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Maternal hyperandrogenism is associated with a higher risk of type 2 diabetes mellitus and overweight in adolescent and adult female offspring: A long-term population-based follow-up study

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Running title: Maternal hyperandrogenism and cardio-metabolic parameters in offspring

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Abstract

Purpose: Adverse intrauterine environment may predispose offspring to cardio-metabolic dysfunction in later life. In this study we aimed to investigate the effects of maternal hyperandrogenism (MH) on cardio-metabolic risk factors in female offspring in later life.

Methods: This prospective population-based study included 211 female offspring with MH and 757 female offspring without MH (controls). Both groups were followed from baseline to the date of incidence of events, censoring, or end of the study period, whichever came first. Age scaled unadjusted and adjusted cox regression models were applied to assess the hazard ratios(HR) and 95% confidence intervals (CIs) for the association of MH with pre-diabetes (pre-DM), type 2 diabetes mellitus (T2DM), overweight and obesity in offspring of both groups. Statistical analysis was performed using the software package STATA; significance level was set at $P < 0.05$.

Results: This study revealed a higher risk of T2DM (unadjusted HR: 2.67, 95% CI: 1.33-5.36) and overweight (unadjusted HR: 1.41, 95% CI: 1.06-1.88) in female offspring with MH, compared to controls. Results remained unchanged after adjustment for potential confounders including body mass index, education, physical activity, mother's age at delivery, birth weight, and childhood obesity. However, no significant difference was observed in the risk of pre-DM and obesity in females with MH, compared to controls in both unadjusted and adjusted models.

Conclusion: This pioneer study with a long-term follow-up demonstrated that MH increases the risk of developing T2DM and being overweight in female offspring in later life. Further long-term population-based studies are needed to confirm these findings.

Key words: Maternal hyperandrogenism, Pre-diabetes (pre-DM), Type 2 diabetes mellitus (T2DM), Overweight, Obesity.

Introduction

Cardio-metabolic disorders are one of the most leading causes of global mortality and morbidity which have been increasing steadily over the course of the last decades [1, 2].

Emerging evidence supports that early intrauterine life environmental factors including hormonal, nutritional and metabolic disturbances are important determinants of human health and disease in adulthood [3]. It is hypothesized that those disturbances may permanently influence molecular and gene expression, physiology or morphology of the developing organs in the fetal period, leading to susceptibility of the occurrence of diseases, such as cardiovascular diseases (CVDs) and endocrine disorders in later life [4].

It is well documented that the fetus is extremely sensitive to steroid hormones exposure during its early development [5]. Researches in animals and human suggested that intrauterine exposure to high androgen levels may contribute to adverse long-lasting reproductive and/or metabolic traits including reduced glucose stimulated insulin secretion, impaired glucose tolerance, dyslipidemia, and hypertension in adulthood [6-11].

Recent evidence supports that women experiencing hyperandrogenism associated with polycystic ovary syndrome (PCOS) or gestational hyperandrogenemia have elevated blood cord androgens concentration compared to non-PCOS women, consequently the fetus also be exposed to high levels of androgens from the maternal circulation origin [12-15]. In this respect, some animal and human studies support that offspring of PCOS subjects exhibit worse metabolic parameters than those born to healthy mothers [7, 11, 16-18]. In a recent published meta-analysis of nine studies, subtle signs of altered cardio-metabolic health such as increased insulin resistance (IR) in male and female children of women with PCOS has been reported [19]. However, long-term prospective studies with well-defined controls from unselected populations are needed to explore whether maternal hyperandrogenism (MH) during the pregnancy period increases the risk of cardio-metabolic disturbances in offspring in later life.

Hence, in the present population-based study with about 2 decades of follow-up, we aimed to investigate the risk of pre-diabetes mellitus (pre-DM), type 2 diabetes mellitus (T2DM), overweight and obesity among female offspring with MH compared to female offspring without MH (controls), in later life.

Materials and methods

This prospective population-based study included a total of 968 offspring of women participated in Tehran Lipid and Glucose Study (TLGS). TLGS as an ongoing and large-scale cohort study, initiated in 1998 to explore the prevalence and risk factors of non-communicable diseases among 15005 both male and female individuals, aged ≥ 3 years who were followed with 3 years intervals. Follow-up visits include demographic, anthropometric and metabolic assessments, general physical examinations as well as laboratory measurements. In addition, a comprehensive reproductive and androgen excess assessment have been considered for all female participants. The details of TLGS are provided elsewhere [20, 21].

For the purpose of the present study, we included female offspring whose mothers' hyper androgenic status was identified and had at least one follow-up visit (n = 968); including 211 female offspring whose mothers were known cases of hyperandrogenism (female offspring whose mothers had hyperandrogenism during the pregnancy period (female offspring with MH)) and 757 female offspring of women without such history of hyperandrogenism (female offspring without MH (controls)). Those with lack of sufficient data about the MH history, taking medication that may influence the cardio-metabolic parameters or suffering from any cardio-metabolic disturbances for each specific events at the baseline were excluded from the study. All included participants were followed-up from baseline to the date of incidence of events, censoring, or end of the study period, whichever came first. The selection process of participants has been shown in Figure 1.

Anthropometrics and biochemical measurements

During face-to-face interviews, using a standard questionnaire, information was documented on demographic variables and personal as well as family medical history. Weight and height were measured, in the standing position with calibrated equipments; body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Participants were asked about their level of physical activity in the past 12 months using the modifiable activity questionnaire. Physical activity status was defined as active for those with three or more days of severe-intensity activity of at least 20 minutes, or ≥ 5 days of moderate-intensity activity or walking at least 30 minutes, or ≥ 5 days of any combination of walking, moderate or severe-intensity activities, reaching at least 600 metabolic equivalent task minutes per week and less active for those not reaching to this threshold.

Overnight fasting venous blood serum samples were collected for biochemical assessments. All blood analyses were performed at the TLGS reference laboratory on the day of blood collection. Plasma glucose (fasting and non-fasting) was measured by the glucose oxidase method on the day of blood collection; inter- and intra-assay coefficients of variations (CVs) were both less than 2.3%.

Outcome and Term Measures

Hyperandrogenic status of mothers was identified according to the clinical manifestations of hyperandrogenism included hirsutism, acne, and androgenic alopecia, and/or biochemical hyperandrogenemia. Hirsutism was diagnosed based on a standardized modified Ferriman-Gallwey scoring system, women who had score ≥ 8 were considered as hirsute [22]. Acne was scored based on its number, type, and distribution. Androgenic alopecia was characterized by moderate to severe hair loss on the temples or diffuse thinning on the crown [23]. Biochemical hyperandrogenism was evaluated as an elevated serum levels of one or more androgens above the 95th percentile, including total testosterone (TT), androstenedione (A4), dehydroepiandrosterone sulfate (DHEAS), and free androgen index (FAI), determined in the

selected healthy non-hirsute eumenorrheic women in the study population; specifically, the upper normal limits were 0.89 ng/ml, 2.9 ng/ml, 179 μg/dl and 5.39 for TT, A4, DHEAS and FAI, respectively [24]. TT, A4 and DHEAS levels were measured by enzyme immunoassay (Diagnostic Biochem Canada). Sex hormone binding globulin (SHBG) was measured by immunoenzymometric assay (Mercodia). All enzyme-linked immunosorbent assay (ELISA) tests were performed using the Sunrise ELISA Reader (Tecan). The FAI was calculated using the following formula: TT (nmol/L) × 100/SHBG (nmol/L). Inter- and intra-assay coefficients of variations (CVs) for all hormones were less than 7%. No androgen excess group was considered as those women without hyperandrogenism and/or oligo/anvoulation. Oligo/anovulation was defined as either regular or irregular menstrual cycles ≥ 34 days or ≤ 8 menstrual cycles in a year [25].

T2DM was defined according to the American Diabetes Association criteria as fasting plasma glucose ≥ 126 mg/dl, or 2-hour plasma glucose ≥ 200 mg/dl, or using medications for a previous diagnosis of T2DM. Pre-DM referred to those with impaired fasting glucose where the fasting plasma glucose levels were 100–125 mg/dl; or impaired glucose tolerance where the 2-hour plasma glucose values in the oral glucose tolerance test (OGTT) were 140–199 mg/dl [26]. Obesity was defined as a BMI ≥ 30 kg/m^2, and a participants was considered overweight for BMI values more 25 and less than 30 kg/m^2 [27].

Statistical analysis

Our study had the power of 80% to detect a hazard ratio equal to 2.0 for the effect of MH on T2DM at a 0.05 significance level, after adjusted for an anticipated event rate of 6% for T2DM as a main outcome.

Continuous variables were checked for the normality based on the one-sample Kolmogorov–Smirnov test; and were presented as mean (standard deviation) if they had a normal distribution, or median with inter-quartile range (IQ25-75) for variables with skewed distribution.

Categorical variables were presented as number and percentages. Demographic and clinical characteristics of female offspring were compared according to MH using the student *t* test or χ^2 test for continuous or categorical data, respectively. The Mann-Whitney test was applied to compare variables with skewed distribution.

We applied Cox regression model to assess the hazard ratios and 95% confidence intervals (CIs) for the association of MH with pre-DM, T2DM, overweight and obesity in female offspring. The event date was considered as when the intended outcome was occurred for the first time, and age at event was computed. We used an attained age scale where the primary time variable in the Cox model is defined by study offspring age at entry into the study (birth) and age at which they experience an event or their follow-up is censored. Use of the attained age scale provides the most flexible control for age effects while avoiding the need to include an effect of age [28].

Both unadjusted and adjusted Cox regression models were applied. Potential confounding factors including BMI, educational status, and physical activity were entered in the multivariate Cox model as time-varying covariates. Other risk factors such as mother's age at delivery, birth weight and childhood obesity were also entered into the model as baseline covariates.

Missing data for repeated measurement data was imputed using multiple imputation method considering time trend of the variable with Amelia package in R [29]. The unadjusted and adjusted survival functions were also plotted.

Statistical analysis was performed using the software package STATA (version 13; STATA Inc., College station, TX, USA) and R version 4.0.3; significance level was set at $P < 0.05$.

Results

Characteristics of mothers and their female offspring according to hyperandrogenic status are presented in Table 1.

Median (IQR) age of entry for female offspring with MH and female offspring without MH (controls) was 12.4 (7.3-16.6) and 15.7 (9.8-20.1) years, respectively. Female offspring with MH and controls reached to the median age of 27.8 (22.1-32.4) and 31.1 (25.2-35.8) at the end of follow-up, respectively.

Table 2 summarizes the results of Cox regression analysis regarding the association between the MH and metabolic consequences. The results of analysis showed that there was a higher risk of T2DM (unadjusted HR: 2.67, 95% CI: 1.33-5.36) and overweight (unadjusted HR: 1.41, 95% CI: 1.06-1.88) in female offspring with MH, compared to controls. The results of multivariate Cox regression model, adjusted for BMI, education, physical activity, mother's age at delivery, birth weight, and childhood obesity, showed that the risk of T2DM (adjusted HR: 2.44, 95% CI: 1.13-5.27) and overweight (adjusted HR: 1.47, 95% CI: 1.10-1.97) significantly increased in female offspring with MH. However, there was no significant difference in the risk of pre-DM and obesity in female offspring with MH compared to controls in both unadjusted and adjusted models.

Figure 2 presents differences in the survival curves for metabolic consequences in terms of T2DM (Figure 2.a), pre-DM (Figure 2.b), overweight (Figure 2.c) and obesity (Figure 2.d) according to MH status. As we saw in the Cox model results, based on the unadjusted model (Figure 2.a), female offspring with MH have a higher risk of T2DM, compared to controls (*P*-value = 0.01), moreover, the adjusted survival curve indicated a significantly higher risk of T2DM (lower survival probability) in female offspring with MH (*P*-value = 0.02). Furthermore, Fig 2.c depicts a significantly higher risk of overweight in female offspring with MH compared to controls in both unadjusted (*P*-value = 0.02) and adjusted (*P*-value = 0.01) curves. However, there was no statistically significant difference in terms of pre-DM and obesity's survival curves.

Discussion

In this large, population-based study with two decades of follow-up, it was demonstrated that the risk of T2DM and being overweight was statistically increased among female offspring with MH compared to controls in later life (adolescence and adult life), the results did not change after adjustment for potential confounders.

The Barker hypothesis implies that organs structure and function undergo programming during embryonic and fetal life, which determines the set point of physiological and metabolic responses that carry into later life [30]. Accordingly, any alterations in endocrine, nutritional, and metabolic milieu during the early stages of fetal development may potentially predispose individuals to the later susceptibility to certain chronic disorders [31].

T2DM as one of the most complex chronic diseases, develops due to the interaction of genetic, epigenetic, intrauterine environment, and lifestyle factors, all of which may lead to organ dysfunction in multiple tissues including pancreas, liver, muscle, adipose tissue, gastrointestinal system and kidneys as well as the brain. It has been shown that fetal growth and differentiation are largely dependent on the maternal and intrauterine hormonal milieu during early development, as a result all factors disrupted this milieu during the fetal ontogeny may have an important role in the development of IR and subsequently T2DM. Therefore, T2DM can develop in subjects who have experienced an adverse intrauterine environment.

It is well documented that developing human fetus is extremely sensitive to sex steroid hormones, particularly androgens. It has been shown that exposure to high levels of androgens during critical periods of fetal development may influence the programming of metabolic tissues (pancreas, liver, muscle, and adipose tissue), potentially resulting in maladaptive long-lasting metabolic dysfunction at early ages and/or in adulthood [6]. Prenatally androgenized animal models showed decreased insulin sensitivity, hyperinsulinemia, IR, disrupted insulin signaling in metabolic tissues, impaired glucose tolerance and subsequently T2DM in later life [8, 9, 11, 18, 32, 33]. Additionally, in human studies, it has been reported that offspring of

women with PCOS who exposed to androgen excess during early stages of development had worse metabolic parameters compared to those born of healthy mothers [16, 17, 34]. In this respect, Gunning et al. in an individual participant data meta-analysis demonstrated that children of PCOS women exhibited significantly increased fasting insulin and IR, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations compared to controls [19]. In agreement with previous studies, our findings revealed that compared to controls, female offspring with MH show increased risk of T2DM and being overweight in later life.

Although the underlying potential etiology are incompletely understood, however, it may be due to some mechanisms as follows: Findings of animal studies suggest a programmed failure of the pancreatic β-cells due to a prolonged exposure to testosterone during fetal development. Androgen overexposure during the fetal life could alter fetal pancreatic development, function and the number of β-cells in females, which may lead to increased insulin secretion, higher risk of IR and impaired response to glucose stimulation. In addition, it may provoke systemic oxidative stress leading to predispose to β-cells injury [8, 35-37]. Moreover, recent evidence suggests that prenatal exposure to androgen excess could increase the risk of development of PCOS in female offspring [38], which *per se* is one of the most important risk factors for T2DM and obesity among reproductive-aged women in later life [22, 25]. Besides, it is widely acknowledged that genetic defects are implicated in many cardio-metabolic diseases [39, 40]. It is suggested that these genetic basis may increase the risk of developing cardio-metabolic disorders due to altered metabolic pathway of intrauterine androgen exposure [41, 42].

Besides genetic factors, epigenetic changes due to exposure to environmental factors may play important role in the pathogenesis of human diseases. Epigenetic is considered as a link between genes, environmental exposure, and disease development. Epigenetics involves alterations in gene expression caused by DNA methylation, histone modification and non-coding RNA activity without requiring an underlying change in genetics.

The epigenetic changes induced by environmental factors, in somatic cells and the developing organs, can lead to occurrence of disease in the individuals exposed directly. While, epigenetic changes in the germ-line is considered as one of the mechanisms for transgenerational inheritance of diseases [43-45]. In addition to the epigenetic marks, RNA molecules (mRNAs, miRNAs, and piRNAs), metabolites and proteins may play important role in transgenerational inheritance through the germ cells. Both intergenerational and transgenerational epigenetic inheritance have been reported in mammals. Inheritance of an induced epigenetic change and associated phenotype from one generation to the next generation is defined as intergenerational epigenetic inheritance, while transgenerational epigenetic inheritance represents the transmission of an induced epigenetic change and associated phenotype from F2, and eventual subsequent generations without requiring further exposure [46]. In other words, for transgenerational epigenetic inheritance two criteria including, exposure to an event in generation F0 and observing an effect of the event in the third or fourth generation (F2 or F3) are needed.

As previously reported, the maternal environment can influence epigenetic processes in the placenta and fetus that program lasting developmental changes associated with CVDs, hypertension, weight gain, obesity, T2DM, endocrine disruption and reproductive anomalies [4, 47, 48]. Intrauterine exposure to androgens can lead to epigenetic changes, and subsequently alterations in the genes expression, in the developing fetus [49]. Epigenetic programming of reproductive and metabolic function in the adult through maternal androgenization of the fetus is well documented. Some studies conducted on animals, have suggested that transient exposure to androgen excess during early development can induce persistent changes in methylation status of DNA, disturb glucose homeostasis and metabolic system in exposed and subsequent generations [49, 50].

Furthermore, one study conducted on human reported that DNA methylation may play an important role in metabolism [51], it may be due to epigenetic changes in candidate genes that affect metabolism. Altered DNA methylation in tissues for glucose homeostasis (pancreas, liver, skeletal muscle, and adipose tissue) has been reported in subjects with T2DM [52]. Increasing evidence shows that epigenetic changes participate in controlling the fate and regulation of β-cells physiological function as well as apoptosis [53].

As well, it has been reported that exposure to androgens during prenatal life could lead to increased proportion of small adipocytes compared to large adipocytes due to failure of small adipocytes transitioning to mature adipocytes, disruption of adipocyte insulin signaling, adipose mass and distribution that resulting in weight gain, obesity and development of IR during adulthood [8, 54-56].

Strengths and limitations of the study

Our study contains a number of strengths. To the best of our knowledge, our study is one of the longest studies evaluated the risk of cardio-metabolic factors in female offspring with MH. Population-based setting of this cohort study possibly reduces the uncertainty regarding selection bias of clinical-based studies. In addition, our adjustment of the potential confounders produced valuable results. The inter observer and/or intra-assay variability for assessment of clinical/biochemical hyperanrogenism in our data is likely to be minimal because all assessments were done by the same person and/or at the same laboratory. Moreover, as an ongoing study, it allows us to monitor the participants for further events. Nevertheless, our study also had its limitations. Despite the importance, we did not measure lifestyle modifications such as dietary habits, which potentially may influence on adverse cardio-metabolic outcomes. In addition, the hormonal profiles and potential biomarkers of prenatal androgen exposure including anogenital distance (AGD) and the ratio of the second to fourth digits of the hand (2D:4D) have not been assessed in the female offspring.

Conclusion

In conclusion, this pioneer study with a long-term follow-up demonstrated that MH increases the risk of developing T2DM and being overweight in adolescent and adult female offspring. Further long-term population-based studies are needed to confirm these findings. As the precise pathophysiological links are not entirely understood and many aspects still require elucidation, an integrated description of the genetic, epigenetic, and environmental influences involved in the concomitant development of diseases are still needed to shed new light on the interlinks between MH and cardio-metabolic disturbances in offspring.

Statements and declarations

Competing interests

The authors declare that there are no conflicts of interest.

Authors' contributions

M. N. contributed substantially to conception and design, analysis and interpretation of data, drafted the article and revised and approved the final version to be published. M. R. contributed substantially to statistical analysis of data, drafted the article and revised and approved the final version to be published. S. BG. contributed substantially to interpretation of data, drafted the article and revised and approved the final version to be published. F.R.T. contributed substantially to conception and design, analysis and interpretation of data, drafted the article and revised and approved the final version to be published.

Ethics approval

The ethics review board of the Research Institute for Endocrine Sciences approved the study proposal (approval number: IR.SBMU.ENDOCRINE.REC.1399.015).

Consent to participate

Written informed consent was signed by all participants, after a full explanation of the purpose of the study to them. Written consent was obtained from their parents, if they were under 18 years old.

Consent for publication

All authors approved the final version of article for publication.

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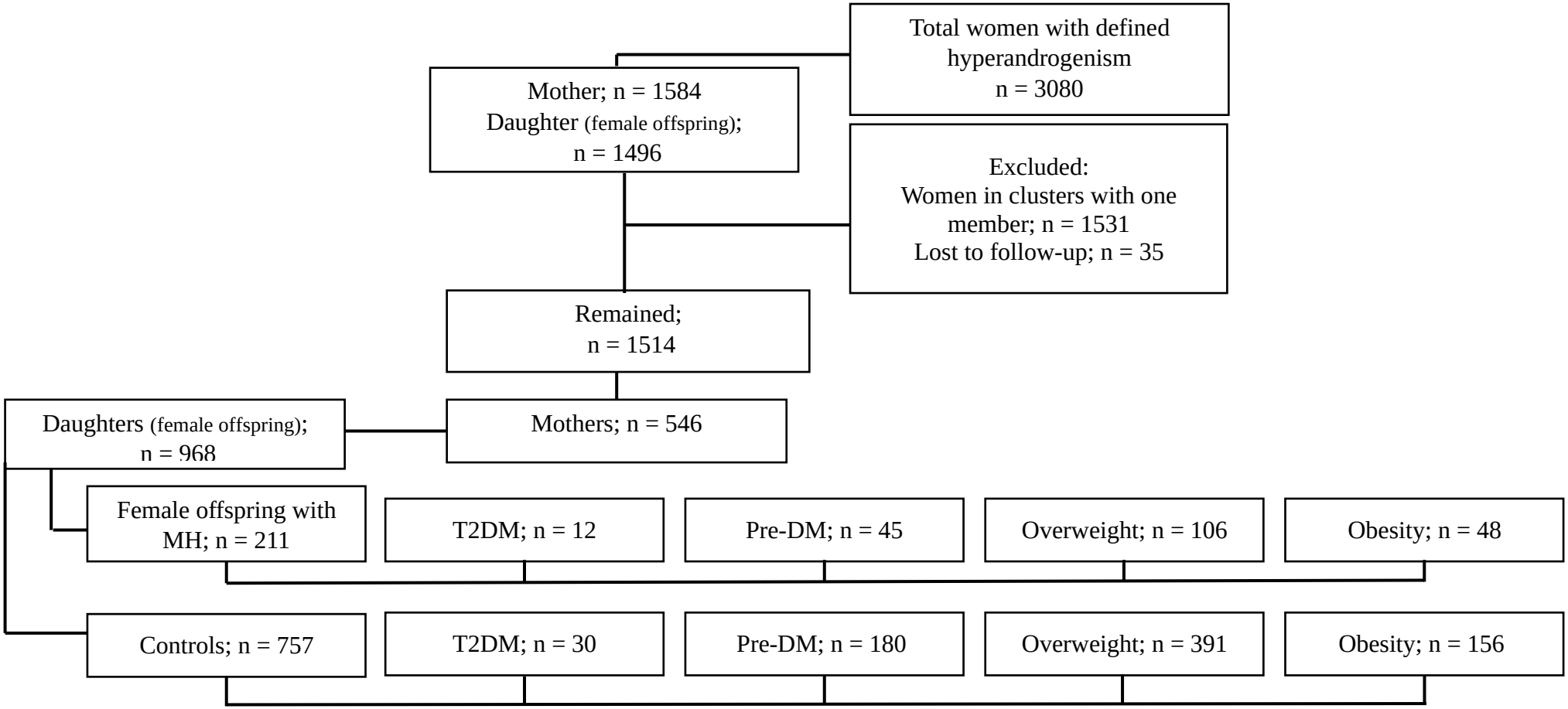


Figure 1. Flowchart of the study.

MH: Maternal hyperandrogenism
Controls: Female offspring without MH
T2DM: Type 2 diabetes mellitus
Pre-DM: Pre-diabetes

Table 1. Characteristics of mothers and female offspring according to hyperandrogenic status.

Mother's characteristics (n = 546)	**With hyperandrogenism history during their pregnancy period (n = 163)**	**Without hyperandrogenism history during their pregnancy period (n = 383)**	***P*-value[a]**
Age at delivery (years)	24.3 ± 6.6	25.5 ± 7.6	0.02
Smoking history (past and current), n (%)	11 (6.7)	25 (6.5)	0.9
Parity	2.8 ± 1.2	3.1 ± 1.5	0.02
Mode of delivery (cesarean section), n (%)	29 (21.8)	56 (19.0)	0.5
Educational level (diploma and upper), n (%)	55 (33.9)	118 (32.6)	0.2
Maternal T2DM, n (%)	30 (18.4)	64 (16.7)	0.6
GDM, n (%)	9 (5.5)	19 (5.0)	0.8
TT (ng/ml)	0.5 (0.3-0.8)	0.4 (0.2-0.6)	0.01
SHBG (nmol/L)	38.0 (28.0-44.0)	45.5 (44.0-55.5)	< 0.001
FAI	5.1 (3.4-7.8)	2.4 (1.5-4.0)	< 0.001
DHEAS (µg/dl)	170 (133.0-220.5)	124.0 (74.0-142.5)	< 0.001
A4 (ng/ml)	1.4 (0.6-2.8)	0.7 (0.5-1.6)	< 0.001
Hyperandrogenism status, n (%)			
Clinical hyperandrogenism,	122 (74.8)	-	-
Biochemical hyperandrogenism	3 (1.84)	-	-
Both clinical and biochemical hyperandrogenism	38 (23.3)	-	-
Daughter's characteristics	**Female offspring with MH (n = 211)**	**Controls (n = 757)**	***P*-value[a]**
Family history of T2DM n (%)	55 (26.1)	265 (35.0)	0.01
Birth weight, n (%)			
Low	13 (6.3)	52 (7.2)	0.8
High	6 (2.9)	25 (3.5)	
Childhood obesity, n (%)	35 (16.8)	108 (14.9)	0.5
T2DM, n (%)	12 (5.7)	30 (4.0)	0.3
Pre-DM, n (%)	45 (21.3)	180 (23.8)	0.4
Overweight, n (%)	106 (50.2)	394 (52.0)	0.6
Obesity, n (%)	48 (22.7)	156 (20.6)	0.5
Age at baseline, median (IQR)	12.4 (7.3-16.6)	15.7 (9.8-20.1)	< 0.001
Age at last follow-up, median (IQR)	27.8 (22.1-32.4)	31.1 (25.2-35.8)	< 0.001
BMI at baseline, median (IQR)	17.8 (15.3-21.5)	19.8 (16.8-22.8)	0.004

BMI at last follow-up, median (IQR)	23.8 (21.5-27.1)	24.5 (21.8-27.9)	0.3
Physical activity at baseline (moderate to high), n (%)	63 (43.4)	268 (45.3)	0.7
Physical activity at last follow-up (moderate to high), n (%)	185 (87.7)	653 (86.3)	0.5
Educational level at baseline (diploma and upper), n (%)	53 (25.1)	234 (30.9)	0.1
Educational level at last follow-up (diploma and upper), n (%)	109 (51.7)	410 (54.2)	0.5

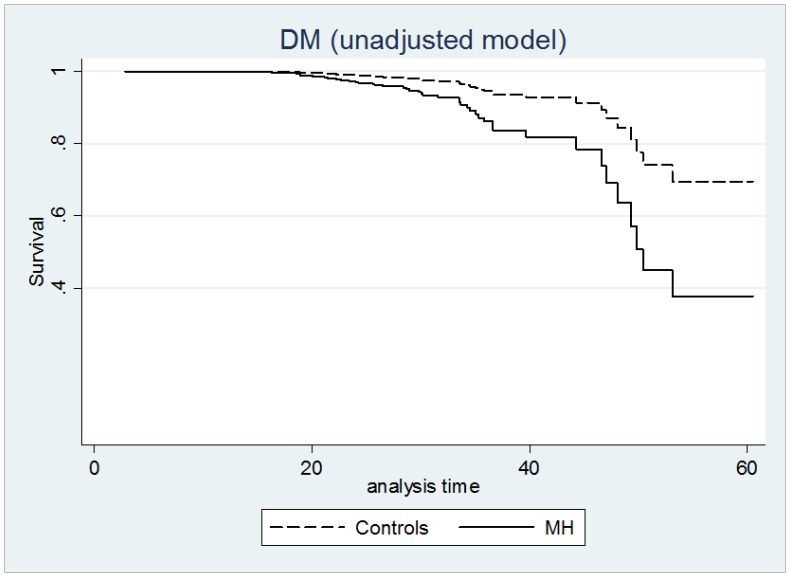
Values are presented as mean (SD), median (IQR, interquartile range) or number (percentage) as appropriate. [a] *P*-value is calculated by independent-samples t-test or Mann-Whitney test for continuous, and χ^2 test for categorical data as appropriate for between group comparison. TT, total testosterone; SHBG, sex hormone binding globulin; FAI, free androgen index; DHEAS, dehydroepiandrosterone sulfate; A4, Androstenedion. T2DM, type 2 diabetes mellitus; Pre-DM, Pre-diabetes mellitus; GDM, gestational diabetes mellitus; MH, maternal hyperandrogenism; Controls, female offspring without MH.

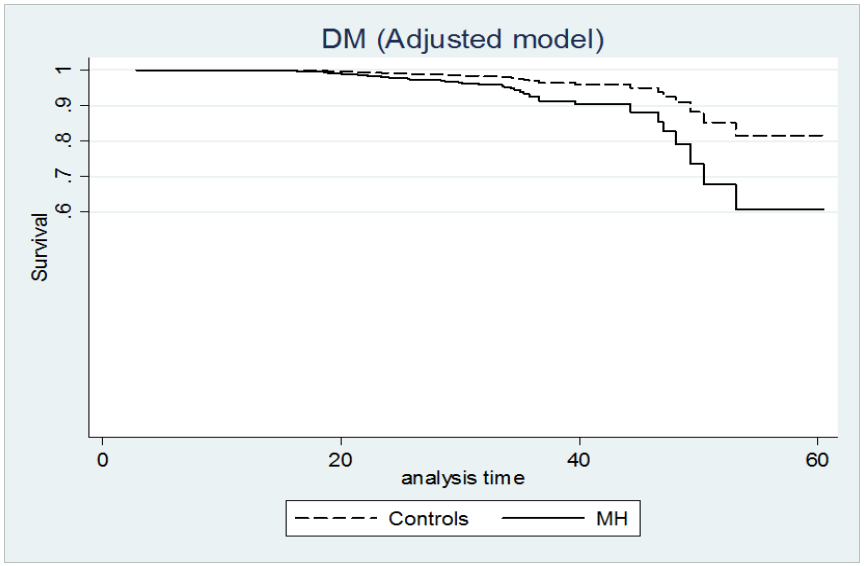
Table 2. Association between maternal hyperandrogenism and metabolic disorders in female offspring.

		T2DM		Pre-DM		Overweight		Obesity	
Model		**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
Unadjusted	Females with MH / Controls	2.67 (1.33-5.36)	0.01	1.31 (0.93-1.83)	0.1	1.41 (1.06-1.88)	0.02	1.47 (0.96-2.25)	0.07
Adjusted*	Females with MH / Controls	2.44 (1.13-5.27)	0.02	1.35 (0.95-1.92)	0.08	1.47 (1.10-1.97)	0.01	1.13 (0.7-1.81)	0.6

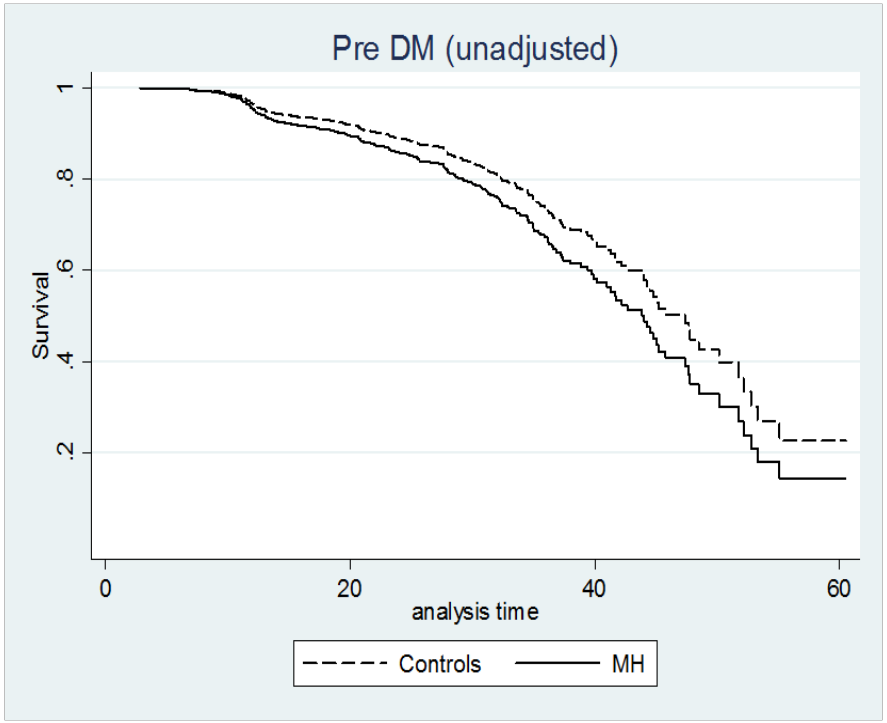
*Adjusted for BMI, education, physical activity, mother's age at delivery, birth weight, and childhood obesity.
T2DM, type 2 diabetes mellitus
Pre-DM, pre-diabetes mellitus
HR, hazard Ratio
CI, confidence interval
MH, maternal hyperandrogenism

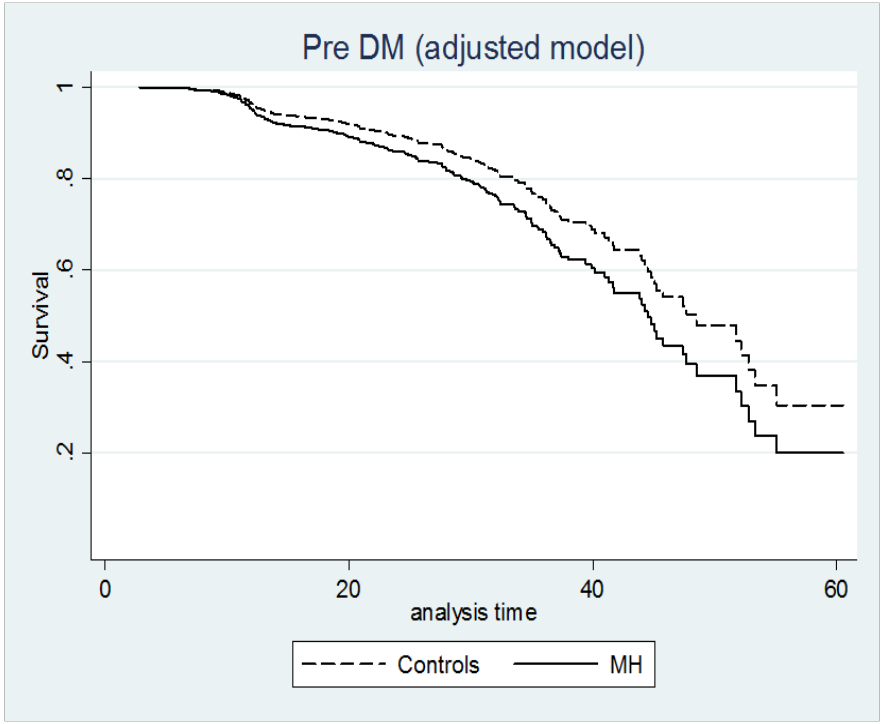
(a)


DM (unadjusted model)
Survival
1
.8
.6
.4
0
20
40
60
analysis time
Controls
MH

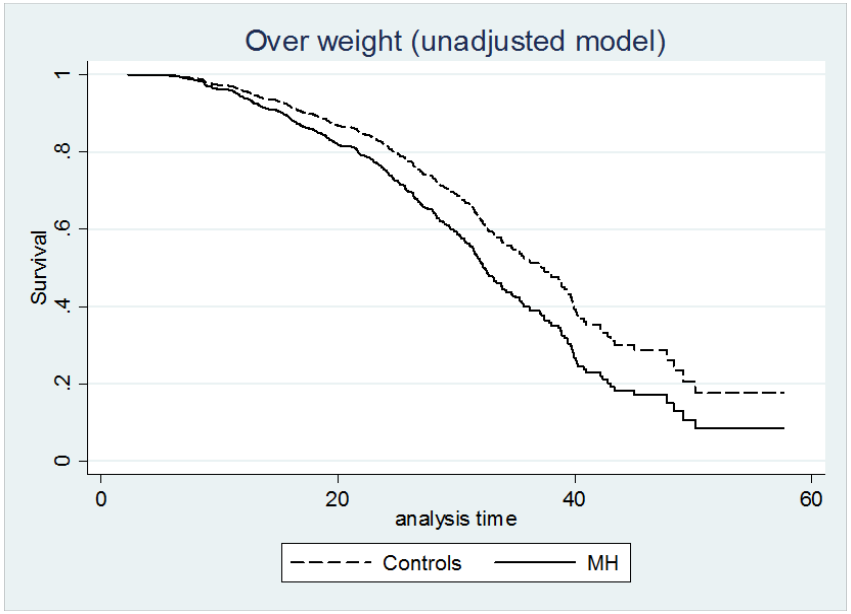

DM (Adjusted model)
Survival
1
.9
.8
.7
.6
0
20
40
60
analysis time
Controls
MH

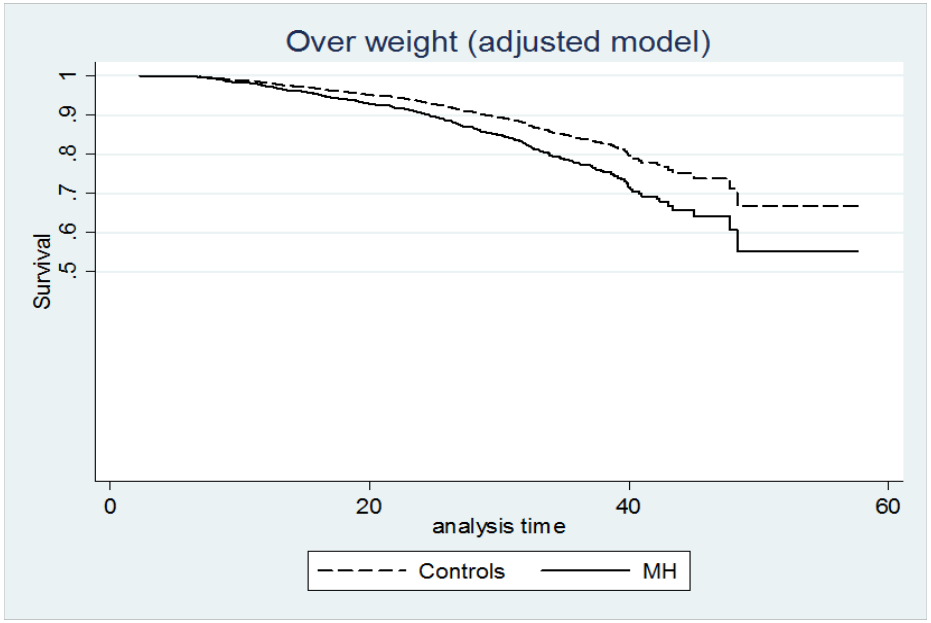
(b)


Pre DM (unadjusted)
Survival
.2
.4
.6
.8
1
0
20
40
60
analysis time
Controls
MH


Pre DM (adjusted model)
Survival
.2
.4
.6
.8
1
0
20
40
60
analysis time
Controls
MH

(c)


Over weight (unadjusted model)
Survival
0
.2
.4
.6
.8
1
0
20
40
60
analysis time
Controls
MH


Over weight (adjusted model)
Survival
.5
.6
.7
.8
.9
1
0
20
40
60
analysis time
Controls
MH

(d)

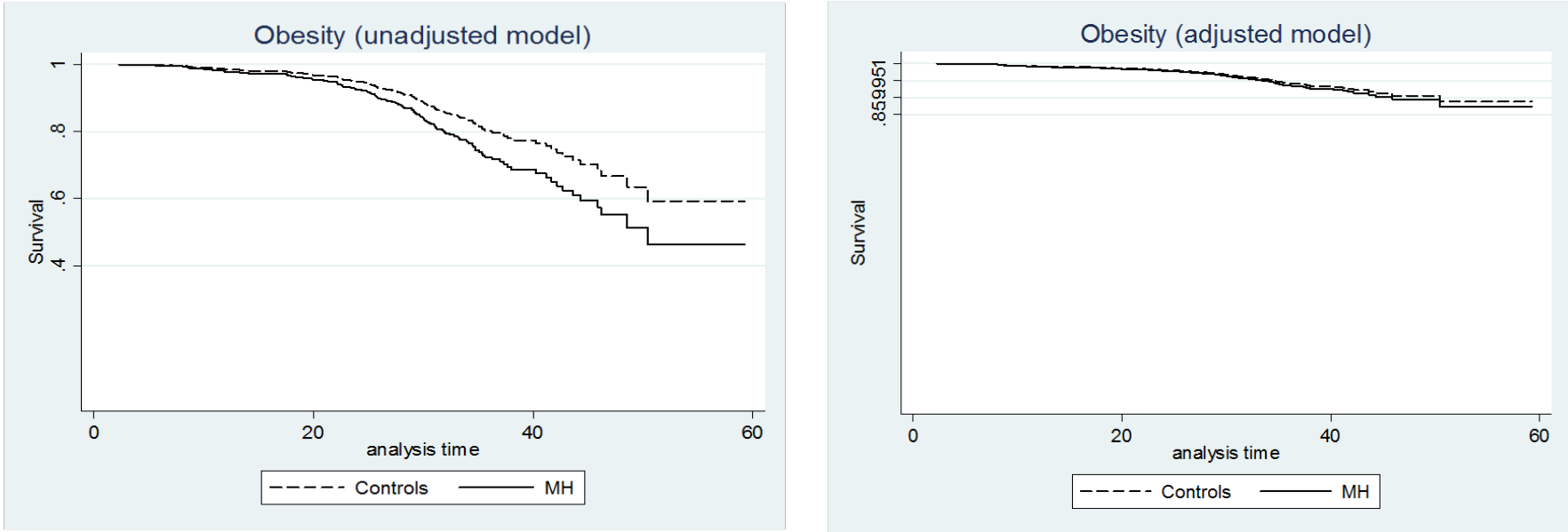


Figure 2: Unadjusted and adjusted survival plots showing survival (time to event) curves for female offspring with maternal hyperandrogenism (MH) and controls (female offspring without maternal hyperandrogenism).