	178	1.1 (0.6-2.1)	1.4 (0.6-3.3)	0.44
2.85-3.24	341	216	67	624	1.0 (0.7-1.3)	0.9 (0.6-1.3)		113	109	23	245	1.5 (0.8-2.6)	1.4 (0.6-3.3)	
3.25-3.76	315	198	69	582	1.0 (0.8-1.4)	1.1 (0.7-1.6)		111	152	39	302	1.7 (1.0-3.0)	1.8 (0.8-4.0)	
>3.77	230	187	64	481	1.2 (0.9-1.6)	1.2 (0.8-1.8)		158	233	57	448	1.7 (1.0-3.0)	1.8 (0.8-3.8)	
Total cholesterol														
<4.19	359	198	75	632	1.0 reference	1.0 reference		73	60	19	152	1.0 reference	1.0 reference	
4.20-4.61	341	231	57	629	1.4 (1.1-1.9)	0.8 (0.5-1.2)	0.13	115	73	17	205	0.6 (0.3-1.0)	0.7 (0.3-1.5)	0.04
4.62-5.0	332	191	64	587	1.0 (0.7-1.3)	0.8 (0.5-1.2)		102	114	27	243	1.3 (0.8-2.2)	1.2 (0.6-2.5)	
5.1-5.63	327	244	72	643	1.3 (0.9-1.7)	0.9 (0.6-1.4)		114	167	37	318	1.5 (0.9-2.5)	1.2 (0.6-2.4)	
>5.64	248	196	66	510	1.3 (0.9-1.7)	1.0 (0.7-1.5)		143	197	53	393	1.2 (0.7-2.0)	1.2 (0.6-2.4)	
TG (Triglyceride)														
<0.66	363	311	69	743	1.0 reference	1.0 reference		66	59	12	137	1.0 reference	1.0 reference	
0.67-0.86	364	248	82	694	0.8 (0.6-1.0)	1.2 (0.5-2.8)	0.04	83	102	18	203	1.7 (1.0 -3.0)	1.2 (0.5-2.8)	<0.001
0.87-1.09	339	208	65	612	0.9 (0.7-1.2)	1.3 (0.7-3.8)		115	85	23	223	1.6 (0.9-2.8)	1.7 (0.7-3.8)	
1.10-1.45	319	156	58	533	0.8 (0.6-1.1)	1.3 (0.9-4.8)		133	146	30	309	2.5 (1.5- 4.4)	2.1 (0.9-4.8)	
>1.46	222	137	59	418	1.0 (0.7-1.4)	2.0 (1.7-7.4)		149	219	70	438	2.8 (1.7- 4.7)	3.5 (1.6-7.4)	
HDL cholesterol														
<1.20	198	70	47	315	0.9 (0.7-1.2)	1.7 (1.1-2.6)		127	191	50	368	1.9 (1.2-3.0)	2.6 (1.3-5.3)	
1.21-1.40	189	138	43	370	0.9 (0.7-1.1)	1.0 (0.6-1.5)	0.05	81	122	31	234	1.7 (1.0-2.8)	2.3 (1.1-4.9)	0.17
1.41-1.60	475	287	93	855	0.8 (0.6-1.1)	1.1 (0.8-1.5)		158	143	37	338	1.4 (0.9-2.2)	1.8 (0.9-3.6)	
1.61-1.84	358	272	74	704	0.7 (0.5-1.0)	1.0 (0.7-1.4)		85	82	18	185	1.4 (0.8-2.4)	1.8 (0.8-4.0)	
>1.85	387	293	77	757	1.0 reference	1.0 reference		96	73	17	186	1.0 reference	1.0 reference	
TG/HDL-c ratio														
<0.39	374	321	70	765	1.0 reference	1.0 reference		67	42	10	119	1.0 reference	1.0 reference	
0.40-0.54	365	245	75	685	0.8 (0.6-1.0)	1.2 (0.8-1.7)	0.04	85	99	17	201	2.2 (1.2-4.0)	1.9 (0.7-4.9)	<0.001
0.55-0.73	365	213	71	649	0.8 (0.6-1.1)	1.2 (0.8-1.8)		101	91	22	214	2.4 (1.3-4.3)	2.2 (0.9-5.5)	
0.74-1.04	281	172	64	517	0.9 (0.7-1.2)	1.6 (1.1-2.4)		138	131	33	302	3.3 (1.9-5.9)	3.1 (1.3-7.4)	
>1.05	222	109	53	384	0.8 (0.6-1.0)	1.8 (1.2-2.8)		155	248	71	474	3.6 (2.1- 6.1)	4.3 (1.9-10.0)	

Number of women: with ≥2 children (n, reference group), nulliparous women (n[a]), one child mothers (n[b]), total women within category (N); *missing data within lipids on 16 cases of LDL, 2 cases of TG and 2 cases of TG/HDL-c ratio; ** Quintiles calculated on a total sample prior to pregnancy.

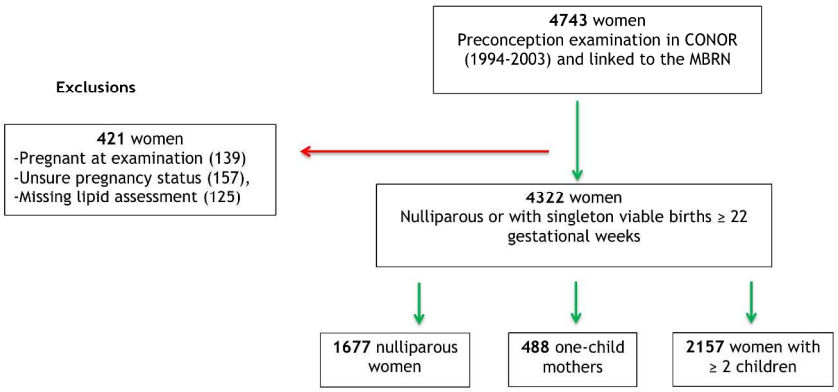

Figure 1

297x209mm (300 x 300 DPI)

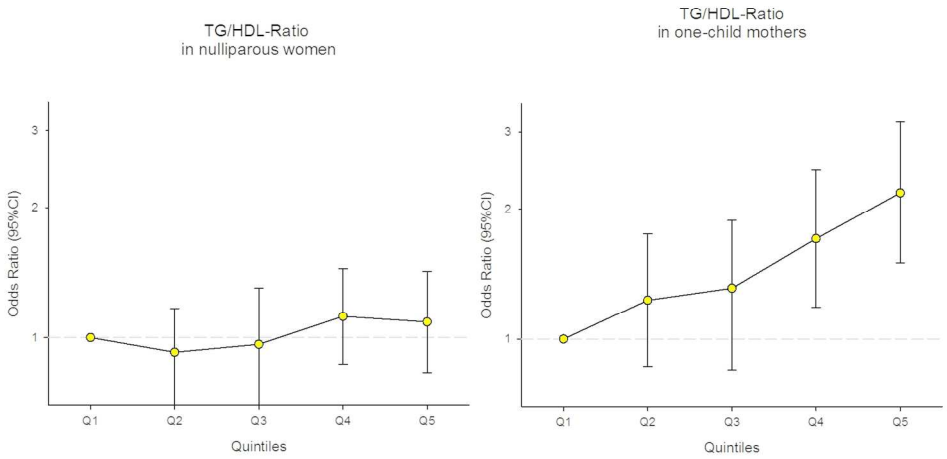

Figure 2

296x209mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4, 5
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6, 7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7, 8
Study size	10	Explain how the study size was arrived at	9, Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7, 9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	6, 7, 9
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	8
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	Included – Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10
		(b) Indicate number of participants with missing data for each variable of interest	9, Tables 1, 2, 3
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	9, 10, 11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9, 10, 11
		(*b*) Report category boundaries when continuous variables were categorized	Tables 1, 2, 3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12, 13, 14, 15, 16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Women's pre-pregnancy lipid levels and number of children: a Norwegian prospective population-based cohort study

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Women's pre-pregnancy lipid levels and number of children: a Norwegian prospective population-based cohort study

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Abstract

Objective: To study pre-pregnancy serum lipid levels and association with number of children.

Design: Prospective population-based cohort.

Setting: Linked data from Cohort of Norway and The Medical Birth Registry of Norway.

Participants: 2 645 women giving birth to their first child during 1994 - 2003 (488 one-child mothers and 2157 women with ≥ 2 births) and 1 677 nulliparous women.

Main outcome measures: Odds ratios (ORs) for no and one lifetime pregnancy (relative to ≥ 2 pregnancies) obtained by multinomial logistic regression; adjusted for age at examination, education, body mass index (BMI), smoking, time since last meal and oral contraceptive use.

Results: Assessed in quintiles, higher pre-pregnant triglyceride (TG) and triglyceride to high density lipoprotein (TG/HDL-c) ratio levels were associated with increased risk of one lifetime pregnancy compared to having ≥ 2 children. Compared to the highest quintile, women in the lowest quintile of HDL cholesterol levels had an increased risk of one lifetime pregnancy (OR 1.7 95% CI 1.2-2.4), as were women with the highest low density lipoprotein (LDL) cholesterol, TG and TG/HDL-c ratio quintiles (compared to the lowest) (OR 1.2 95% CI 0.8-1.7; OR 2.2 95% CI 1.5-3.2; and OR 2.2 95% CI 1.5-3.2, respectively). Similar effects were found in women with BMI ≥ 25 and the highest LDL and total cholesterol levels in risk of lifetime nulliparity.

Conclusion: Women with unfavorable pre-pregnant lipid profile had higher risk of having no or only one child. These findings substantiate an association between pre-pregnant serum lipid

levels and number of children. Previously observed associations between low parity and increased cardiovascular mortality may in part be due to preexisting cardiovascular disease lipid risk factors.

Key words: Pre-pregnant lipid levels, TG/HDL ratio, maternal health, parity, female fertility.

Strengths and limitations of this study

- Large population-based study with data collected before pregnancy.
- Linkage with the Medical Birth Registry of Norway providing complete registration of total reproduction.
- Limitations include lack of data on family planning, dietary intake, duration of oral contraceptive use, APOE genotype, low-grade inflammation and thyroid status.
- Non-fasting lipid measurements were used, however, adjustments in our analyses for time since last meal did not change the results.

Introduction

Cardiovascular disease (CVD) is an important public health problem and remains as the number one cause of death in women.(1) Reproductive history is important in evaluating health risks in women, as pregnancy may unmask a woman's predisposition for CVD.(1) Several studies have reported increased CVD mortality among women with no or only one lifetime pregnancy.(2, 3, 4) Efforts to elucidate the association between number of children and the risk of female CVD have been inconclusive.(1, 3) Proposed explanations are lifestyle risk factors associated with childrearing (5), sex hormone fluctuations, protective effect of future pregnancies,(3) lifestyle factors prior to conception such as elevated blood pressure and obesity (6) as well as metabolic irregularities triggered by gestation.(1) Detection of high density lipoprotein (HDL) cholesterol and apolipoprotein B (ApoB) in follicular fluid from oocytes (7, 8) suggests a relation between lipids and female reproductive function. More recent studies have reported associations between lipids and fertility in both sexes.(9) Low parity (as a feature of subfecundity) and cardiovascular events may share common pathophysiological mechanisms.(10)

While the role of serum lipids in cardio metabolic health is well established, showing low HDL and high triglycerides (TGs) to be strong predictors of CVD,(11) their role in reproduction is uncertain. It is also uncertain whether women with no or one lifetime pregnancy have a higher CVD risk to begin with, or whether future pregnancies may reduce the CVD risk.

We pursued this question by exploring the extent to which pre-pregnant serum lipid levels of total, HDL and low density lipoprotein (LDL) cholesterol, TG and TG/HDL-c ratio are associated with having no and one lifetime pregnancy.

Materials and Methods

Study Design and Population

We used linked data from Cohort of Norway (CONOR) and the Medical Birth Registry of Norway (MBRN). CONOR is a population-based collection of health data and blood samples provided by participators older than 20 years of age residing in several different regions in Norway during 1994 to 2003.(12) Our subset included women with no children at the time of examination with standardized measurements of height, weight, and non-fasting lipids levels. Lifestyle factors were obtained through an extensive questionnaire that collected self-reported information on smoking, oral contraceptive (OC) use, self-reported status on receipt of social security benefits, attained level of education and various life style factors.(12) Education in Norway consists of primary school (7 years), lower secondary school (3 years), upper secondary school (3 years) and higher education. The first 10 years are obligatory.

The MBRN has since 1967 recorded data on all deliveries in the country after 16th week of gestation.(13) Based on mandatory notification, midwives and doctors report information using standard forms throughout pregnancy and at the time of delivery. The registry includes demographic information, mother's health prior and during pregnancy, complications in pregnancy and perinatal outcome. Using the unique national identification number given to all Norwegian citizens, each woman was linked to all her subsequent births (if any) after participating in CONOR. Women reporting no children in CONOR at the time of examination and with no valid records in the MBRN were considered having no pregnancies.

Women with baseline assessment of lifestyle factors in CONOR were linked to the MBRN. We defined one-child mothers as women being 6 years out from their first pregnancy and with no additional births registered in the MBRN.

Preconception measurements

Non-fasting blood samples were analyzed on a Hitachi 911 Autoanalyzer (Hitachi, Mito, Japan).(12) Applied reagents were from Boehringer Mannheim (Manheim, Germany). Serum concentrations of total cholesterol, HDL cholesterol and TG were analyzed subsequent to sampling. The total cholesterol, HDL cholesterol levels and TGs were measured by an enzymatic method. The day-to-day coefficients of variation were 2.4% and 0.7-1.3% for total cholesterol, HDL cholesterol and TG, respectively. To calculate LDL, we used the Friedewald formula (14): Total serum cholesterol minus HDL cholesterol minus one fifth of the triglyceride concentration. LDL cholesterol levels were calculated only for participants with TG concentrations below 4.5mmol/l.(6, 14) Accordingly, TG/HDL-c ratio was expressed as mmol/l.

Trained personnel measured height and weight with the participants wearing light clothes and no shoes; measurements were taken as follows: - height to the nearest 1.0 cm and weight to the nearest 0.5 kg. Body mass index (BMI) was calculated as weight in kilogram/(height in meters)^2.

Patients and Public Involvement

Patients or public were not directly involved in this study. The detailed explanation of the recruitment process and the obtainment of written informed consent for CONOR was provided elsewhere.(12)

Statistical analyses

Characteristics of the analyzed women were presented as means with standard deviations for continuous data and as number with percentages for categorical data. Differences between nulliparous women, one-child mothers and mothers with two or more children, as well as pre-pregnant health status were analyzed by Chi-square tests and T tests where appropriate. Linear associations across pre-pregnant lipid levels (in quintiles) for no and one lifetime pregnancy were assessed by p-values for trend. Odds ratios (OR) of no and one lifetime pregnancy by lipid levels and TG/HDL-c ratio, when compared to women with two or more pregnancies were calculated using multinomial logistic regression and adjusted for mother's age at examination, level of education (categorized in: <11 years and >11 years of education), smoking (current smoker: yes, no), time since last meal, OC use (now, previously, never) and BMI (linear term). To extend each woman's likelihood of completing her birth record, we separately examined women who were 7 years out from their first pregnancy. About 95% of Norwegian women will complete their second pregnancy within 7 years.(4) To test the effect of (pre-pregnant) BMI, we stratified main analyses by BMI (<25 and ≥ 25). To avoid influence from age at first delivery on number of children, we excluded women older than 34 years at the time of first delivery in a sub analysis. Additionally, we performed sensitivity analyses including only mothers who were 22-30 years old at the time of first delivery. Using presence of a partner (ever) as a proxy for exposure to pregnancy among nulliparous women, we performed logistic regression in a sub-analysis (nulliparous vs. women with ≥ 2 births)

including only women with a reported partner (ever). All analyses were performed using The Statistical Package for Social Sciences (SPSS, version 22.0 and 23.0, Chicago, Illinois).

Results

There were 4 743 women with baseline assessment of lifestyle factors in CONOR (1994-2003) that were linked to the MBRN. We excluded 421 women with pregnancy at the time of examination (n =139), unsure pregnancy status (n =157) and missing lipid assessments (n =125). Thus, 4 322 women were included in the analyses (1 677 nulliparous, 488 one-child mothers and 2 157 women with ≥ 2 births, see Figure 1). Sub-analyses included only women with reported partners (228 nulliparous and 216 mothers with ≥ 2 births).

Characteristics of the included women are given in Table 1. Nulliparous women were older at the time of examination, had higher BMI and were more frequent smokers compared to women with two or more births. A higher proportion of nulliparous women had >11 years of education. One-child mothers had higher mean age both at examination and at delivery (29.5 vs. 26.7 and 32.3 vs. 29.9, respectively), were more often smokers and had lower education than mothers with ≥ 2 births. The mean BMI prior to pregnancy was higher in one-child

mothers (24.2 vs. 23.5), whereas mean years from examination to first delivery were similar for the two groups. Women with no and one child were less frequent users of OCs at the time of examination compared to mothers with ≥ 2 births (27.4%, 34.6% and 48.9%, respectively).

The proportion of diabetes at first delivery in one-child mothers was higher than in women with two or more births (1.4% versus 0.9%, p =0.30). Polycystic Ovary Syndrome (PCOS) was rare and we only had three cases in our material. A significantly higher number of one-child mothers had in-vitro fertilization (IVF) in their first pregnancy (7.2% versus 2.6% in women with ≥ 2 births, p< 0.001) (data not shown). This latter finding remained after excluding mothers older than 34 years at first delivery.

OR's with 95% CI's for no and one lifetime pregnancy (vs. ≥ 2 lifetime pregnancies) by lipid levels (in quintiles) are presented in Table 2 and Figure 2. Significant trends in ORs for one lifetime pregnancy across TG and TG/HDL-c ratio quintiles were observed (p trend = 0.01). OR for having one lifetime pregnancy for women with the highest TG quintile compared to the lowest quintile was 2.2 (95% CI 1.5-3.2). ORs for having one lifetime pregnancy for women with TG/HDL-c ratio levels in the two highest quintiles were 1.7 (95% CI 1.2-2.5) and 2.2 (95% CI 1.5-3.2), respectively, compared to the lowest quintile. There were no significant trends for LDL cholesterol, total cholesterol or HDL cholesterol, although ORs of one lifetime pregnancy for the lowest HDL quintile were 1.7 (95% CI 1.2-2.4) and for the highest LDL quintile 1.2 (95% CI 0.8-1.7). We found no increased risk of being nulliparous by serum lipid levels except for the highest LDL and total cholesterol levels and these estimates were not persuasive (ORs 1.2 [95% CI 0.9-1.6] and 1.2 [95% CI 0.9-1.5], respectively). Truncation of data to extend the time for each woman to complete her birth record (to 7 years) did not appreciably alter the results, neither did exclusion of women older than 34 years at the time of first delivery nor the additional restriction of our analyses to mothers aged 22-30 years at first pregnancy. The similar effects of pre-pregnant lipids as in

one child mothers were observed when sub analysis (nulliparous vs. ≥ 2 births) were performed on women who had a partner (as a proxy for ever being exposed to pregnancy). For women with partner, the risk of having no children was increased among the women in the highest quintiles of TG and TH/HDL-c ratio (compared to the lowest quintiles) and also for those in the lowest HDL quintile (compared to the highest) (OR 1.9, 95% CI 0.9-4.2; OR 2.0, 95% CI 1.0-4.1; and OR 1.6, 95% CI 0.7-3.6, respectively).

Stratified analyses by BMI are presented in Table 3. In women with BMI ≥ 25 there were significant trends in ORs of having no children or one child across increasing levels of pre-pregnant total cholesterol, TG and TG/HDL-c ratio quintiles (p trend= 0.04 and < 0.001, respectively). The adjusted ORs of one lifetime pregnancy for women with BMI ≥ 25 and TG levels in the two highest quintiles were 2.1 (95% CI 0.9-4.8) and 3.5 (95% CI 1.6-7.4) and for the two highest TG/HDL-c ratio quintiles 3.1 (95% CI 1.3-7.4) and 4.3 (95% CI 1.9-10.0) compared to women in the lowest respective quintile. The risk of one lifetime pregnancy was also significantly increased for women with BMI ≥ 25 and the highest LDL and total cholesterol as well as the lowest HDL quintiles (ORs 1.8 [95% CI 0.8-3.8]; 1.2 [95% CI 0.6-2.4] and 2.6 [95% CI 1.3-5.3], respectively). Similarly, ORs of having no pregnancy (in women with BMI ≥ 25) were 1.7 (95% CI 1.0-3.0), 2.8 (95% CI 1.7-4.7) and 3.6 (95% CI 2.1-6.1) for women with the highest LDL, TG and TG/HDL-c ratio quintiles, respectively, compared to women with the lowest quintile. Increased risk of having no children was also found for the overweight and obese women with the lowest HDL quintile (OR 1.9, 95%CI 1.2-3.0). Unlike in one-child mothers, risk of having no pregnancy among overweight and obese women with higher total cholesterol levels only slightly changed from the main results. In women with pre-pregnant BMI < 25, there were significant trends in risk of having one lifetime pregnancy across increasing levels of pre-pregnant TG (p trend=0.04), TG/HDL-c

ratio (p trend=0.04) and HDL quintiles (p trend=0.05). There were increased risks of one lifetime pregnancy in the highest TG quintile (OR 1.9, 95% CI 1.2-3.0) and the two highest TG/HDL-c ratio quintiles (OR 1.6, 95% CI 1.0-2.4 and 1.8, 95% CI 1.2-2.8, respectively), as well as the lowest HDL quintile (OR: 1.7, 95% CI: 1.1-2.6). Risks of no and one lifetime pregnancy with higher LDL and total cholesterol levels only slightly changed compared to our main results.

Discussion

Pre-pregnant lipid levels were associated with having one lifetime pregnancy. Women with high levels of LDL, TG and TG/HDL-c ratio as well as low HDL levels, measured years before conception, were at increased risk of having only one lifetime pregnancy. High levels of LDL and total cholesterol were associated with having no children, while in overweight and obese women this was true for all the lipids examined.

These findings provide a possible biological underpinning for a joint mechanistic pathway for reduced fertility and cardiovascular conditions.(10) Our study suggests that the previously observed association between low parity and increased CVD risk may be confounded by preexisting adverse lipid levels. This does not support the hypothesis that having additional pregnancies reduces CVD risk.(3) Rather, unfavorable lipid profiles may be related to both subfertility and later cardiovascular disease.

There is a lack of studies evaluating the relation between preconception lipid levels and fertility in women. The LIFE study found concentrations of free cholesterol to be associated with fecundity in both sexes.(9) In contrast to our study, TGs and total cholesterol were not found to be significant in individual and couple-based adjusted models (as well as two other measured lipid components: phospholipids and total lipids), however, authors used different study design and lipid measurement methods. In accordance with our findings is the Framingham Heart Study, which detected a trend towards TG elevation and lower HDL serum levels among women with self-reported infertility (as not achieving pregnancy for ≥ 1 year).(15) The presence of HDL cholesterol and ApoB in follicular fluid from human oocytes, suggests that these lipids play a direct role in reproduction.(7, 8, 16) Previous animal studies have reported association between dyslipidemia and infertility.(17) Posed explanations have been that abnormalities in HDL metabolism including change in structure, concentration or function compromise female fertility.(7, 8, 16) It has been suggested that genetic polymorphisms that alter function in proteins engaged in cholesterol metabolism may affect human fertility.(18, 19) One of the possible molecular mechanisms could be through a mediating role of HDL on Paraoxonase 1 (PON1) activity. Paraoxonase (PON) is an HDL-associated enzyme that inhibits LDL oxidation, and thus protects cells from oxidative stress.(20) Its stability and binding affinity is strongly influenced by changes in shape and size of HDL particles.(21) These changes may lead to decreased antioxidative capacity and consecutively – oxidative stress. Oxidative stress is associated with adverse cardiovascular and fertility outcomes, including atherosclerosis, PCOS, preeclampsia, endometriosis and infertility .(19, 22) A recent study in women of reproductive age with upper normal ranges of thyroid-stimulating hormone has suggested a direct link between unfavorable lipid profile and increased oxidative membrane damage.(23)

Recent insights suggest TG/HDL-c ratio to be a reliable marker of insulin resistance and atherogenicity,(24) highlighting its ability to identify insulin resistance in apparently healthy individuals.(25) Observed higher levels of TG/HDL-c ratio in our study are indicative of possible preexisting metabolic risk factors among women with one lifetime pregnancy, as well as subpopulation of nulliparous women (overweight, obese and with reported partners – as a proxy for exposed to pregnancy). This is also consistent with increasing rates of infertility in both sexes among population with metabolic syndrome.(9) The higher proportion of diabetes in this group of women further supports this notion. In agreement, the Japan Nurses Health study reported significant increase in risk of diabetes in young nulliparous women (<45 years of age) with ovarian infertility.(26) Accordingly, the Framingham Heart Study found infertile premenopausal women to have increased odds of diabetes and obesity.(15) Given the accompanying metabolic irregularities among major causes of female infertility,(15, 27) substantially higher proportion of IVF treatment among one-child mothers indirectly supports metabolic implications. The latter finding remains after exclusion of women older than 34 years at the time of first delivery.

Dyslipidemia is associated with PCOS (28, 29, 30). However, we only identified 3 women with PCOS in our study sample. Thus, presence of subclinical forms or underreporting may be present.

In accordance with the literature,(27, 31) risk of having no and only one child showed strong effects in overweight and obese women (BMI ≥ 25) in stratified analyses (Table 3). Nevertheless, the higher risk of having only one child remained in normal weight women (BMI< 25) with the lowest HDL quintile and the highest TG and TG/HDL-c ratio quintiles. These findings mirror observations from the literature of metabolic irregularities among normal weight women as independent risk factor for future fertility impairments.(32, 33) The

Life Study reported both female and male lipid concentrations to affect fecundity, irrespective of their BMI.(9)

Compared to women with two or more pregnancies, total cholesterol levels above clinically recommended range were associated with risk of having no children, irrespective of BMI. The Life Study reported a higher percentage of women with a history of irregular menstrual cycles in the highest quartile of free cholesterol.(9) The Japan Nurses Health Study found women with ovarian infertility to be at high risk of hypercholesterolemia.(26) In our study, total cholesterol levels were not associated with risk of having one lifetime pregnancy, except among overweight and obese women. This could suggest that total cholesterol levels play varied roles in different subfecundity or infertility sub-types. In addition, nulliparous women in our study were older at examination and had higher BMI. Both age and obesity are associated with systemic oxidative stress.(19, 22) It is possible that in such physiologic environment clinically abnormal levels of certain lipids might activate additional pathologic processes that adversely affect reproductive function.(28)

In our study, women with one lifetime pregnancy had poorer lifestyle factors (BMI, smoking), were older and less educated. Lower mean education among one-child mothers is in agreement with a Nordic demographic study,(34) which shows that later onset of childbearing is related to lower number of children finally born in women with low education. Given that educational level and occupation are key indicators of socioeconomic status,(35) observed lower parity among women with low education could also reflect unfavorable socioeconomic position as a limiting factor to further pregnancies. However, a study exploring age at first

birth, parity and post-reproductive mortality suggests that late childbearing in itself may be a signal of preexisting poor health of a woman.(36)

The observed risk differences between nulliparous women and one-child mothers in our main results (Figure 2, Table 2) could be explained by heterogeneity of causes for childlessness among nulliparous women in this cohort. The risk may, therefore, be diluted by low risk groups of women who are voluntary childless (37) or have not been exposed to pregnancy (ever). Given the lack of information on women's reproductive planning in our data, we tried to address this in a sub analysis including only women with reported partner (ever) as a proxy for being exposed to pregnancy. Here we found that the results for nulliparous women were similar to our main results for one-child mothers. Women with reported partner had higher risk of having no children (compared to partnered women with ≥ 2 births) if their TG and TG/HDL-c ratio levels were in the highest quintiles and HDL in the lowest quintile (OR 1.9, 0.9-4.2; OR 2.0, 95% CI 1.0-4.1; and OR 1.6, 95% CI 0.7-3.6, respectively). These findings support the role of serum lipids in lifetime nulliparity among women with partners.

Strengths and limitations

Our subset of women was from a large population-based health study with pre-pregnant health data. Linked data from the MBRN provided complete registration of total reproduction. The prospective design minimized the potential for bias. A weakness is that blood sampling was performed in non-fasting state. Studies show that TG levels are sensitive to recent food intake, while cholesterol levels seem to be less affected.(38) We addressed this by adjusting our analyses for time since last meal and the main results were unchanged, suggesting that

non-fasting lipids are not likely to introduce a systematic bias. Non-fasting lipids have successfully been used in lipid and CVD research.(9, 39, 40)

The study lacked data on Apolipoprotein E genotype, CRP and thyroid tests/thyroid antibodies, factors that all are found to affect female fertility.(23, 41) No assessments of duration or temporal proximity of OC use, dietary intake or stress were available, therefore unmeasured confounding cannot be ruled out. Smoking adversely influences female fertility,(42) with most of its effect attributed to HDL cholesterol decrease.(43) We accounted for this in our analyses; however, smoking status of participants was only available at enrollment. The ethnic homogeneity of the included women may reduce generalizability of our findings.

Unfavorable pre-pregnant lipid levels were associated with having no and one lifetime pregnancy. Women's metabolic homeostasis is important for reproduction and also has cardio metabolic implications.(32, 44) Preexisting poor lipid and metabolic profiles could represent one of the possible linkages between previously observed reduced fertility and later cardiovascular disease.

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Contributors

All authors had full access to the data and are responsible for the integrity of the data. AP, RS, LD and NHM designed the study. AP and NHM conducted the analyses; NHM created the

figure and AP created the tables and the flow chart. AP drafted the manuscript. NHM, RS and LD reviewed the preliminary analyses and initial draft and provided critical comments.

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Competing interest statement

None declared. All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author).

Ethical approval

The study was approved by the regional ethical review board REK-Vest (Ref number 2013/118) and access to data was granted by the Steering Committee for CONOR and by the MBRN.

Data sharing statement

This data set contains personal data and cannot be made public due to confidentiality requirements according to Norwegian legislations. Researchers who are interested in

analyzing data from CONOR or the MBRN may apply to the appropriate organizations after having obtained all necessary approvals according to Norwegian legislations.

Figure Legends

Figure 1

Norwegian women examined in Cohort of Norway (CONOR) before conception of their first pregnancy and with linked data from the Medical Birth Registry of Norway (MBRN).

Figure 2

Odds ratios (OR) with 95% confidence interval (95%CI) for no and one lifetime pregnancy (reference: women with ≥ 2 pregnancies) by TG/HDL-c ratio quintiles in 4 322 women in Cohort of Norway, 1994-2003. The estimates were obtained by multinomial logistic regression and adjusted for age at examination, educational level, smoking, time since last meal, oral contraceptive use and body mass index (linear term).

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Table 1 Characteristics of 4 322 Norwegian women in Cohort of Norway, 1994-2003 with: no, one or ≥ 2 children. Values are numbers (percentages) unless stated otherwise.

Mean values	1 677 no child	488 one child	2 157 ≥2 children
Age (SD) at examination	30.5 (2.1)	29.5 (5.2)	26.7 (4.0)
Age (SD) at first delivery	-	32.3 (4.9)	29.9 (3.8)
Years (SD)-examination to first pregnancy	-	3.7 (2.1)	4.1 (2.3)
Body mass index (SD) at examination*	24.8 (5.1)	24.2(4.5)	23.5 (3.4)
Oral contraceptive use*			
now	455 (27.4)	168 (34.6)	1047 (48.9)
previously	724 (43.5)	239 (49.2)	779 (36.4)
never	484 (29.1)	79 (16.3)	317 (14.8)
Smoking at examination*			
yes	537 (32.2)	182 (37.4)	462 (21.5)
no	1 132 (67.8)	304 (62.6)	1 685 (78.5)
Education*			
<11 years	312 (18.8)	127 (26.3)	300 (14.1)
≥11 years	1 344 (81.2)	356 (73.7)	1 834 (85.9)

SD = standard deviation; *missing data on smoking: 8 nulliparous, 2 one child mothers and 10 women with ≥ 2 children,; education: 21 nulliparous, 5 one child mothers and 23 women with ≥ 2 children; BMI: 10 nulliparous; OC use: 4 nulliparous, 2 one child mothers and 14 women with ≥2 children.

Table 2 Odds ratios (OR) with 95% confidence interval (95%CI) for no and one lifetime pregnancy (reference: women with ≥ 2 pregnancies) by pre-pregnant lipid quintiles in 4 322 women in Cohort of Norway, 1994-2003. The estimates were obtained by multinomial logistic regression and adjusted for age at examination, educational level, smoking, time since last meal, oral contraceptive use and body mass index (linear term).

Lipid quintiles** in mmol/l	n (%)	n[a] (%)	n[b] (%)	N	Nulliparous OR (95%CI)	One child mothers OR (95%CI)	P for trend
LDL cholesterol*							
<2.42	449 (56.9)	261 (33.0)	80 (10.1)	790	1.0 reference	1.0 reference	
2.43-2.84	433 (52,3)	309 (37.4)	85 (10.3)	827	1.1 (0.9-1.4)	1.0 (0.7-1.4)	
2.85-3.24	454 (52.2)	325 (37.4)	90 (10.4)	869	1.0 (0.8-1.3)	0.9 (0.7-1.3)	0.82
3.25-3.76	426 (48.0)	353 (39.8)	108 (12.2)	887	1.1 (0.7-1.2)	1.1 (0.8-1.6)	
>3.77	391 (41.9)	421 (45.1)	121 (13.0)	933	1.2 (0.7-1.1)	1.2 (0.8-1.7)	
Total cholesterol							
<4.19	432 (55.0)	259 (33.0)	94 (12.0)	785	1.0 reference	1.0 reference	
4.20-4.61	456 (54.6)	304 (36.4)	75 (9.0)	835	1.2 (0.9-1.5)	0.8 (0.5-1.1)	
4.62-5.0	434 (52.2)	306 (36.8)	91 (11.0)	831	1.0 (0.8-1.3)	0.9 (0.6-1.2)	
5.1-5.63	442 (45.7)	415 (43.0)	109 (11.3)	966	1.3 (1.0-1.7)	1.0 (0.7-1.4)	0.26
>5.64	393 (43.4)	393 (43.4)	119 (13.1)	905	1.2 (0.9-1.6)	1.0 (0.7-1.4)	
TG (Triglyceride)							
<0.66	429 (48.6)	372 (42.2)	81 (9.2)	882	1.0 reference	1.0 reference	
0.67-0.86	447 (49.8)	350 (39.0)	100 (11.1)	897	0.9 (0.7-1.1)	1.2 (0.8-1.7)	
0.87-1.09	455 (54.4)	294 (35.1)	88 (10.5)	837	0.9 (0.7-1.2)	1.3 (0.9-1.9)	0.01
1.10-1.45	452 (53.6)	303 (35.9)	88 (10.4)	843	1.0 (0.8-1.3)	1.4 (1.0-2.0)	
>1.46	373 (43.3)	358 (41.6)	130 (15.1)	861	1.1 (0.9-1.5)	2.2 (1.5-3.2)	
HDL cholesterol							
<1.20	326 (47.5)	263 (38.3)	97 (14.1)	686	1.0 (0.8-1.3)	1.7 (1.2-2.4)	
1.21-1.40	271 (44.7)	260 (42.9)	75 (12.4)	606	1.0 (0.8-1.2)	1.2 (0.8-1.7)	
1.41-1.60	634 (53.0)	431 (36.1)	130 (10.9)	1195	1.0 (0.7-1.3)	1.2 (0.9-1.6)	0.18
1.61-1.84	443 (49.7)	356 (40.0)	92 (10.3)	891	0.9 (0.7-1.2)	1.1 (0.8-1.5)	
>1.85	483 (51.2)	367 (38.9)	94 (10.0)	944	1.0 reference	1.0 reference	

Number of women: with ≥2 children (n, reference group), nulliparous women (n[a]), one child mothers (n[b]), total women within category (N); *missing data within lipids on 16 cases of LDL and 2 cases of TG; ** Quintiles calculated on a total sample prior to pregnancy.

Table 3 Odds ratios (OR) with 95% confidence interval (95%CI) for no and one lifetime pregnancy (reference: women with ≥ 2 pregnancies) by pre-pregnant lipid quintiles in 4 322 women in Cohort of Norway, 1994-2003. The estimates were obtained by multinomial logistic regression, presented stratified by body mass index (BMI) and adjusted for age at examination, educational level, smoking, time since last meal and oral contraceptive use.

	Women with pre-pregnant BMI < 25							Women with pre-pregnant BMI ≥ 25						
Lipid quintiles ** in mmol/l	n	n[a]	n[b]	N	Nulliparous OR (95%CI)	One child mothers OR (95%CI)	p for trend	n	n[a]	n[b]	N	Nulliparous OR (95%CI)	One child mothers OR (95%CI)	p for trend
LDL cholesterol*														
<2.42	377	219	69	665	1.0 reference	1.0 reference		72	41	11	124	1.0 reference	1.0 reference	
2.43-2.84	343	240	64	647	1.1 (0.9-1.5)	0.9 (0.6-1.4)	0.84	90	68	20	178	1.1 (0.6-2.1)	1.4 (0.6-3.3)	0.44
2.85-3.24	341	216	67	624	1.0 (0.7-1.3)	0.9 (0.6-1.3)		113	109	23	245	1.5 (0.8-2.6)	1.4 (0.6-3.3)	
3.25-3.76	315	198	69	582	1.0 (0.8-1.4)	1.1 (0.7-1.6)		111	152	39	302	1.7 (1.0-3.0)	1.8 (0.8-4.0)	
>3.77	230	187	64	481	1.2 (0.9-1.6)	1.2 (0.8-1.8)		158	233	57	448	1.7 (1.0-3.0)	1.8 (0.8-3.8)	
Total cholesterol														
<4.19	359	198	75	632	1.0 reference	1.0 reference		73	60	19	152	1.0 reference	1.0 reference	
4.20-4.61	341	231	57	629	1.4 (1.1-1.9)	0.8 (0.5-1.2)	0.13	115	73	17	205	0.6 (0.3-1.0)	0.7 (0.3-1.5)	0.04
4.62-5.0	332	191	64	587	1.0 (0.7-1.3)	0.8 (0.5-1.2)		102	114	27	243	1.3 (0.8-2.2)	1.2 (0.6-2.5)	
5.1-5.63	327	244	72	643	1.3 (0.9-1.7)	0.9 (0.6-1.4)		114	167	37	318	1.5 (0.9-2.5)	1.2 (0.6-2.4)	
>5.64	248	196	66	510	1.3 (0.9-1.7)	1.0 (0.7-1.5)		143	197	53	393	1.2 (0.7-2.0)	1.2 (0.6-2.4)	
TG (Triglyceride)														
<0.66	363	311	69	743	1.0 reference	1.0 reference		66	59	12	137	1.0 reference	1.0 reference	
0.67-0.86	364	248	82	694	0.8 (0.6-1.0)	1.2 (0.5-2.8)	0.04	83	102	18	203	1.7 (1.0 -3.0)	1.2 (0.5-2.8)	<0.001
0.87-1.09	339	208	65	612	0.9 (0.7-1.2)	1.3 (0.7-3.8)		115	85	23	223	1.6 (0.9-2.8)	1.7 (0.7-3.8)	
1.10-1.45	319	156	58	533	0.8 (0.6-1.1)	1.3 (0.9-4.8)		133	146	30	309	2.5 (1.5- 4.4)	2.1 (0.9-4.8)	
>1.46	222	137	59	418	1.0 (0.7-1.4)	2.0 (1.7-7.4)		149	219	70	438	2.8 (1.7- 4.7)	3.5 (1.6-7.4)	
HDL cholesterol														
<1.20	198	70	47	315	0.9 (0.7-1.2)	1.7 (1.1-2.6)		127	191	50	368	1.9 (1.2-3.0)	2.6 (1.3-5.3)	
1.21-1.40	189	138	43	370	0.9 (0.7-1.1)	1.0 (0.6-1.5)	0.05	81	122	31	234	1.7 (1.0-2.8)	2.3 (1.1-4.9)	0.17
1.41-1.60	475	287	93	855	0.8 (0.6-1.1)	1.1 (0.8-1.5)		158	143	37	338	1.4 (0.9-2.2)	1.8 (0.9-3.6)	
1.61-1.84	358	272	74	704	0.7 (0.5-1.0)	1.0 (0.7-1.4)		85	82	18	185	1.4 (0.8-2.4)	1.8 (0.8-4.0)	
>1.85	387	293	77	757	1.0 reference	1.0 reference		96	73	17	186	1.0 reference	1.0 reference	
TG/HDL-c ratio														
<0.39	374	321	70	765	1.0 reference	1.0 reference		67	42	10	119	1.0 reference	1.0 reference	
0.40-0.54	365	245	75	685	0.8 (0.6-1.0)	1.2 (0.8-1.7)	0.04	85	99	17	201	2.2 (1.2-4.0)	1.9 (0.7-4.9)	<0.001
0.55-0.73	365	213	71	649	0.8 (0.6-1.1)	1.2 (0.8-1.8)		101	91	22	214	2.4 (1.3-4.3)	2.2 (0.9-5.5)	
0.74-1.04	281	172	64	517	0.9 (0.7-1.2)	1.6 (1.1-2.4)		138	131	33	302	3.3 (1.9-5.9)	3.1 (1.3-7.4)	
>1.05	222	109	53	384	0.8 (0.6-1.0)	1.8 (1.2-2.8)		155	248	71	474	3.6 (2.1- 6.1)	4.3 (1.9-10.0)	

Number of women: with ≥2 children (n, reference group), nulliparous women (n[a]), one child mothers (n[b]), total women within category (N); *missing data within lipids on 16 cases of LDL, 2 cases of TG and 2 cases of TG/HDL-c ratio; ** Quintiles calculated on a total sample prior to pregnancy.

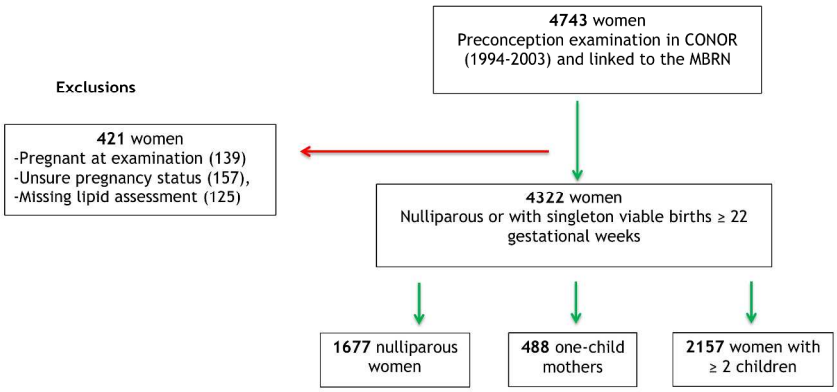

Figure 1

297x209mm (300 x 300 DPI)

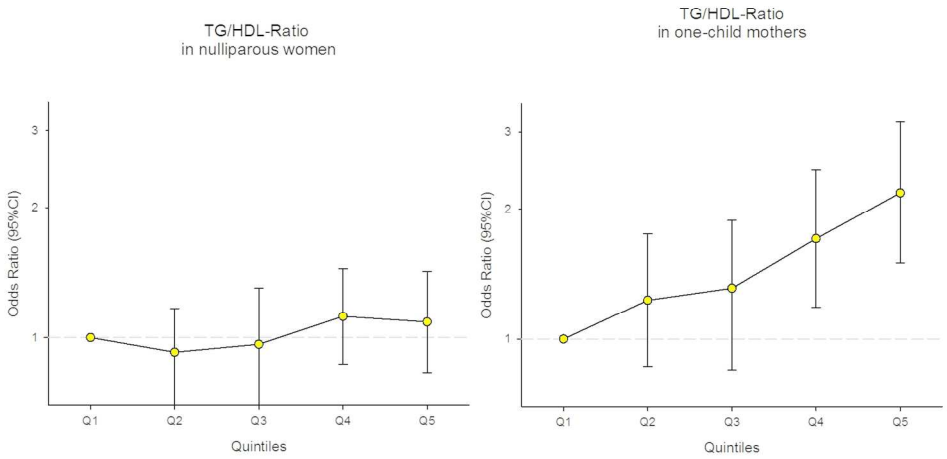

Figure 2

296x209mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4, 5
Methods			
Study design	4	Present key elements of study design early in the paper	6, 7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6, 7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6, 7
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7
Bias	9	Describe any efforts to address potential sources of bias	7, 8
Study size	10	Explain how the study size was arrived at	9, Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 7, 9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	8
		(*c*) Explain how missing data were addressed	6, 7, 9
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	8
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	Included – Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 10
		(b) Indicate number of participants with missing data for each variable of interest	9, Tables 1, 2, 3
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	9, 10, 11
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9, 10, 11
		(*b*) Report category boundaries when continuous variables were categorized	Tables 1, 2, 3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12, 13, 14, 15, 16
Generalisability	21	Discuss the generalisability (external validity) of the study results	16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.