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ORIGINAL ARTICLE ***Reproductive epidemiology***

Body mass index and subfertility: multivariable regression and Mendelian randomization analyses in the Norwegian Mother, Father and Child Cohort Study

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STUDY QUESTION: What is the association between BMI and subfertility?

SUMMARY ANSWER: We observed a J-shaped relationship between BMI and subfertility in both sexes, when using both a standard multivariable regression and Mendelian randomization (MR) analysis.

WHAT IS KNOWN ALREADY: High BMI in both women and men is associated with subfertility in observational studies and this relationship is further substantiated by a few small randomized controlled trials of weight reduction and success of assisted reproduction. Women with low BMI also have lower conception rates with assisted reproduction technologies.

STUDY DESIGN, SIZE, DURATION: Cohort study (the Norwegian Mother, Father and Child Cohort Study), 28 341 women and 26 252 men, recruited from all over Norway between 1999 and 2008.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women (average age 30, average BMI 23.1 kg/m^2) and men (average age 33, average BMI 25.5 kg/m^2) had available genotype data and provided self-reported information on time-to-pregnancy and BMI. A total of 10% of couples were subfertile (time-to-pregnancy $\geq$12 months).

MAIN RESULTS AND THE ROLE OF CHANCE: Our findings support a J-shaped association between BMI and subfertility in both sexes using multivariable logistic regression models. Non-linear MR validated this relationship. A 1 kg/m^2 greater genetically predicted BMI was linked to 18% greater odds of subfertility (95% CI 5% to 31%) in obese women ($\geq$30.0 kg/m^2) and 15% lower odds of subfertility (−24% to −2%) in women with BMI <20.0 kg/m^2. A 1 kg/m^2 higher genetically predicted BMI was linked to 26% greater odds of subfertility (8–48%) among obese men. Low genetically predicted BMI values were also related to greater subfertility risk in men at the lower end of the BMI distribution. A genetically predicted BMI of 23 and 25 kg/m^2 was linked to the lowest subfertility risk in women and men, respectively.

LIMITATIONS, REASONS FOR CAUTION: The main limitations of our study were that we did not know whether the subfertility was driven by the women, men or both; the exclusive consideration of individuals of northern European ancestry; and the limited amount of participants with obesity or BMI values <20.0 kg/m^2.

WIDER IMPLICATIONS OF THE FINDINGS: Our results support a causal effect of obesity on subfertility in women and men. Our findings also expand the current evidence by indicating that individuals with BMI values <20 kg/m^2 may have an increased risk of subfertility. These results suggest that BMI values between 20 and 25 kg/m^2 are optimal for a minimal risk of subfertility.

STUDY FUNDING/COMPETING INTEREST(S): The MoBa Cohort Study is supported by the Norwegian Ministry of Health and Care Services and the Norwegian Ministry of Education and Research. This project received funding from the European Research Council under the European Union's Horizon 2020 research and innovation program (grant agreement No 947684). It was also partly supported by the Research Council of Norway through its Centres of Excellence funding scheme, project number 262700. Open Access funding was provided by the Folkehelseinstituttet/Norwegian Institute of Public Health. D.A.L. is a UK National Institute for Health Research Senior Investigator (NF-SI-0611-10196) and is supported by the US National Institutes of Health (R01 DK10324) and a European Research Council Advanced Grant (DevelopObese; 669545). The funders had no role in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. D.A.L. receives (or has received in the last 10 years) research support from National and International government and charitable bodies, Roche Diagnostics and Medtronic for research unrelated to the current work. The rest of the authors declare that no competing interests exist.

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Key words: BMI / subfertility / Mendelian randomization / multivariable regression / MoBa

Introduction

Body weight is associated with the ability to reproduce (Sallmén *et al.*, 2006; Silvestris *et al.*, 2018). In observational studies, high BMI in women is associated with greater risk of subfertility, commonly defined as trying to conceive without success for ≥12 months (Ramlau-Hansen *et al.*, 2007; van der Steeg *et al.*, 2008), or a lower success of assisted reproductive technology (Pinborg *et al.*, 2011). In addition, women with BMI <18.5 kg/m^2 have a lower chance of assisted reproduction success (Xiong *et al.*, 2020), supporting the hypothesis of a non-linear relationship between BMI and subfertility. Men with BMI ≥30 kg/m^2 are also more prone to present reduced fertility and fecundity rates (National Institute for Health and Care Excellence, 2013; Sundaram *et al.*, 2017) and experience decreased success of assisted reproductive technology (Campbell *et al.*, 2015; Mushtaq *et al.*, 2018). In addition, a non-linear, J-shaped association between BMI and surrogate indicators of subfertility such as oligozoospermia and azoospermia has been reported in previous studies (Sermondade *et al.*, 2013). However, findings from intervention trials contrast with those from observational studies. Weight loss after lifestyle modifications did not improve the success of assisted reproduction in two large trials in Nordic populations (Mutsaerts *et al.*, 2016; Einarsson *et al.*, 2017) although previous smaller studies suggested a beneficial effect (Best *et al.*, 2017). Moderate weight loss has only been shown to increase the rate of live births in spontaneous conceived pregnancies in one of these trials (Einarsson *et al.*, 2017) and particularly among women with anovulation due to polycystic ovary syndrome (Norman and Mol, 2018). Finally, although massive weight loss after bariatric surgery is linked to normalization of hormonal axes in women and men (Lee *et al.*, 2019; Snoek *et al.*, 2021), improvement in some surrogate indicators of fertility (more regular menstrual cycles in women, less erectile dysfunction in men) (Lee *et al.*, 2019; Snoek *et al.*, 2021) and a decrease in subfertility risk in women (Snoek *et al.*, 2021), its effects on fertility have been little evaluated in intervention trials in women (Grzegorczyk-Martin *et al.*, 2020) and it has been related to a reduction in sperm quality in men (Wood *et al.*, 2020).

BMI is closely linked to a broad range of other characteristics that are also related to subfertility (Collins and Rossi, 2015; Hart, 2016). In addition, although female and male BMI have shown independent effects on fertility (Ramlau-Hansen *et al.*, 2007; Sundaram *et al.*, 2017), the partner's BMI may also confound the role of BMI on this outcome as individuals with greater BMI values are more likely to have a partner with elevated BMI (assortative mating) (Silventoinen *et al.*, 2003). Thus, the independent causal relationship of female and male BMI on subfertility remains unclear. The use of complementary methodological approaches could contribute to a better understanding of this matter. Mendelian randomization (MR) uses genetic variants that are robustly related to an exposure (e.g. BMI) to retrieve the unconfounded effect of that exposure on an outcome (e.g. subfertility) (Lawlor *et al.*, 2008). Results from MR are less likely to be confounded by the socioeconomic and behavioral factors that commonly affect conventional regression analyses but, at the same time, are susceptible to bias due to weak instruments and horizontal pleiotropy (Davey Smith and Hemani, 2014). Given the different sources of bias between multivariable regression and MR, when findings agree, it increases confidence in the consistent results reflecting a causal effect (Lawlor *et al.*, 2016).

Our aim was to investigate the association between BMI and subfertility in women and men using multivariable logistic regression and MR.

Materials and methods

The Norwegian Mother, Father and Child Cohort Study

Our study included participants in the Mother, Father and Child Cohort Study (MoBa) (Magnus *et al.*, 2006; 2016). The MoBa Study is a population-based pregnancy cohort study conducted by the Folkehelseinstituttet/Norwegian Institute of Public Health. Participants were recruited from all over Norway from 1999 to 2008. The women consented to participation in 41% of the pregnancies. The cohort now

includes 114 500 children, 95 200 mothers and 75 200 fathers. The current study is based on version #12 of the quality-assured data. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data Protection Agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now based on regulations related to the Norwegian Health Registry Act.

For the current study, we defined a subsample of parents with available genotype data and pre-pregnancy information on BMI. The genotype data used in this study come from blood samples obtained from both parents during pregnancy (Paltiel *et al.*, 2014) and followed the pipeline described by Helgeland *et al.* (2019) regarding genotype calling, imputation and quality control. We have described our work according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting MR and cohort studies.

BMI

Maternal and paternal pre-pregnancy weight and height were reported in the questionnaire completed at recruitment and used to calculate BMI as weight in kilograms divided by the squared height in meters. Extreme BMI values <15 or $>60\,kg/m^2$ were excluded.

Genetic risk score for BMI

We used the results from the most recent genome-wide association study (GWAS) of BMI to create the genetic instrument in our analysis (Yengo *et al.*, 2018). This GWAS included approximately 700 000 individuals of European ancestry (none of them participated in the MoBa cohort) that yielded 941 independent single-nucleotide polymorphisms (SNPs) associated with BMI (Yengo *et al.*, 2018). Eight hundred ninety-six of the 941 SNPs were available in the MoBa genotype data. We computed a weighted genetic risk score (GRS) by multiplying the number of risk alleles by the effect estimate of each variant and dividing by the total number of SNPs (Choi *et al.*, 2020).

Subfertility

At the time of recruitment, women were asked whether the pregnancy was planned, and to provide information on how many months it had taken them to conceive (Magnus *et al.*, 2006). The answer options were less than 1 month, 1–2 months and 3 or more months. If the mother had used $\geq$3 months, she was asked to further specify exactly how many months the couple had been trying to conceive. Subfertility was defined as time-to-pregnancy $\geq$12 months or having used assisted reproductive technologies. Those reporting a time-to-pregnancy $<$12 months were included in the reference group. Participants with unplanned pregnancies were excluded from the main analyses.

Other variables

From the MoBa questionnaires, we gathered information on age (continuous), educational level (years of education equivalent to the US system (Rietveld *et al.*, 2013; Barrabés, 2016), continuous), cigarette smoking (never smokers, former smokers, having quitted smoking by 12th (mothers) or 18th gestational week (fathers) or being a current smoker) and previous number of deliveries (0, 1, 2 or $\geq$3).

Ethical approval

The MoBa cohort is conducted according to the Declaration of Helsinki for Medical Research involving Human Subjects. The data collection in MoBa is approved by the Norwegian Data Inspectorate. Participants provided a written informed consent before joining the cohort. This project was approved by the Regional Committee for Medical and Health Research Ethics of South/East Norway (reference: 2017/1362).

Statistical analyses

We used means and SDs to describe normally distributed continuous variables, medians and 1st–3rd quartiles for non-normally distributed continuous variables, and proportions for categorical variables. We assessed differences in baseline characteristics among subfertile and non-subfertile parents using *t*-tests for normally distributed continuous variables, Mann–Whitney *U*-tests for non-normally distributed continuous variables, and chi-squared tests in categorical variables.

We first evaluated the presence of a linear relationship between BMI and subfertility in women and men separately by standard logistic regressions. We examined the evidence for a non-linear association by assessing the relationship between a $1\,kg/m^2$ increase in measured BMI and subfertility odds in BMI categories defined by current WHO guidelines: underweight and normal-low weight ($<20.0\,kg/m^2$), normal weight ($20.0–24.9\,kg/m^2$), overweight ($25.0–29.9\,kg/m^2$) and obesity ($\geq30.0\,kg/m^2$). We also assessed whether a model using smoothed cubic splines ($K+4$ degrees of freedom) to model the relationship between BMI and subfertility fitted the data better than a simple linear term using a likelihood ratio test. All logistic regression models were adjusted for age, education years, smoking and number of previous deliveries. Models further adjusted for the partner's BMI were additionally performed as sensitivity analyses to minimize bias due to assortative mating. Clustered standard errors were computed in all models to account for dependency between women/men who participated with more than one pregnancy.

In the MR analyses, we used a linear regression model to obtain a genetically predicted BMI using the GRS for BMI as a predictor. We assessed the linear relationship between genetically predicted BMI and subfertility by logistic regression models. We explored non-linear associations by investigating the association between a $1\,kg/m^2$ increase in the genetically predicted BMI and subfertility within residual BMI categories using WHO definitions as previously described. Residual BMI is defined as the participant's reported BMI minus the genetically predicted BMI. The stratification according to residual BMI allows the comparison of participants who would have a similar BMI if they had the same genetic information and is a strategy to minimize collider bias (Sun *et al.*, 2019). A more detailed description of this methodology is available in Supplementary Materials and Methods.

We also applied a fractional polynomial method to calculate non-linear MR estimates of BMI on subfertility odds. In this procedure, we first divided the population into 100 strata of equal number of participants according to the residual BMI. We then calculated the linear MR estimate in each stratum (the association of the GRS with the outcome divided by the association of the GRS with the exposure). Finally, we performed a meta-regression of these estimates against the mean value of the reported BMI in each of the 100 strata using a fractional polynomial model as previously described (Sun *et al.*, 2019;

Rogne et al., 2020). We also calculated a fractional polynomial test, which assessed if the model using fractional polynomials to model the relationship between genetically predicted BMI and subfertility fitted the causal effect estimates better than a model with a simple linear term. The fundamentals of this non-linear MR approach are further explained in Supplementary Materials and Methods.

Three assumptions must be met in a valid MR study: the genetic instrument is robustly associated with the exposure, the genetic instrument is only linked to the outcome through the exposure of interest, and there is no confounding of the genetic instrument–outcome associations (Burgess et al., 2019). The strength of the genetic instrument (the association between the GRSs and BMI) was assessed in women and men separately using linear regressions, *F*-statistics and R^2 coefficients of determination. Regarding the second assumption, a common cause of violation is horizontal pleiotropy (i.e. genetic instrumental variables influence other risk factors for the outcome in addition to the exposure of interest) (Davey Smith and Hemani, 2014). To check this bias, we assessed the associations between quartiles of the GRS and predefined risk factors for subfertility (age, educational levels, smoking and number of previous pregnancies). Whenever we found indication of pleiotropic effects, we performed: (i) multivariable MR analyses if a valid genetic instrument could be calculated, i.e. if there were GWAS or meta-analyses of GWAS whose summary data were available (Burgess and Thompson, 2015); or (ii) stratified analyses. We identified summary GWAS data that enabled us to conduct multivariable MR analyses for educational level and smoking initiation and conducted stratified analyses according to age (below vs. over the median). For the multivariable MR accounting for educational level, we used the results from the most recent GWAS of education, which included approximately 1.1 million individuals and reported 1271 independent SNPs (Lee et al., 2018). We estimated the genetically predicted years of education using a GRS based on the 1159 available SNPs in the MoBa genotype data. For the multivariable MR accounting for smoking, we used the summary results of the most recent GWAS, which included more than 1.2 million participants and reported 378 SNPs associated with smoking initiation (Liu et al., 2019). In this case, we estimated the genetically determined risk of starting to smoke by a GRS based on the 355 available SNPs in the MoBa genotype data. In both multivariable MR analyses, we estimated the genetically predicted BMI values also including the GRS for education and the GRS for smoking initiation. Similarly, the genetically predicted number of educational years and likelihood of starting to smoke were estimated considering the GRS for BMI in addition to the GRS for the covariate of interest. Finally, we assessed the association between the genetically predicted BMI and subfertility as previously described using models further adjusted for the genetically predicted education years and likelihood of starting to smoke. Finally, regarding the third MR assumption (lack of confounding of the genetic instrument-outcome associations), all the one sample MR analyses were adjusted for 10 ancestry-informative principal components to account for population stratification (Wang et al., 2015).

We further explored unbalanced horizontal pleiotropy by methods developed for use in two sample MR (Bowden et al., 2015, 2016; Hemani et al., 2018). We first carried out two GWASs (one for women and one for men) to find out which SNPs were linked to subfertility in the MoBa cohort (full details are provided in the Supplementary Materials and Methods). We then searched the SNPs associated with BMI in the GWAS summary data, extracted the information about their relationship with subfertility, and harmonized both datasets to create a two sample MR framework. We performed the two sample MR by different methodologies: inverse variance weighted regression, MR-Egger, weighted median and weighted mode methods. We checked the presence of horizontal pleiotropy by: estimating the MR-Egger intercept (a deviation from zero would suggest horizontal pleiotropy); comparing the causal estimates obtained in the inverse variance weighted regression, the MR-Egger and the weighted median and mode methods (a divergence among them would also suggest horizontal pleiotropy); and generating a scatterplot as a visual check for potentially pleiotropic outliers in the variant-specific causal estimates (Bowden et al., 2015, 2016; Hemani et al., 2018). We also estimated between SNP heterogeneity (by the Cochran's *Q* and the Rücker's *Q'* statistics according to the inverse variance weighted regression and MR-Egger methods, respectively).

As additional sensitivity analyses: (i) we included parents reporting not having planned their pregnancies in the reference group (total sample: 34 157 women and 31 496 men); and (ii) we removed the conceptions by assisted reproductive technologies from the case group (706 and 670 in women and men, respectively (21% of the overall subfertile cases)).

All analyses were performed in R Software version 4.0.3 (packages: *compareGroups*, *estimatr*, *ggplot2*, *miceadds* and *TwoSampleMR*). Code for data management and statistical analysis is available here: https://github.com/alvarohernaez/MR_BMI_subfertility_MoBa/blob/main/syntax.

Results

Study population

Our study population consisted of 28 341 women (30 years old on average, mean pre-pregnancy BMI 23.1 kg/m^2) and 26 252 men (33 years old on average, mean BMI pre-pregnancy 25.5 kg/m^2) with singleton pregnancies and information on both BMI and genotype (Fig. 1). A total of 10% of the couples were subfertile. Women and men who were subfertile were older, had a lower educational level, were more likely to be current/former smokers, and more likely to be trying for a first pregnancy, and had on average greater BMI (Table I).

Association between reported BMI and subfertility: multivariable logistic regressions

The means ± SDs of the GRSs were 851 ± 22.5 and 851 ± 22.3 in women and men, respectively. In the standard multivariable linear association, each 1 kg/m^2 increase in BMI was linked to 4% greater odds of subfertility in women (odds ratio (OR) 1.04, 95% CI 1.04 to 1.05, $P < 0.001$) and men (OR 1.04, 95% CI 1.02 to 1.05, $P < 0.001$). However, a non-linear model based on restricted cubic splines fitted the data better than a linear term in both sexes (likelihood ratio tests: $P_{women} < 0.001$, $P_{men} = 0.024$; Fig. 2). These relationships were J-shaped, with a positive association from BMI values of 22.1 and 22.6 kg/m^2 onwards in women and men, respectively. A 1 kg/m^2 increase in BMI was linked to 4% greater odds of subfertility in women

with a BMI between 20.0 and 24.9 kg/m^2 (OR 1.04, 95% CI 1.00 to 1.08, $P = 0.050$), 10% increased odds in overweight women (OR 1.10, 95% CI 1.04 to 1.17, $P < 0.001$) and 3% greater odds in obese women (OR 1.03, 95% CI 1.00 to 1.06, $P = 0.027$). On the contrary, a 1 kg/m^2 increment in BMI was associated with 15% lower odds of subfertility in women with BMI <20.0 kg/m^2 (OR 0.85, 95% CI 0.73 to 0.97, $P = 0.021$) (Fig. 2A). In men, a 1 kg/m^2 increase in BMI was linked to 5% greater odds of subfertility in participants with a BMI between 20.0 and 24.9 kg/m^2 (OR 1.05, 95% CI 0.99 to 1.10, $P = 0.094$), 7% increased odds in overweight men (OR 1.07, 95% CI 1.02 to 1.11, $P = 0.004$) and 8% greater odds in obese men (OR 1.08, 95% CI 1.03 to 1.12, $P < 0.001$), and there was no evidence of an association in those with BMI values <20.0 kg/m^2 (OR 0.86, 95% CI 0.53 to 1.40, $P = 0.538$) (Fig. 2B).

When adjusting for the partner's BMI, these associations were still present and of a similar magnitude (Supplementary Fig. S1 and Table SI).

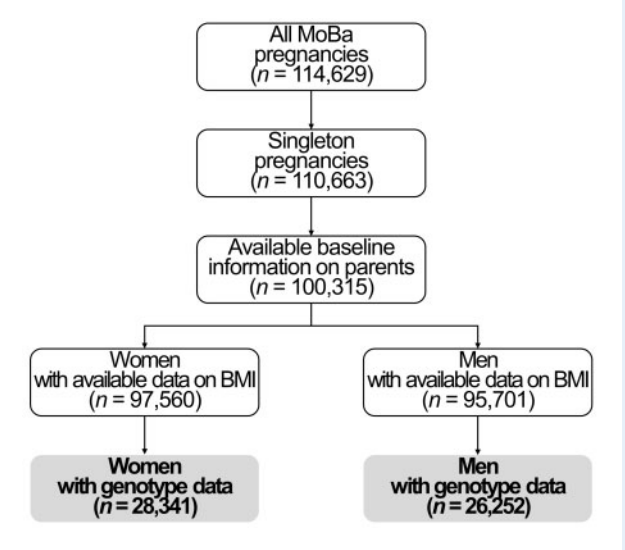

Figure 1. Study flow chart. MoBa, the Norwegian Mother, Father and Child Cohort Study.

MR analyses on BMI and subfertility in women

Each one unit increase in the GRS was linked to a BMI increase of 0.044 kg/m^2 (95% CI 0.041 to 0.046, $P < 0.001$, 5.65% of BMI variation explained, F-statistic = 1208). There was evidence of a J-shaped relationship between the genetically predicted BMI and subfertility in women (fractional polynomial test P-value for non-linearity = 0.030), which was positive for BMI values ≥ 22.8 kg/m^2 (Fig. 3). A 1 kg/m^2 increase in genetically predicted BMI was linked to 15% greater odds of subfertility in obese women (OR 1.18, 95% CI 1.05 to 1.31,

Table I Population characteristics.

	Women				Men			
	All (n = 28 341)	Subfertility reported (n = 3412)	No subfertility reported (n = 24 929)	*P*-value	All (n = 26 252)	Subfertility reported (n = 3173)	No subfertility reported (n = 23 079)	*P*-value
Age at delivery, years (mean ± SD)	30.3 ± 4.14	31.5 ± 4.35	30.1 ± 4.08	<0.001	32.7 ± 4.90	34.1 ± 5.34	32.5 ± 4.80	<0.001
Education years (mean ± SD)	17.5 ± 3.11	17.0 ± 3.32	17.6 ± 3.07	<0.001	16.6 ± 3.49	16.2 ± 3.53	16.6 ± 3.48	<0.001
Tobacco use (*n*, %)				<0.001				<0.001
Never smokers	15 313 (54.2%)	1725 (50.7%)	13 588 (54.7%)		19 565 (74.8%)	2247 (71.0%)	17 318 (75.3%)	
Former smokers	7546 (26.7%)	902 (26.5%)	6644 (26.8%)		885 (3.38%)	118 (3.73%)	767 (3.34%)	
Quitters before 12th (♀) or 18th week (♂)	3397 (12.0%)	440 (12.9%)	2957 (11.9%)		425 (1.62%)	58 (1.83%)	367 (1.60%)	
Current smokers	1973 (6.99%)	334 (9.82%)	1639 (6.60%)		5281 (20.2%)	740 (23.4%)	4541 (19.7%)	
Previous pregnancies (*n*, %):				<0.001				<0.001
0	12 803 (45.2%)	2011 (59.0%)	10 792 (43.4%)		11 962 (45.6%)	1870 (59.0%)	10 092 (43.8%)	
≥1	15 500 (54.8%)	1397 (41.0%)	14 103 (56.6%)		14 260 (54.4%)	1299 (41.0%)	12 961 (56.2%)	
BMI, kg/m^2 (median, 1st–3rd quartile)	23.1 (21.2–25.9)	23.7 (21.5–27.2)	23.1 (21.1–25.7)	<0.001	25.5 (23.7–27.7)	25.8 (23.9–28.1)	25.4 (23.7–27.7)	<0.001
BMI categories (*n*, %)				<0.001				<0.001
<20 kg/m^2	3401 (12.0%)	395 (11.6%)	3006 (12.1%)		299 (1.14%)	41 (1.29%)	258 (1.12%)	
20.0–24.9 kg/m^2	16 151 (57.0%)	1722 (50.5%)	14 429 (57.9%)		11 200 (42.7%)	1235 (38.9%)	9965 (43.2%)	
25.0–29.9 kg/m^2	6260 (22.1%)	804 (23.6%)	5456 (21.9%)		12 090 (46.1%)	1481 (46.7%)	10 609 (46.0%)	
≥30.0 kg/m^2	2529 (8.92%)	491 (14.4%)	2038 (8.18%)		2663 (10.1%)	416 (13.1%)	2247 (9.74%)	

Differences in baseline characteristics among subfertile and non-subfertile parents were assessed by *t*-tests in normally distributed continuous variables, Mann–Whitney *U* tests in non-normally distributed continuous variables, and chi-squared tests in categorical variables.

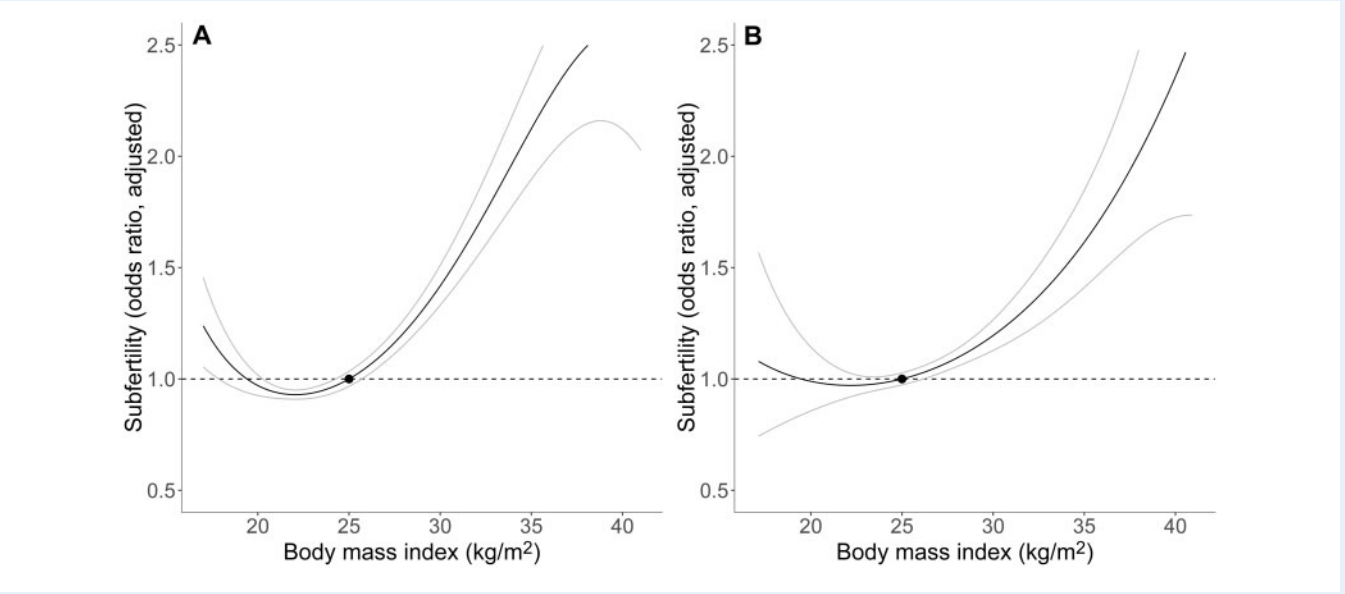

Figure 2. Association between reported body mass index and subfertility in women (A) and men (B). Non-linear logistic regression analyses (smoothed cubic splines) adjusted for age, education level, smoking and number of previous pregnancies. A BMI of 25 kg/m^2 was set as reference (black dot). Gray lines represent 95% confidence intervals.

$P = 0.004$), 14% lower odds in women with BMI <20.0 kg/m^2 (OR 0.86, 95% CI 0.76 to 0.98, $P = 0.022$), and unrelated to subfertility in those with BMI values between 20.0 and 24.9 kg/m^2 (OR 1.01, 95% CI 0.96 to 1.06, $P = 0.834$) and in overweight women (OR 1.04, 95% CI 0.96 to 1.12, $P = 0.304$).

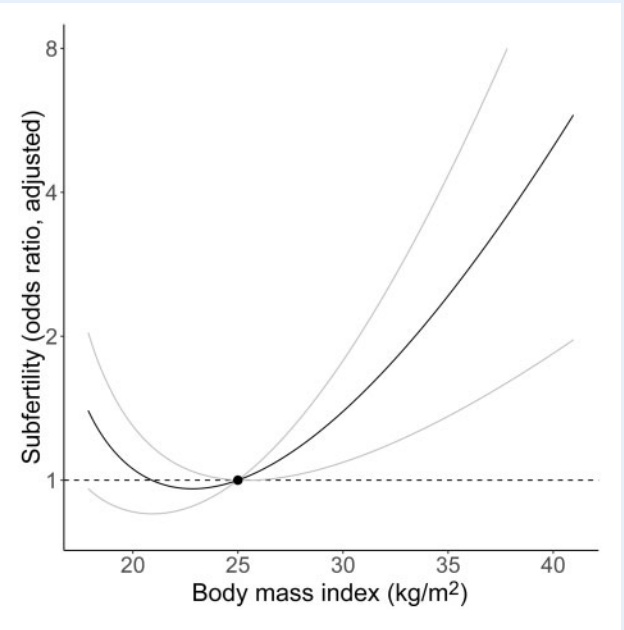

Figure 3. Mendelian randomization analysis of body mass index and subfertility in women. A BMI of 25 kg/m^2 was set as reference (black dot). Gray lines represent 95% confidence intervals.

MR analyses on BMI and subfertility in men

Each one unit increase in the GRS was linked to a BMI increase of 0.033 kg/m^2 in men (95% CI 0.031 to 0.035, $P < 0.001$, 5.18% of BMI variation explained, F-statistic = 1061). We observed a non-linear, J-shaped association between genetically predicted BMI and subfertility in men (P-value for non-linearity = 0.014), which was positive for BMI values ≥25.0 kg/m^2 (Fig. 4). A 1 kg/m^2 increment in genetically predicted BMI was linked to 26% greater odds of subfertility in obese men (OR 1.26, 95% CI 1.08 to 1.48, $P = 0.003$). As observed in Fig. 4, low genetically predicted BMI values were also related to greater subfertility risk in men at the lower end of the BMI distribution (although only 1.14% of all men presented BMI values <20 kg/m^2). Genetically predicted BMI was unrelated to subfertility in men with BMI values between 20.0 and 24.9 kg/m^2 (OR 0.95, 95% CI 0.87 to 1.04, $P = 0.281$) and overweight participants (OR 1.02, 95% CI 0.94 to 1.10, $P = 0.653$).

Verification of MR assumptions

Regarding horizontal pleiotropy, we observed an inverse relationship of GRS for BMI with education and age, and there was a lower proportion of never smokers in participants with high GRS values in both women (Supplementary Table SII) and men (Supplementary Table SIII). In both sexes, we observed similar J-shaped associations between BMI and subfertility in the multivariable MR accounting for education and smoking to those observed in the main analyses (Tables II and III; Supplementary Figs S2 and S3). In relation to age, we stratified our analyses into participants below and over the median age (30 years in women, 32 years in men). Genetically predetermined BMI had a similar non-linear, J-shaped associations with subfertility in both age groups as seen in the main analyses (Tables II and III, Supplementary Fig. S4).

Further sensitivity analyses using a two sample MR framework indicated no evidence of a linear relationship between BMI and subfertility, no horizontal pleiotropy according to different methods with various assumptions, and no SNP heterogeneity (Supplementary Table SIV and Fig. S5).

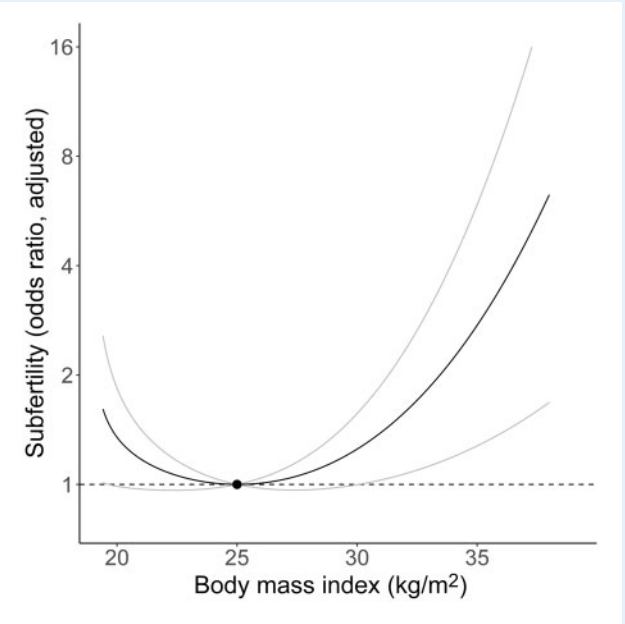

Figure 4. Mendelian randomization analysis of body mass index and subfertility in men. A BMI of 25 kg/m^2 was set as reference (black dot). Gray lines represent 95% confidence intervals.

Other sensitivity analyses

Genetically predetermined BMI presented similar non-linear, J-shaped associations with subfertility in both women and men also when including parents with non-planned pregnancies in the reference group (Supplementary Table SV and Fig. S6) and after excluding assisted reproduction technology users (Supplementary Table SV and Fig. S7).

Discussion

Our findings from multivariable and MR analyses indicate that BMI has a J-shaped association with subfertility in both women and men. Both participants with BMI values <20.0 kg/m^2 and ≥30.0 kg/m^2 had an increased risk of subfertility. The consistency of the results between multivariable regression and MR, and across several sensitivity analyses, increases confidence in these findings being causal.

Although a positive association between BMI and subfertility has been reported in observational studies (Ramlau-Hansen *et al.*, 2007; van der Steeg *et al.*, 2008; Pinborg *et al.*, 2011; Campbell *et al.*, 2015; Mushtaq *et al.*, 2018), an improvement in assisted reproduction success has not been observed in all randomized controlled trials of weight loss after lifestyle modifications (Mutsaerts *et al.*, 2016; Best *et al.*, 2017; Einarsson *et al.*, 2017; Norman and Mol, 2018) or bariatric surgery (Grzegorczyk-Martin *et al.*, 2020; Wood *et al.*, 2020). Our data suggest that increases in BMI from 23 and 25 kg/m^2 in women and men, respectively, are linked to greater odds of subfertility. These

Table II Multivariable and age-stratified MR analyses in women.

	MR: main analyses	Multivariable MR considering education years	Multivariable MR considering smoking initiation	Stratified MR: age of delivery < median	Stratified MR: age of delivery > median
Linear MR					
OR for Δ1 kg/m^2 (whole population)	1.04 (1.00 to 1.08)	1.03 (0.99 to 1.07)	1.04 (1.00 to 1.08)	1.01 (0.96 to 1.08)	1.07 (1.01 to 1.12)
Non-linear MR					
Fractional polynomial test (*P*-value for non-linearity)	0.030	0.027	0.033	0.165	0.007
OR for Δ1 kg/m^2 (stratified analyses)					
<20.0 kg/m^2	0.85 (0.76 to 0.98)	0.84 (0.73 to 0.97)	0.85 (0.74 to 0.97)	0.79 (0.66 to 0.95)	0.91 (0.76 to 1.09)
20.0–24.9 kg/m^2	1.01 (0.96 to 1.06)	1.00 (0.95 to 1.06)	1.00 (0.95 to 1.05)	0.98 (0.91 to 1.06)	1.02 (0.95 to 1.09)
25.0–29.9 kg/m^2	1.04 (0.96 to 1.12)	1.04 (0.96 to 1.13)	1.05 (0.97 to 1.13)	1.08 (0.96 to 1.21)	1.05 (0.95 to 1.16)
≥30.0 kg/m^2	1.18 (1.05 to 1.31)	1.17 (1.04 to 1.32)	1.19 (1.06 to 1.34)	1.07 (0.92 to 1.24)	1.28 (1.08 to 1.51)
BMI with lowest subfertility odds	22.8 kg/m^2	22.8 kg/m^2	23.1 kg/m^2	24.7 kg/m^2	22.1 kg/m^2

MR, Mendelian randomization; OR, odds ratio.

Table III Multivariable and age-stratified MR analyses in men.

	MR: main analyses	Multivariable MR considering education years	Multivariable MR considering smoking initiation	Stratified MR: age of delivery < median	Stratified MR: age of delivery > median
Linear MR					
OR for Δ1 kg/m^2 (whole population)	1.02 (0.97 to 1.08)	1.01 (0.95 to 1.07)	1.03 (0.97 to 1.10)	1.06 (0.97 to 1.15)	1.01 (0.94 to 1.08)
Non-linear MR					
Fractional polynomial test (*P*-value for non-linearity)	0.014	0.042	0.010	0.011	0.090
OR for Δ1 kg/m^2 (stratified analyses)					
<20.0 kg/m^2	0.81 (0.47 to 1.41)	0.68 (0.39 to 1.21)	0.80 (0.42 to 1.50)	0.85 (0.47 to 1.52)	0.48 (0.20 to 1.16)
20.0–24.9 kg/m^2	0.95 (0.87 to 1.04)	0.95 (0.87 to 1.04)	0.97 (0.88 to 1.07)	0.97 (0.85 to 1.11)	0.96 (0.86 to 1.08)
25.0–29.9 kg/m^2	1.02 (0.94 to 1.10)	1.01 (0.92 to 1.09)	1.02 (0.94 to 1.12)	1.05 (0.93 to 1.20)	1.01 (0.91 to 1.11)
≥30.0 kg/m^2	1.26 (1.08 to 1.48)	1.25 (1.06 to 1.48)	1.26 (1.05 to 1.50)	1.32 (1.06 to 1.66)	1.24 (1.00 to 1.53)
BMI with lowest subfertility odds	25.0 kg/m^2	26.9 kg/m^2	24.8 kg/m^2	24.1 kg/m^2	24.5 kg/m^2

MR, Mendelian randomization; OR, odds ratio.

associations appeared unaffected by assortative mating or horizontal pleiotropy. The concordance of the findings from multivariable regressions and MR increase our confidence that this association is causal (Lawlor *et al.*, 2016). A possible explanation for the divergence between our findings and those from randomized trials is that MR considers small but lifelong changes in risk factors, whereas trials consider larger magnitudes of change but are only able to measure short-term effects (Burgess *et al.*, 2012). This lack of concordance could be particularly expected for interventions that are qualitatively very different to the effects of the genetic variants on BMI (such as bariatric surgery).

Several biological mechanisms can explain a potential association between high BMI and subfertility. Obesity is linked to biochemical disruptions (insulin resistance, adipocyte hyperactivation, greater levels of non-esterified fatty acids in plasma, increased hepatic triglyceride synthesis) (Amiri and Ramezani Tehrani, 2020). These are in turn linked to impaired endocrine responses in women (lower synthesis of estrogens and luteinizing hormone, a greater production of androgens, and a decay in sex hormone binding globulins) and men (decreased testosterone levels, increased estrogen production in adipose tissue, defective hypothalamic pituitary gonadal regulation and decreased concentrations of sex hormone binding globulins) (Amiri and Ramezani Tehrani, 2020). These endocrine alterations and other conditions linked to high BMI values, such as low-grade inflammation in reproductive tissues and some sex-dependent alterations (menstrual abnormalities, increased testicular heat, greater risk of erectile dysfunction), may finally compromise fecundity (Broughton and Moley, 2017; Silvestris *et al.*, 2018; Amiri and Ramezani Tehrani, 2020; Salas-Huetos *et al.*, 2021).

The J-shaped association between BMI and subfertility also support that participants with low BMI may have a greater risk of subfertility. A decrease in BMI was linked to greater subfertility in women with a BMI <20 kg/m^2, and we observed a similar tendency among men. Our results agree with previous observational studies reporting decreased fertility in women with low body weight who have undergone assisted reproductive technologies (Xiong *et al.*, 2020). Low BMI values could be linked to subfertility because they are intimately related to undernutrition, which is associated with an impaired function of the reproductive system (Cai *et al.*, 2017), defective concentrations of adipocyte-related regulators of endocrine processes such as leptin (Mitchell *et al.*, 2005), and increased risk or pregnancy complications (Dickey *et al.*, 2013).

Our work presents some limitations. First, subfertility is a couple-dependent measure and was reported by mothers in the cohort (if a women was classified as subfertile, this condition was extrapolated to her partner). Thus, we are unable to determine whether subfertility was driven by the women, men or both. In addition, there is previous evidence of assortative mating on BMI (Silventoinen *et al.*, 2003), which could also confound the association between BMI and subfertility. Second, MoBa is a pregnancy cohort, and only includes couples who eventually conceived. Additional studies which are also able to include couples who never conceived are warranted. Third, the BMI GRS was associated with some predefined risk factors of subfertility, indicating that some horizontal pleiotropy may be present. However, multivariable MR and stratified analyses confirmed a robust association between BMI and subfertility, and additional sensitivity analyses found no evidence of horizontal pleiotropy in our data. Fourth, most of the

associations with subfertility were found in the participants with extreme BMI values and therefore should be interpreted with caution. Fifth, we were unable to use standard BMI categories for underweight (WHO threshold $<18.5\,kg/m^2$), as this only included 2.69% and 0.14% of the female and male participants, respectively. We decided to group underweight with low-normal weight participants ($18.5–20.0\,kg/m^2$), to be able to estimate more robust ORs. Therefore, further studies involving larger populations in the lower end of the BMI distribution are warranted. Finally, our study sample (couples who eventually conceived and were of a northern European ancestry) limits the generalizability of our conclusions to other populations. Nevertheless, our work also has several strengths. To our knowledge, studies exploring non-linear associations between BMI and subfertility using multivariable regressions and an MR approach have been lacking. Both present different sources of bias (multivariable regression could be biased by residual confounding, whilst MR could be biased by unbalanced horizontal pleiotropy), but the consistency in the findings according to both approaches increases confidence that these findings may be causal (Davey Smith and Hemani, 2014; Sun *et al.*, 2019). This was facilitated by having large numbers of well-characterized participants with genome-wide and subfertility data coming from a relatively homogeneous population with northern European ancestry. This last aspect minimized the risk of confounding due to population stratification in our MR analyses, as well as the further adjustment for 10 ancestry-informative principal components (Wang *et al.*, 2015). Finally, our genetic instrument is robust (Burgess and Thompson, 2011; Evans *et al.*, 2013) and has been successfully used in several other MR studies (Cheung *et al.*, 2019; Takahashi *et al.*, 2019; Rogne *et al.*, 2020).

In conclusion, we observed a J-shaped relationship between BMI and subfertility in both sexes, when using both a standard multivariable regression and MR analysis. Taken together, our results support a causal role of BMI on subfertility. These results suggest that BMI values between 20 and $25\,kg/m^2$ are optimal for a minimal risk of subfertility.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The consent given by the participants does not allow for storage of data on an individual level in repositories or journals. Researchers who want access to data sets for replication should submit an application to datatilgang@fhi.no. Access to data sets requires approval from the Regional Committee for Medical and Health Research Ethics in Norway and an agreement with MoBa. Source data of the GWAS on BMI (Yengo *et al.*, 2018) are available in the Genetic Investigation of ANthropometric Traits (GIANT) Consortium website (https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files#GWAS_Anthropometric_2015_BMI_Summary_Statistics). Source data of the GWAS on education years (Lee *et al.*, 2018) are available in the Supplementary Tables of the article (https://www.nature.com/articles/s41588-018-0147-3#Sec34). Finally, source data of the GWAS on smoking initiation (Liu *et al.*, 2019) are available in the Supplementary Tables of the article (https://www.nature.com/articles/s41588-018-0307-5#Sec14).

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Authors' roles

M.C.M. conceived and designed the study, obtained funding and coordinated the project. A.H. and M.C.M. are responsible for the data curation and the formal analysis. T.R., K.H.S. and C.M.P. provided support in data analysis, software use and visualization of results. A.H., T.R., K.H.S., S.E.H., C.M.P., A.F., S.B., D.A.L. and M.C.M. were involved in the definition of the methodology of the study and the interpretation of data. A.H. prepared the first draft of the manuscript and T.R., K.H.S., S.E.H., C.M.P., A.F., S.B., D.A.L. and M.C.M. revised it critically.

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the writing of the report; or in the decision to submit the article for publication.

Conflict of interest

D.A.L. receives (or has received in the last 10 years) research support from National and International government and charitable bodies, Roche Diagnostics and Medtronic for research unrelated to the current work. The rest of the authors declare that no competing interests exist.

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