Evidence for metabolic and reproductive phenotypes in mothers of women with polycystic ovary syndrome

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Dyslipidemia is a feature of polycystic ovary syndrome (PCOS), but its pathogenesis remains controversial. We performed this study of mothers of women with PCOS to test the hypothesis that dyslipidemia is a heritable trait in families of women with PCOS and to investigate the impact of age on reproductive and metabolic phenotypes. Fasting blood was obtained in 215 non-Hispanic white mothers of women with PCOS and 62 control women. The prevalence of metabolic syndrome was compared with that in non-Hispanic white women of comparable age from the National Health and Nutrition Examination Survey III. Mothers had higher total ($P < 0.001$) and low-density lipoprotein (LDL) cholesterol levels ($P = 0.007$), whereas high-density lipoprotein and triglyceride levels did not differ compared with control women. The only predictors of LDL levels in mothers were their daughters' LDL levels ($r^2 = 0.11$, $P < 0.001$) and their own unbound testosterone levels ($r^2 = 0.04$, $P = 0.03$). The prevalence of metabolic syndrome was increased in obese (body mass index ≥ 30 kg/m^2) mothers compared with obese non-Hispanic white women from the National Health and Nutrition Examination Survey III ($P = 0.04$). Thirty-one percent of mothers reported a history of menstrual irregularity. These mothers had higher androgen levels, markers of insulin resistance, and LDL levels than mothers with regular menses. LDL levels are increased in mothers of women with PCOS, suggestive of a heritable trait. A history of menstrual irregularity identifies mothers with features of PCOS. Obese mothers have a very high prevalence of metabolic syndrome. These findings suggest that both the reproductive and metabolic abnormalities persist with age in PCOS.

hyperandrogenemia | hypertriglyceridemia | insulin resistance | low-density lipoprotein cholesterol | menstrual irregularity

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in premenopausal women (1) characterized by disordered gonadotropin secretion and hyperandrogenism (2). Women with PCOS frequently have substantial insulin resistance (3) and are at increased risk for type 2 diabetes mellitus (DM2) (4, 5). Affected women also have other risk factors for cardiovascular disease, including obesity and dyslipidemia (6–8). Studies of women with PCOS, by definition, have been limited to women in their reproductive years, and little is known about the reproductive or metabolic phenotypes as these women age. Paradoxically, the only controlled study of older women with PCOS suggested that lipid abnormalities normalize in the fifth decade of life as the prevalence of these changes increases in the normal population (7). The long-term health consequences of PCOS are of considerable importance because of the well established increase in cardiovascular disease risk conferred by DM2 and the emerging data on risk conferred by other disorders associated with insulin resistance. Moreover, the full burden of these associated disorders may not become evident until affected women are in their sixth to eighth decade of life, given the later onset of cardiovascular disease in women (9, 10).

Familial clustering of PCOS consistent with a genetic susceptibility to the disorder is well documented (11–14). Limited studies of mothers of women with PCOS have reported increased androgen levels (12, 14), insulin resistance, and an increased prevalence of glucose intolerance (13, 14), suggesting that these abnormalities are heritable. We performed this study of mothers of women with PCOS to test the hypothesis that dyslipidemia is a heritable trait in families of women with PCOS and to investigate the impact of age on reproductive and metabolic phenotypes. In addition, because menstrual irregularity appears to be an excellent surrogate for PCOS (15, 16), we sought to determine whether a history of irregular menses could be used to identify mothers with other features of PCOS.

Results

Clinical and Reproductive Features. Mothers tended to be older and heavier than control women, but these changes did not achieve statistical significance (Table 1). There were no differences in waist circumference or blood pressure measurements between the two groups (Table 1). Testosterone (T) levels tended to be higher, and unbound T (uT) levels were higher in mothers than in control women (Table 1). Dehydroepiandrosterone sulfate and sex hormone-binding globulin levels did not differ between the two groups (Table 1). Seventy-three mothers were premenopausal, 79 were postmenopausal, and 63 had unknown menopausal status. Thirty-one control women were premenopausal, 20 were postmenopausal, and 11 had unknown menopausal status. There was a trend toward higher uT levels in premenopausal mothers compared with premenopausal control women (mothers, 0.24 ± 0.20 nmol/liter, vs. control women, 0.16 ± 0.09 nmol/liter; $P = 0.06$), but total T levels did not differ. There were no differences in total T and uT levels between postmenopausal mothers and postmenopausal control women (data not shown).

Thirty-one percent of mothers ($n = 67$) reported a history of irregular menses. There were no differences in age or body mass index (BMI) between these mothers and those with a history of regular menses (data not shown). Total T and uT levels were higher in mothers with a history of irregular menses than in those with a history of regular menses and in control women (Fig. 1). When hyperandrogenemia was defined categorically (11, 17), 3% of mothers fulfilled the National Institute of Child Health and Human Development diagnostic criteria for PCOS (18), and 6% of mothers had hyperandrogenemia with a history of regular menses.

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Abbreviations: PCOS, polycystic ovary syndrome; DM2, type 2 diabetes mellitus; LDL, low-density lipoprotein; NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; HOMA IR, homeostatic index of insulin resistance; T, testosterone; uT, unbound T; HDL, high-density lipoprotein.

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Table 1. Clinical and biochemical features

Features	Mothers (n = 215)	Control (n = 62)	P value*
Age, years	52 ± 8	49 ± 10	0.07
BMI, kg/m^2	29.9 ± 7.0	28.4 ± 7.2	0.15
Waist circumference, cm	92 ± 16	89 ± 15	0.18
Systolic blood pressure, mmHg†	125 ± 17	120 ± 15	0.26
Diastolic blood pressure, mmHg	74 ± 10	74 ± 9	0.86
T, nmol/liter	1.0 ± 0.7	0.8 ± 0.3	0.09
uT, nmol/liter	0.2 ± 0.2	0.1 ± 0.1	0.003
DHEAS, μmol/liter	2.8 ± 1.6	2.8 ± 1.5	0.27
SHBG, nmol/liter	124 ± 74	118 ± 59	0.55
Fasting glucose, mmol/liter	5.3 ± 1.2	4.9 ± 0.5	0.02
Fasting insulin, pmol/liter	103 ± 58	82 ± 32	0.09
HOMA IR	3.6 ± 3.1	2.8 ± 1.3	0.02
Cholesterol, mmol/liter	5.65 ± 1.06	4.99 ± 0.84	<0.001
LDL, mmol/liter	3.58 ± 0.97	3.11 ± 0.66	0.007
HDL, mmol/liter	1.24 ± 0.56	1.24 ± 0.44	0.75
Triglycerides, mmol/liter	1.89 ± 1.69	1.48 ± 0.96	0.19

Data are presented as unadjusted mean ± SD. DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin.
*Analysis of covariance adjusted for age and BMI.
†1 mmHg = 133 Pa.

Metabolic Features. Fasting glucose levels were higher, and fasting insulin levels tended to be higher in mothers, but fasting glucose:insulin ratios did not differ between the two groups (Table 1). The homeostatic index of insulin resistance (HOMA IR) was higher in mothers than in control women (Table 1). Among 45 mothers who underwent an oral glucose tolerance test, eight mothers had impaired glucose tolerance, and two had newly diagnosed DM2, for an overall prevalence rate of glucose intolerance of 22%. Furthermore, seven additional mothers who did not undergo an oral glucose tolerance test were found to have newly diagnosed DM2, and 10 were found to have impaired fasting glucose based on fasting glucose levels by American Diabetes Association criteria (4). Levels of total and low-density lipoprotein (LDL) cholesterol were higher in mothers than in control women (Table 1), even after adjustment for tobacco, alcohol, and exercise habits. Our study had 99% power to detect this difference, with a two-sided significance level of 0.05. LDL levels remained significantly increased in mothers when those with glucose intolerance were excluded from the analysis (n = 33) and when the analysis was repeated in a subgroup of mothers with documented normal glucose tolerance and no prior history of diabetes (n = 34). Triglyceride and high-density lipoprotein (HDL) levels did not differ between the two groups (Table 1). Total cholesterol levels were higher in premenopausal mothers than in control women, and LDL levels tended to be higher in premenopausal mothers than in control women (Table 2). Total and LDL cholesterol levels were higher in postmenopausal mothers than in control women (Table 2).

Mothers with a history of irregular menses had higher fasting glucose (Fig. 1) and total and LDL cholesterol levels than did mothers with a history of regular menses (P = 0.03 for total and LDL cholesterol) and control women ($P < 0.001$ for total and LDL cholesterol). HOMA IR was higher in mothers with a history of irregular menses than in control women (Fig. 1). In

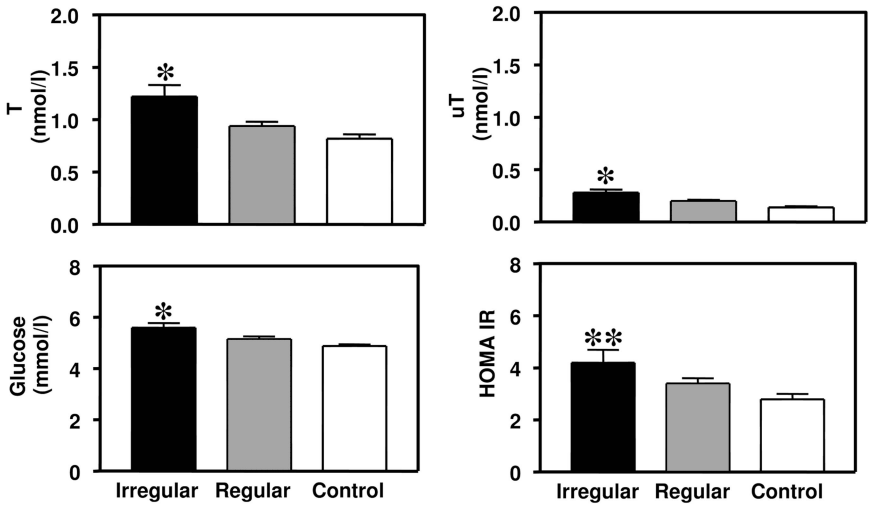

Fig. 1. Mothers with irregular menses had higher total T, uT, and fasting glucose levels compared with mothers with regular menses (P = 0.007 for total T, P = 0.01 for uT, and P = 0.02 for fasting glucose) and compared with control women (P = 0.002 for total T, $P < 0.001$ for uT, and $P < 0.001$ for fasting glucose). Mothers with irregular menses had higher HOMA IR compared with control women (P = 0.001). Black bars, mothers with irregular menses; gray bars, mothers with regular menses; white bars, control women.

Table 2. Changes in lipid parameters with menopause

	Premenopausal		Postmenopausal	
Lipid parameters	Mothers (n = 73)	Control (n = 31)	Mothers (n = 79)	Control (n = 20)
Cholesterol, mmol/liter	5.26 ± 0.94*	4.55 ± 0.67	5.98 ± 0.99†	5.26 ± 0.73
LDL, mmol/liter	3.37 ± 0.93‡	2.89 ± 0.62	3.79 ± 0.96§	3.19 ± 0.64
HDL, mmol/liter	1.24 ± 0.36	1.18 ± 0.26	1.32 ± 0.76	1.48 ± 0.30
Total triglycerides, mmol/liter	1.58 ± 1.04	1.50 ± 1.11	2.04 ± 1.22‡	1.29 ± 0.60

Data are presented as mean ± SD. Analysis was performed by analysis of covariance adjusted for age and BMI. *, P = 0.02, mothers vs. controls; †, P = 0.007, mothers vs. controls; ‡, P = 0.07, mothers vs. controls; §, P = 0.01, mothers vs. controls.

mothers with a history of regular menses, total cholesterol and LDL levels were higher than those of control women (P = 0.002 for total and P = 0.03 for LDL cholesterol) whereas fasting glucose levels and HOMA IR did not differ.

There was a positive linear correlation between LDL levels in mothers and those in their proband daughters with PCOS (r^2 = 0.19, $P < 0.001$). In a multivariate regression analysis, the only predictors of LDL levels in mothers were their daughters' LDL levels (r^2 = 0.11, $P < 0.001$) and mothers' own uT levels (r^2 = 0.04, P = 0.03). Age, BMI, HOMA IR, tobacco use, alcohol intake, and exercise were not significant predictors of LDL levels in mothers.

Prevalence of Metabolic Syndrome in PCOS Mothers. Forty-seven percent of mothers had metabolic syndrome, compared with 32% of non-Hispanic white women from the National Health and Nutrition Examination Survey (NHANES) III ($P < 0.001$). This difference was due primarily to an increased prevalence of metabolic syndrome in obese mothers compared with obese women from NHANES (Fig. 2) (P = 0.04 compared with NHANES III). Obese mothers were similar to obese women from NHANES III with regard to distribution of age and BMI (data not shown). These findings did not change when the 1999–2000 updated NHANES data were used in the analysis (data not shown). The frequency of individual components of metabolic syndrome in mothers was as follows: low HDL levels (73%), increased waist (56%), increased triglyceride levels (50%), increased blood pressure (40%), and increased fasting glucose levels (13%).

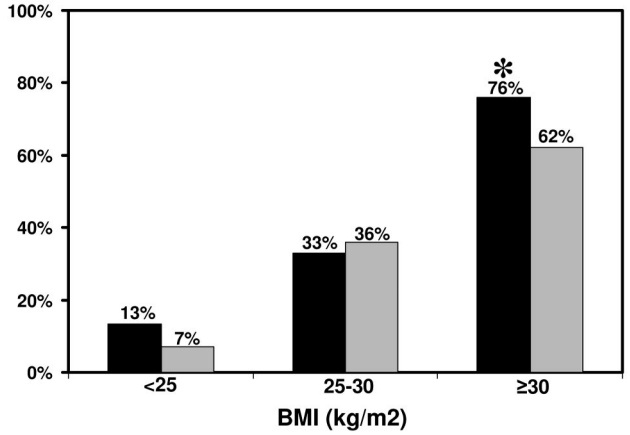

Fig. 2. The prevalence of metabolic syndrome is shown in mothers of women with PCOS compared with women from NHANES III after stratification based on BMI. Obese mothers had higher prevalence of metabolic syndrome compared with obese women from NHANES III (P = 0.04). Black bars, mothers of women with PCOS; gray bars, women from NHANES III.

Discussion

PCOS is associated with a markedly increased prevalence of DM2 in premenopausal women (4, 5), but little is known about the long-term health outcomes of these women. Given the evidence for genetic susceptibility to the reproductive and metabolic features of the syndrome (11, 12, 14), we examined mothers of affected women to gain insight into the changes in these phenotypes with age. We found that mothers of women with PCOS had elevated LDL levels and markers of insulin resistance as well as an increased prevalence of the metabolic syndrome. As a group, mothers also had elevations in bioavailable (unbound) T levels, consistent with previous reports (12, 14). Furthermore, it was possible to identify a subset of mothers with a history of irregular menses who had other features of PCOS: hyperandrogenemia (3), markers of insulin resistance (3), and higher LDL levels (8). Our results suggest that both the reproductive and metabolic features of PCOS are heritable and persist with aging.

We have previously reported that elevated LDL levels are the predominant lipid abnormality in women with PCOS, independent of obesity (6, 8), analogous to our findings in mothers. In mothers this abnormality was also independent of glucose tolerance. In one prospective study of women with PCOS, however, differences in LDL levels disappeared in the fifth decade as LDL levels increased in control women (7). In our population, LDL levels were increased in postmenopausal mothers. Our inclusion of older women and our larger sample size may account for the discrepant findings between the present study and the previous study (7). The strongest predictor of LDL levels in mothers was their proband daughters' LDL levels, which accounted for 11% of the variation in mothers' LDL levels. The mothers' own androgen levels (uT) accounted for only 4% of the variation in LDL levels. Neither body weight nor markers of insulin resistance predicted LDL levels in mothers, so 85% of the variation in LDL levels remained unexplained by any known factors. These observations are consistent with studies in women with PCOS that suggest a role for androgens rather than insulin resistance in the pathogenesis of alterations in LDL levels (19, 20). For example, blocking androgen action with the receptor antagonist flutamide resulted in significant decreases in total and LDL cholesterol levels in women with PCOS, independent of changes in body weight and insulin sensitivity (19, 20). Conversely, improving insulin sensitivity with a thiazolidendione (21) or with a hypocaloric diet alone or combined with metformin did not alter LDL levels in affected women (20).

Prior studies suggest that a self-reported history of menstrual irregularity identifies women with an endocrine profile characteristic of PCOS (15, 16, 22). Thirty-one percent of mothers reported a history of menstrual irregularity. These mothers also had higher levels of androgens, glucose, and LDL compared with mothers with a history of regular menses, suggesting that these mothers may have had PCOS. Indeed, 3% of mothers fulfilled the National Institute of Child Health and Human Development

diagnostic criteria for classic PCOS (18), and another 6% had a history of regular menses and hyperandrogenemia. The prevalence of hyperandrogenemia was likely underestimated in this study because of a general decline in ovarian function with age leading to lower circulating androgen levels (23). The finding of hyperandrogenemia with and without irregular menses is similar to our finding in the reproductive-age sisters of women with PCOS where we identified two affected phenotypes: (*i*) irregular menses and hyperandrogenemia (classic PCOS) and (*ii*) regular menses and hyperandrogenemia (11). Our study supports the utility of menstrual history as a marker for PCOS in both epidemiologic (24, 25) and genetic (26, 27) studies.

We compared the prevalence of the metabolic syndrome in mothers with that in women from the NHANES III survey because we specifically excluded features of the metabolic syndrome in our control women. There was a significant increase in the prevalence of metabolic syndrome in obese mothers compared with obese women from NHANES III. It is likely that the actual prevalence of metabolic syndrome in mothers was underestimated because we excluded mothers receiving medications for hypertension, diabetes, or hyperlipidemia. The use of the NHANES III data is constrained by the fact that the data were collected from 1988 to 1994. The prevalence of metabolic syndrome has increased in the women enrolled in the updated population between 1999 and 2000 (28). However, the increased prevalence was not statistically significant in women between the ages of 40 and 69 years (28), the age range of mothers. Furthermore, our findings in the updated NHANES from 1999 to 2000 did not differ from the comparison to the original NHANES III cohort. In addition, both mothers and the NHANES III cohort were recruited from across the United States.

We were not able to determine the exact prevalence of abnormal glucose tolerance in our population because we excluded mothers who were receiving antidiabetic medications, and only a small fraction of mothers underwent glucose tolerance testing. Among the ≈21% of mothers who underwent the glucose tolerance test, the prevalence of glucose intolerance was similar to that in women of comparable age and ethnicity in NHANES III cohort (29). This finding is in contrast to earlier studies from other investigators, who had reported an increase in the prevalence of glucose intolerance in parents of women with PCOS (13, 14).

In summary, mothers of women with PCOS had elevated LDL levels consistent with a heritable trait. They also had elevated androgen levels and markers of insulin resistance. Mothers with a history of irregular menses were more severely affected. Obese mothers had an increase in the prevalence of the metabolic syndrome compared with the general population of U.S. women. Taken together, these findings suggest that mothers of women with PCOS may be at increased risk for cardiovascular disease and that menstrual history may identify those mothers at greatest risk.

Methods

Study Population. The study was approved by the Institutional Review Boards of Brigham and Women's Hospital, Northwestern University Feinberg School of Medicine, the Pennsylvania State University College of Medicine, and the University of Pennsylvania Medical Center, and subjects were enrolled between 1993 and 2004. Written informed consent was obtained from all participants. We used the National Institute of Child Health and Human Development diagnostic criteria for PCOS (18): no more than six menses per year and either total T $>$ 2.0 nmol/liter or nonsex hormone-binding globulin-bound T (uT) $>$0.5 nmol/liter, levels greater than two standard deviations above the mean value that we have established in reproductively normal women aged 18–40 years in the early follicular phase of the menstrual cycle (11, 17). Other causes of anovulation and hyperandrogenemia were excluded by appropriate tests (11, 17).

The mothers of women with PCOS fulfilling the above criteria were invited to participate in this study. We limited our analysis to non-Hispanic white women because of the potential confounding effects of ethnicity on insulin sensitivity and lipid levels (30). Control women of comparable age, weight, and ethnicity were studied. The selection criteria for control women were as follows: (*i*) regular 27- to 35-day menstrual cycles throughout their reproductive life, (*ii*) no history of hypertension or personal or family history of diabetes mellitus, (*iii*) no clinical or biochemical evidence of hyperandrogenism, and (*iv*) normal glucose tolerance according to the World Health Organization criteria (31). We did not examine the daughters of control women for features of PCOS because our family studies have shown that ≈50% of sisters of women with PCOS are affected (11). Therefore, even if reproductive-age daughters of control women were available for study, an unaffected daughter would not exclude genetic susceptibility to PCOS in that family. Furthermore, inclusion of control women with daughters who have PCOS would bias the results toward the null hypothesis (32). We assumed a background prevalence of 7% for PCOS among daughters of control women. None of the subjects was receiving hormonal (33), antihypertensive (34), or antidiabetic (35) medications, including 6 mothers with a history of diabetes and 27 with a history of hypertension.

Data Collection. Mothers were evaluated at one of the four study sites (on-site) or in a local laboratory (off-site). All control women were studied on-site. All subjects were requested to complete a questionnaire on their reproductive history, exercise habits, tobacco use, and alcohol intake: 99% of mothers and 100% of control women provided information on menses, 96% of mothers and 100% of control women provided information on menopause, 91% of mothers and 97% of control women provided information on exercise, and 55% of mothers and 93% of control women provided information on tobacco and alcohol use. Mothers were considered to have a history of (*i*) regular menses if they reported menses occurring every 27–35 days between the ages of 20 and 35 years regardless of current menopausal status; (*ii*) irregular menses if they reported a chronic history of menses $>$35 days between the ages of 20 and 35 years regardless of current menopausal status (24). Three mothers did not report menstrual history and were assigned unknown menstrual status and excluded from all analyses based on menstrual history. Hyperandrogenemia in mothers was defined by the same criteria used in the probands with PCOS (11, 17). We did not record self-reported hirsutism because we have found that such self-assessments are unreliable (unpublished observations), an observation that has also been confirmed by other investigators (36).

Mothers and control women were considered postmenopausal if their last menstrual cycle was $>$1 year before enrollment and their follicle-stimulating hormone levels were ≥40 units/liter (37). Women were considered premenopausal if they still experienced menstrual cycles and their follicle-stimulating hormone levels were $<$40 units/liter (37). Women who reported recent changes in their menstrual cycle pattern, had ceased to have menses but their last cycle was within 1 year, or in whom it was not possible to evaluate menopausal status because of a hysterectomy and/or oophorectomy were considered to have unknown menopausal status and were not included in the analyses for menopausal status.

Height, weight, blood pressure, and waist measurements were obtained as previously reported for the on-site subjects (11, 17). For the off-site subjects, the height and weight were self-reported as previously validated (11, 17). Waist circumference

was self-reported for 31% of mothers, who were provided with a calibrated tape measure for this determination. Self-measured waist circumference correlates well with measurements performed by a trained technician (38). A morning blood sample was obtained after an overnight fast from all subjects as previously reported (11, 17). All control women and 21% of mothers ($n = 45$) underwent a 75-g oral glucose tolerance test with fasting and 2-h postchallenge blood sampling for glucose and insulin levels.

Metabolic syndrome was defined according to National Cholesterol Education Program Adult Treatment Panel III guidelines (39). This assessment was possible only in mothers ($n = 125$) who had waist circumference and blood pressure determinations. Because several features of the metabolic syndrome were specifically excluded in our selection criteria for control women, we compared the prevalence of metabolic syndrome in mothers with that in 40- to 69-year-old non-Hispanic white women from the NHANES III survey (www.cdc.gov/nchs/nhanes.htm). The NHANES III survey was conducted from 1998 through 1994 and provides national estimates of the health and nutritional status of the United States population. Because prevalence rates of metabolic syndrome have recently been reported to increase in the updated NHANES survey from 1999 to 2000 (28), we also compared the prevalence of metabolic syndrome in mothers with that in the updated NHANES survey from 1999 to 2000. We compared prevalence rates after stratification by BMI: <25 kg/m^2, nonobese; 25–30 kg/m^2, overweight; ≥ 30 kg/m^2, obese.

Assays. Plasma glucose, insulin, follicle-stimulating hormone, T, uT, dehydroepiandrosterone sulfate, sex hormone-binding globulin, total cholesterol, HDL, LDL, and triglyceride levels were determined as previously reported (8, 11, 17). Non-sex hormone-binding globulin T (uT) was obtained from serum total T and non-sex hormone-binding globulin fraction by ammonium sulfate precipitation, a method that correlates well with measurements of free T by equilibrium dialysis (40).

Statistical Methods. We designed this study to detect at least a 10% difference in mean LDL levels between mothers and control women by a two-sample t test. A sample of 45 control women would provide the study with a statistical power of 80% to detect this difference compared with our sample of ≈ 200 mothers with a two-sided significance level of 0.05. HOMA IR was calculated according to the following formula: [fasting glucose (mmol/liter) $\times$ fasting insulin (microunits/ml)]/22.5 (41). Continuous variables were compared by analysis of covariance adjusted for age and BMI because there was a trend toward higher age and BMI in mothers, and this analysis was repeated in mothers ($n = 84$) and control women ($n = 56$) with further adjustment for tobacco, alcohol, and exercise history. For comparison of LDL levels we repeated the analysis after exclusion of mothers with a history of prior diabetes who were not on antidiabetic medications ($n = 6$) as well as mothers who were found to have glucose intolerance during the study: impaired glucose tolerance ($n = 8$), impaired fasting glucose ($n = 10$), or DM2 ($n = 9$). The analysis for LDL cholesterol was also repeated in the subset of mothers with normal glucose tolerance based on World Health Organization criteria with oral glucose tolerance testing during the study and who did not have a prior history of diabetes ($n = 34$). Differences among the groups were assessed by Tukey's post hoc test. Data were log-transformed to achieve homogeneity when necessary. Linear relationships between mothers and their proband daughters were assessed by Pearson correlation coefficients. A multivariate regression analysis was performed with LDL levels in mothers as the dependent variable and age, BMI, uT levels, and HOMA IR in mothers and LDL levels in their proband daughters as the independent variables; this analysis was repeated in mothers ($n = 84$) with the addition of tobacco, alcohol, and exercise history as independent variables. Categorical variables were compared by χ^2 analysis. Statistical analyses were performed by using the SAS 8.2 (SAS Institute, Cary, NC) and SPSS 12.0 (SPSS, Chicago) data analysis software. Differences were considered to be significant at $P \leq 0.05$. Data are presented as the untransformed mean $\pm$ SD.

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