# Pre-pregnancy adverse lipid profile and subsequent risk of gestational diabetes

Emily S. Han, MPH,[1], Ronald M. Krauss, MD,[2], Fei Xu, MA,[1], Sneha B. Sridhar, MPH,[1], Assiamira Ferrara, PhD,[1], Charles P. Quesenberry, PhD,[1], Monique M. Hedderson, PhD[1]

[1]Division of Research, Kaiser Permanente Northern California, 2000 Broadway, Oakland, CA; [2]Children's Hospital Oakland Research Institute

**Context:** Lower LDL peak diameter and a predominance of small, dense LDL are associated with type 2 diabetes, but it is unclear whether they are a risk factor for gestational diabetes mellitus (GDM).

**Objective:** To evaluate whether pre-pregnancy lipid profile predicts development of GDM during pregnancy.

**Design:** A nested case-control study among women who participated in a multiphasic health exam where blood was collected and stored between 1984 and 1996 and then had a subsequent pregnancy between 1984 and 2009.

**Setting:** Kaiser Permanente Northern California.

**Participants:** Cases were 254 women who developed GDM. Two controls were selected for each case and matched for year of blood draw, age at baseline, age at pregnancy, and number of intervening pregnancies.

**Main Outcome Measures:** Pre-pregnancy LDL peak diameter and pre-pregnancy lipid subfraction concentrations grouped according to size, and odds of developing GDM.

**Results:** Women in the lowest quartiles of LDL peak diameter and HDL had increased odds of GDM compared with women in the highest quartiles [OR (95% CI): 2.60 (1.37–4.94) and 1.98 (1.01–3.86), respectively], in multivariable adjusted models. Being in the highest quartile of small and very small LDL sub-fractions also increased the odds of GDM [2.61 (1.35–5.03) and 2.44 (1.22–4.85), respectively].

**Conclusions:** Lower LDL peak diameter size and HDL levels and higher levels of small and very small LDL subfraction groups were present years before pregnancy in women who developed GDM. A pre-pregnancy atherogenic lipid profile may help identify women at risk of GDM to target for prevention.

Women with gestational diabetes mellitus (GDM) are at increased risk for perinatal morbidities (1–3) and developing type 2 diabetes (4), and their offspring are at increased risk of childhood obesity and later diabetes as well (5, 6). However, it has not been well established what biomarkers can be used to detect risk of GDM before pregnancy, to help prevent adverse pregnancy and metabolic outcomes in both mothers and their children. Prepregnancy biomarkers may provide valuable insight into understanding the etiology of GDM, which can in turn inform GDM prevention strategies.

Having a predominance of small, dense low density lipoprotein (LDL) particles and a small LDL peak particle diameter has been associated with insulin resistance, type

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA
Copyright © 2016 by the Endocrine Society
Received November 10, 2015. Accepted March 30, 2016.

Abbreviations:



The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.

JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM
EARLY RELEASE:
ENDOCRINE SOCIETY

2 diabetes, and cardiovascular disease (7–10), but it is unclear whether this adverse lipid profile is also a risk factor for GDM. Past studies (11, 12) have only been able to assess LDL particle size and lipid concentrations during pregnancy, when lipid profile is known to change in response to the hormonal and metabolic changes induced by pregnancy (13). These two previous studies also had limited sample size, but they suggest that women with GDM are more likely to have a smaller mean LDL particle diameter (11) and higher concentrations of small, dense LDL subfractions during pregnancy (12). To clarify the temporal sequence of this association, it is important to study prospectively how the prepregnancy LDL particle profile is related to subsequent risk of GDM. In addition, new techniques make it possible to assess the entire spectrum of LDL, high density lipoprotein (HDL), and other lipoprotein particles and to better characterize and group lipid subfractions by size (14). Therefore the objective of this study was to evaluate whether LDL peak diameter and specific LDL subfractions, HDL, Very Low Density Lipoprotein (VLDL), and Intermediate Density Lipoprotein (IDL) measured years before pregnancy are associated with development of GDM.

## Materials and Methods

The setting is Kaiser Permanente Northern California (KPNC), an integrated health care delivery system that provides medical care for about one third of the underlying population in the San Francisco Bay Area. KPNC subscribers are representative of the region (15).

The source population consisted of female KPNC members who completed a voluntary Multiphasic Health Checkup (MHC) at the Kaiser Permanente Oakland Medical Center between 1984 and 1996. KPNC members at this facility were invited to complete a comprehensive health checkup upon enrollment. The MHC consisted of a clinic visit for the completion of questionnaires and clinical measurements, including blood pressure (BP), weight, and serum glucose and cholesterol (measured in serum obtained from a random blood draw). An extra serum sample was collected and stored at −40°C for future use. The goal of the MHC was to provide health maintenance through early diagnosis (16).

Among women 15–45 years of age (median age of 34) who participated in the MHC from 1985–1996 (n = 27,743 with clinical and questionnaire data, as well as an extra serum sample), we identified 4098 women who subsequently delivered an infant as of 2010 by searching the KPNC hospitalization database and the Pregnancy Glucose Tolerance and GDM Registry (17), an active surveillance registry that annually identifies all pregnancies resulting in a livebirth or stillbirth among KPNC members. Women with recognized prepregnancy diabetes (18) are excluded from the GDM Registry; therefore, women who had been diagnosed with diabetes prior to the index pregnancy were not eligible to be included. The Pregnancy Glucose Tolerance and GDM Registry captures the results of all screening and diagnostic tests for GDM from KPNC's electronic laboratory database (data available since 1994).

### Study Design

This is a nested case-control study, within a cohort of 4098 women who took part in an MHC examination, had an extra tube of serum stored for future use, and had a subsequent pregnancy, on average, 7 years after the MHC examination. All cohort members who went on to develop GDM were included as cases; 2 controls were selected for each case from among women not meeting the GDM case definition.

### GDM case definition

267 women with GDM were identified through the KPNC electronic databases. Cases had either 1) glucose values obtained during a standard 100-g, 3-hour oral glucose tolerance test (OGTT) that met the Carpenter-Coustan plasma glucose thresholds for GDM (19) in the laboratory database (n = 228), or 2) a hospital discharge diagnosis of GDM in the electronic hospital discharge database for pregnancies occurring before the electronic laboratory data were available (prior to 1994; n = 39). Standardized medical chart review was conducted by trained abstractors to confirm that all 267 cases had a 100-g, 3-hour OGTT meeting the Carpenter-Coustan criteria (19) for GDM [plasma glucose thresholds: fasting, 5.3 mmol/l (95 mg/dl); 1-hour, 10.0 mmol/l (180 mg/dl); 2-hour, 8.6 mmol/l (155 mg/dl); 3-hour, 7.8 mmol/l (140 mg/dl)] and to assess possible ineligibility. Cases were excluded if, at the time of the MHC examination, they had a random glucose $>$ 200 mg/dl (n = 6) or no indication of GDM during the index pregnancy (n = 5). In addition 2 cases were excluded because of an unmeasurable lipid sample (n = 2), leaving a total of 254 confirmed cases of GDM with valid lipid measurements.

### Control Selection and Matching Criteria

Among women without an indication of GDM, controls were randomly selected; two controls were individually matched to each case on year of MHC serum collection date (±3 months), age at MHC serum collection (±2 years), number of intervening pregnancies (0, 1, ≥2), and age at delivery of the index pregnancy (±2 years). Matching for the year of serum collection was required to account for any potential degradation in the quality of the serum over time, thereby assuring the sample storage time was approximately the same for cases and controls. Since GDM is more common in older women, age at serum collection and age at delivery were used for matching. To account for any differences in pregnancies between baseline examination and the index pregnancy, cases and controls were matched by number of pregnancies. Controls were excluded from the analysis if they had glucose values diagnostic of GDM found during medical chart abstraction (n = 5), had an abnormal screening glucose but no follow-up diagnostic glucose test (n = 5), had one abnormal glucose value on the diagnostic glucose test suggestive of 'mild' GDM (n = 5), or an unmeasurable lipid sample (n = 7). Of the 508 matched controls identified, 490 were eligible.

### Exposure Variables

Lipoprotein particle analysis was performed by Dr. Ronald Krauss's lab at the Children's Hospital Oakland Research Institute. Lipid subfraction concentrations (nmol/L) were measured by ion mobility, as was peak LDL diameter (Å) (20). LDL sub-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.

fractions were grouped as a function of particle diameter as described previously: LDL-Large (22.0 –23.3 nm); LDL-Medium (21.4–22.0 nm); LDL-Small (20.8–21.4 nm); LDL-Very Small (18.0–20.8 nm). Subfractions of IDL, VLDL, and HDL were grouped together and analyzed as the following size ranges: Total HDL as both HDL-Small and HDL-Large (7.7–14.5 nm), IDL as both IDL-Small and IDL-Large (23.3–29.6 nm), and VLDL as VLDL-Small, VLDL-Medium, and VLDL-Large (29.6–52.0 nm) (21).

### Covariate data

Body mass index (BMI) at the time of MHC examination was calculated as kg/m$^2$; height was measured using a stadiometer and weight using a balance beam scale. To calculate weight change (kg/y) from the MHC examination to the start of pregnancy, prepregnancy weight was abstracted from the medical record, or self-reported prepregnancy weight was used if measured was unavailable. Information on age, race/ethnicity, family history of diabetes, alcohol consumption ($\geq$1 drink/d vs $<$ 1 drink/d), and time since food ingestion (divided into 2-hour increments since last food ingestion at the time of the MHC up to $\geq$ 10 hours) was collected using self-administered questionnaires (16). Total cholesterol was assessed using a Kodak Ektachem Chemistry analyzer by the regional laboratory of KPNC at the time of the MHC. This laboratory participates in the College of American Pathologists' accreditation and monitoring program. Serum samples were thawed, aliquoted and transported in batches on dry ice to Dr. Peter Havel's laboratory at the University of California, Davis, for measurement of insulin by radioimmunoassay (RIA) (Millipore). The intra-assay and inter-assay CVs are $<$ 4.0% and $<$ 10%, respectively. Insulin resistance was calculated based on the homeostasis model assessment-estimated insulin resistance (HOMA-IR) using the following equation: (fasting glucose x fasting insulin)/22.5, where glucose was measured in mmol/liter and insulin in $\mu$U/ml (22).

### Statistical Analysis

Conditional logistic regression was used to obtain odds ratios (ORs) to estimate the risk of GDM continuously by 1 standard deviation (SD) change in lipid size or concentration, and by quartile of concentration for each lipid subfraction group of interest (see Table 1 for list). LDL peak diameter was modeled in the same manner. We chose potential confounders a priori including: race/ethnicity, prepregnancy BMI, alcohol use, family history of diabetes, HOMA-IR, and time since last food intake (in 2-hour increments with the final category $\geq$ 10 hours fasting), all assessed at the time of the MHC. To examine the effect of weight gain during pregnancy up to the time of GDM diagnosis, this variable was also added to the adjusted conditional logistic regression model. P-values for tests for trend for each lipid group were obtained to examine if there were significant trends with increasing or decreasing quartiles in the adjusted models.

To assess the potential modifying effects of prepregnancy BMI [overweight or obese ($\geq$25 kg/m$^2$) vs not overweight or obese ($<$25 kg/m$^2$)], race-ethnicity (white, Asian, Hispanic and African American), and median time since MHC examination ($\geq$6.2 years vs $<$ 6.2 years), we included appropriate interaction terms in the fully adjusted regression model with 1 standard deviation decrease of LDL peak diameter.

This study was approved by the Institutional Review Board of the Kaiser Foundation Research Institute.

## Results

Table 1 summarizes the demographic, anthropometric, reproductive, and metabolic characteristics of the study participants by case/control status. There were higher proportions of Asians and Hispanics among cases. Compared to controls, women with GDM had higher levels of several cardiometabolic risk factors, including a family history of diabetes, a higher prepregnancy BMI, and higher weight gain before pregnancy. Cases had higher glucose, cholesterol, insulin, and calculated HOMA-IR values at their MHC examination conducted on average 7 years before pregnancy. Prepregnancy LDL peak diameter was, on average, 1.4 Å smaller in cases (230.6 $\pm$ 5.6 Å) compared to controls (232.0 $\pm$ 4.7 Å). Cases had higher concentrations of all LDL subfractions and lower concentrations of total HDL compared to controls.

Table 2 displays the associations between prepregnancy lipoprotein particle concentrations and peak particle diameter with GDM risk obtained from conditional logistic regression models. Continuous and quartile models were similarly significant. Women with a prepregnancy LDL peak diameter in the lowest quartile had 2.6 times the odds of developing GDM compared to the reference quartile (highest quartile) (Odds Ratio (OR): 2.60, 95% Confidence Interval (CI): 1.37–4.94), in the fully adjusted model. There was a significant trend of increasing odds of GDM with decreasing quartile of LDL peak diameter.

In analysis related to lipoprotein subfractions grouped according to particle diameter (Table 2), increasing concentration of the LDL-Smallest particles led to increased odds of developing GDM (P-trend $<$ 0.01). The highest two quartiles of LDL-Smallest particle concentrations were significantly associated with increased odds of developing GDM (OR: 1.99, 95% CI: 1.05–3.75 for quartile 3; and OR: 2.44, 95% CI: 1.22–4.85 for quartile 4) compared to the first quartile. LDL-Medium had increased odds of GDM with increasing quartile concentration of each (P-trend $<$ 0.01). However, unlike in the quartile model, in the adjusted continuous model, LDL-Small was not significant and did not have a significant P-trend. While none of the quartiles were significant for LDL-Large, the adjusted continuous model was significant, reflecting the significant P-trend of increasing risk of GDM with increasing concentration of LDL-Large particles. Women in the lowest two quartiles of total HDL particle concentration had nearly two times higher odds of GDM compared to women in the highest HDL quartile (OR: 1.98; 95% CI: 1.01–3.86 for Quartile 1 and OR: 1.92; 95% CI 1.02–3.62 for Quartile 2). Neither total IDL or VLDL particle concentrations were significantly associated with GDM.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.

**Table 1.** Characteristics of gestational diabetes case women and control women

| | GDM cases ($n$ = 254) | Controls ($n$ = 490) | $P$-value[1] |
|---|---|---|---|
| Age at MHC clinic visit (years) | 27.8 ± 5.5 | 27.9 ± 5.2 | 0.069 |
| Age at delivery (years) | 35.0 ± 5.1 | 34.6 ± 4.9 | 0.002 |
| Time between exam and delivery (years) | 7.1 ± 4.4 | 6.7 ± 4.4 | <0.001 |
| Race/Ethnicity | | | <0.001 < |
| Non-Hispanic White | 50 (19.7) | 183 (37.4) | 0.001 |
| African American | 90 (35.4) | 180 (36.7) | |
| Asian/Pacific Islander | 80 (31.5) | 84 (17.1) | |
| Hispanic | 34 (13.4) | 43 (8.8) | |
| Alcohol use (Occasional or more drinks/day) | 147 (57.9) | 341 (69.6) | <0.001 |
| Family History of Diabetes | 151 (59.5) | 187 (38.2) | <0.001 |
| Pre-pregnancy Body Mass Index (kg/m$^2$) | 26.1 ± 6.5 | 23.7 ± 4.6 | <0.001 |
| Weight change from MHC to pregnancy (kg) | 8.2 ± 9.9 | 4.4 ± 8.1 | <0.001 |
| Time since last food ingestion at MHC | | | 0.355 |
| <2 h | 19 (7.5) | 32 (6.5) | |
| 2-<4 h | 42 (16.5) | 85 (17.4) | |
| 4-<6 h | 41 (16.1) | 86 (17.6) | |
| 6–8 h | 17 (6.7) | 40 (8.2) | |
| 8-<10 h | 113 (44.5) | 194 (39.6) | |
| ≥10 h | 18 (7.1) | 29 (5.9) | |
| Glucose (mg/dL) | 89.7 ± 13.5 | 83.6 ± 8.4 | <0.001 |
| Cholesterol (mg/dL) | 182.9 ± 33.3 | 176 ± 32.6 | 0.006 |
| HOMA-IR index | 6.1 ± 8.1 | 3.7 ± 4.2 | <0.001 |
| Insulin ($\mu$Units/ml) | 25.9 ± 28.7 | 17.4 ± 16.8 | <0.001 |
| LDL peak diameter (Å) | 230.6 ± 5.6 | 232.0 ± 4.7 | <0.001 |
| **Sub-fraction groups (nmol/liter)[2]** | | | |
| HDL[3] | 4180.4 ± 1524.9 | 4650.8 ± 1605.5 | <0.001 |
| LDL-Large[4] | 369.9 ± 178.2 | 334.6 ± 146 | 0.003 |
| LDL-Medium[5] | 94.3 ± 70.2 | 77.2 ± 47.8 | <0.001 |
| LDL-Small[6] | 61.1 ± 41.5 | 53.7 ± 31.5 | 0.006 |

(*Continued*)

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.

**Table 1.** Continued

| | GDM cases ($n$ = 254) | Controls ($n$ = 490) | $P$-value[1] |
|---|---|---|---|
| LDL-Very Small[7] | 110.2 ± 43 | 101.9 ± 36.9 | 0.003 |
| VLDL[8] | 134.4 ± 44.5 | 130.3 ± 43.5 | 0.168 |
| IDL[9] | 371.7 ± 125.5 | 386.8 ± 119.9 | 0.112 |

Data are mean ± SD or $N$ (%), unless otherwise indicated. [1]P-values from conditional logistic regression.. [2]Groups for analysis assigned as following: [3]HDL-Small + HDL-Large (7.7–14.5 nm). [4]LDL-Large (22.0–23.3 nm). [5]LDL-Medium (21.4–22.0 nm). [6] LDL-Small (20.8–21.4 nm). [7]LDL-Very Small (18.0–20.8 nm). [8]VLDL-Small + VLDL-Medium + VLDL large (29.6–52.0 nm). [9]IDL-Small + IDL-Large (23.3–29.6 nm).

There was no significant effect modification by BMI, race-ethnicity, or time since MHC examination for any of the associations examined.

## Discussion

In this case-control study, women who developed GDM had a smaller LDL peak diameter, lower average HDL concentrations, and higher average concentrations of small, dense LDL particles on average 7 years before pregnancy compared to controls. Smaller LDL peak diameter, lower HDL levels, and higher levels of small, dense LDL particles were associated with subsequent development of GDM, independently of known risk factors including BMI, weight gain before pregnancy, age, and race-ethnicity, as well as markers of insulin resistance and family history of diabetes. Our findings are among the first to suggest that smaller LDL peak diameter and an adverse lipid profile consisting of low HDL levels and smaller, denser, LDL subfractions may predict GDM years before pregnancy.

The relationship between LDL particle size and type 2 diabetes and insulin resistance has been well-studied. Haffner et al found that decreasing LDL size is associated with insulin resistance in individuals without diabetes (23), and Krayenbeuhl et al found that in type 2 persons with diabetes, insulin resistance was correlated with smaller LDL particle size (R = 0.61) (24). Suh et al found that 203 Korean type 2 diabetes patients had significantly smaller LDL mean particle size (26.32 nm vs. 26.49 nm) and a higher percentage of small, dense LDL to total LDL (25). In terms of prospective studies, Mora et al found that small LDL was associated with incident diabetes with a hazard ratio (HR) of 4.04 (95% CI: 3.21–5.09) after adjustment (26). However, less is known about the role of the lipid profile in GDM risk.

Our findings of the prospective association between LDL subfractions and GDM are generally consistent with findings from nonprospective studies. Qiu et al found that GDM cases had a lower mean LDL particle size when measured during delivery compared with controls and a nonsignificant but nearly twofold higher odds of GDM for each 10 Å decrease in LDL mean particle size (11). Rizzo et al examined LDL size in the second trimester of pregnancy and found that overall LDL size was decreased in GDM cases (12). However, these prior studies did not examine other lipoproteins such as HDL, and their samples were obtained during pregnancy and may have been influenced by GDM status. During pregnancy women experience hormonal and metabolic changes; concentrations of VLDL, IDL, and triglycerides increase as do concentrations of small, dense LDL (13). Therefore, assessing the associations prepregnancy allowed us to examine the temporal association between the lipoprotein profile and GDM. We found that decreasing prepregnancy LDL peak diameter and having higher levels of smaller, denser LDL particles was associated with increasing odds of subsequently developing GDM. We also found that lower levels of prepregnancy HDL were associated with developing GDM, similar to what has been found in the CARDIA study (27).

While the present results support the possibility that small, dense LDL particles may play a role in the development of GDM, the mechanistic basis for such an effect remains speculative. The underlying etiology of GDM is believed to be diminished $\beta$-cell function coupled with increased insulin resistance (28), leading to an inability to compensate for the increased insulin resistance induced by pregnancy. Small LDL particles have reduced LDL receptor affinity, are more susceptible to uptake by arterial walls, and are more susceptible to oxidation (14, 29), leading to increased free radical activity. Oxidative stress induces insulin resistance in peripheral tissue and impairs insulin secretion from pancreatic $\beta$-cells (30, 31). Hence, such an effect of small, dense LDL could contribute to increased likelihood of developing GDM.

The strengths of this study include a large and diverse study cohort, with strong representation from several racial-ethnic groups and a large number of GDM case patients with matched controls. To our knowledge this is the

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.

**Table 2.** Odds ratios (OR) and 95% confidence intervals (CI) for GDM associated with pre-pregnancy lipids.

| | Conditional logistic regression models | | |
|---|---|---|---|
| | Crude | Multivariable adjusted[1] | *P*-trend[1,2] |
| **Pre-pregnancy risk factor** | | | |
| LDL Peak Diameter (Å)[3] | | | |
| Continuous Model | **1.37 (1.17–1.61)** | **1.39 (1.10–1.74)** | |
| Quartile1 (214.6–229.3) | **1.98 (1.27–3.10)** | **2.60 (1.37–4.94)** | **0.005** |
| Quartile2 (229.4–232.5) | 1.34 (0.87–2.06) | 1.20 (0.67–2.12) | |
| Quartile3 (232.6–234.5) | 0.84 (0.52–1.34) | 1.05 (0.57–1.96) | |
| Quartile4 (234.6–243.9) | 1.00 | 1.00 | |
| HDL Group (nmol/liter)[3] | | | |
| Continuous Model | **1.40 (1.18–1.67)** | **1.33 (1.05–1.70)** | |
| Quartile1 (1189.2–3497.3) | **2.28 (1.40–3.72)** | **1.98 (1.01–3.86)** | **0.021** |
| Quartile2 (3497.4–4431.9) | **2.14 (1.32–3.48)** | **1.92 (1.02–3.62)** | |
| Quartile3 (4432.0–5696.6) | **1.68 (1.02–2.75)** | 1.59 (0.83–3.07) | |
| Quartile4 (5696.7–11 303.8) | 1.00 | 1.00 | |
| IDL Group (nmol/liter)[3] | | | |
| Continuous Model | 1.13 (0.97–1.32) | 1.10 (0.89–1.37) | |
| Quartile1 (98.9–303.7) | 1.35 (0.88–2.09) | 1.42 (0.76–2.63) | 0.365 |
| Quartile2 (303.8–376.4) | 1.23 (0.79–1.91) | 1.09 (0.59–1.99) | |
| Quartile3 (376.5–460.8) | 1.10 (0.71–1.71) | 1.28 (0.70–2.35) | |
| Quartile4 (460.9–863.7) | 1.00 | 1.00 | |
| LDL-Smallest (nmol/liter)[4] | | | |
| Continuous Model | **1.28 (1.09–1.50)** | **1.31 (1.04–1.64)** | |
| Quartile1 (35.1–78.8) | 1.00 | 1.00 | **0.020** |
| Quartile2 (78.9–93.9) | 0.99 (0.61–1.59) | 1.50 (0.78–2.88) | |
| Quartile3 (94.0–116.9) | 1.56 (0.99–2.47) | **1.99 (1.05–3.75)** | |
| Quartile4 (117.0–327.4) | **1.93 (1.20–3.12)** | **2.44 (1.22–4.85)** | |
| LDL-Small (nmol/liter)[4] | | | |
| Continuous Model | **1.22 (1.06–1.41)** | 1.15 (0.94–1.40) | |
| Quartile1 (14.7–36.5) | 1.00 | 1.00 | 0.182 |
| Quartile2 (36.6–47.9) | 1.17 (0.73–1.87) | 1.73 (0.90–3.35) | |
| Quartile3 (48.0–59.6) | 1.45 (0.93–2.27) | 1.75 (0.94–3.26) | |
| Quartile4 (59.7–365.0) | **1.77 (1.11–2.81)** | **2.61 (1.35–5.03)** | |
| LDL-Medium (nmol/liter)[4] | | | |
| Continuous Model | **1.32 (1.14–1.51)** | **1.31 (1.07–1.60)** | |
| Quartile1 (16.5–51.0) | 1.00 | 1.00 | **0.009** |
| Quartile2 (51.1–67.5) | 0.98 (0.63–1.54) | 1.30 (0.70–2.41) | |
| Quartile3 (67.6–87.9) | 1.13 (0.72–1.78) | 1.48 (0.81–2.70) | |
| Quartile4 (88.0–467.7) | **1.59 (1.02–2.49)** | **1.99 (1.04–3.80)** | |
| LDL-Large (nmol/liter)[4] | | | |
| Continuous Model | **1.26 (1.09–1.47)** | **1.44 (1.14–1.83)** | |
| Quartile1 (66.2–226.0) | 1.00 | 1.00 | **0.003** |
| Quartile2 (226.1–308.2) | 0.84 (0.53–1.33) | 0.87 (0.47–1.61) | |
| Quartile3 (308.3–420.5) | 1.02 (0.65–1.61) | 1.27 (0.69–2.34) | |
| Quartile4 (420.6–1174.5) | 1.54 (0.98–2.41) | 1.79 (0.93–3.45) | |
| VLDL Group (nmol/liter)[4] | | | |
| Continuous Model | 1.11 (0.96–1.29) | 1.14 (0.93–1.40) | |
| Quartile1 (33.7–100.1) | 1.00 | 1.00 | 0.219 |
| Quartile2 (100.2–123.9) | 1.50 (0.96–2.33) | 1.84 (0.98–3.47) | |
| Quartile3 (124.0–153.2) | 1.29 (0.82–2.02) | 1.49 (0.79–2.83) | |
| Quartile4 (153.3–308.3) | 1.52 (0.98–2.37) | 1.52 (0.83–2.78) | |

Data are Odds Ratio (95% Confidence Interval). [1]Adjusted for race/ethnicity, pre-pregnancy BMI, family history of diabetes, alcohol use at time of the MHC examination (one or more *vs.* less than one drink/day), HOMA-IR, time since last food ingestion, and weight change from MHC exam to pregnancy. [2] P-value from a continuous linear model. [3]-1 SD [4] +1 SD

first study to utilize prepregnancy measurements to examine the relationship of specific lipoprotein subfractions to development of GDM.

This study also has several important limitations. First, over half our samples were nonfasting, and there can be changes in LDL size in nonfasting individuals (32–34). To account for this, we adjusted for time since last food ingestion as a proxy for fasting status in 2-hour increments with the final category ≥ 10 hours fasting. We were unable to assess triglyceride levels which may influence LDL par-

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.

ticle size; at the time of the MHC only total cholesterol was measured (35). This meant that we were also unable to assess the atherogenic lipoprotein phenotype, which is characterized by elevated triglycerides and LDL, and low HDL levels (7). In addition, we lacked information on diet and physical activity at the baseline examination and the subsequent pregnancy and these factors may impact LDL particle size and subfraction concentrations. Additionally, there may be other confounders that we did not measure and control for that may have resulted in bias of our point and interval estimates of association. Therefore, we were unable to determine whether the impact of smaller LDL particle size was independent of lifestyle on GDM risk in this study.

In conclusion, a lipoprotein profile including smaller LDL peak particle diameter, lower HDL levels and higher levels of small, dense LDL, determined on average 7 years before pregnancy, is associated with increased likelihood for developing GDM. While a causal mechanism for this association remains to be identified, our findings are consistent with the possibility that improving the cardiometablic risk profile in women of reproductive age may reduce the risk of GDM. LDL size and subfraction measurements in reproductive-aged women may be helpful to identify those at risk of GDM to target for early treatment and prevention efforts. Future studies designed to assess the sensitivity and specificity of LDL subfractions in predicting GDM will be valuable to help further assess the clinical utility of these biomarkers.

## Acknowledgments

Address all correspondence and requests for reprints to: Emily Han, MPH, Division of Research, Kaiser Permanente Northern California, 2000 Broadway, Oakland, CA 94612, Telephone: 916–317-3510, Fax: 510–891-3606, Email: Emily.Han@kp.org.

**Disclosure Statement**: The authors have nothing to disclose.

This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development grant R01-HD-065904 to M.M.H.

## References

1. **Ferrara A, Weiss NS, Hedderson MM et al.** Pregnancy plasma glucose levels exceeding the American Diabetes Association thresholds, but below the National Diabetes Data Group thresholds for gestational diabetes mellitus, are related to the risk of neonatal macrosomia, hypoglycaemia and hyperbilirubinaemia. *Diabetologia*. 2007;50:298–306.
2. **Hedderson MM, Ferrara A, Sacks DA.** Gestational diabetes mellitus and lesser degrees of pregnancy hyperglycemia: association with increased risk of spontaneous preterm birth. *Obstet Gynecol*. 2003; 102(4):850–856.
3. **Wendland EM, Torloni MR, Falavigna M et al.** Gestational diabetes and pregnancy outcomes–a systematic review of the World Health Organization (WHO) and the International Association of Diabetes in Pregnancy Study Groups (IADPSG) diagnostic criteria. *BMC Pregnancy Childbirth*. 2012;12:23.
4. **Bellamy L, Casas JP, Hingorani AD, Williams D.** Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet*. 2009;373(9677):1773–1779.
5. **Silverman BL, Rizzo TA, Cho NH, Metzger BE.** Long-term effects of the intrauterine environment. The Northwestern University Diabetes in Pregnancy Center. *Diabetes Care*. 1998;21 Suppl 2:B142–B149.
6. **Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ.** Childhood obesity and metabolic imprinting: the ongoing effects of maternal hyperglycemia. *Diabetes Care*. 2007;30(9): 2287–2292.
7. **Selby JV, Austin MA, Newman B et al.** LDL subclass phenotypes and the insulin resistance syndrome in women. *Circulation*. 1993;88(2): 381–387.
8. **Austin MA, Selby JV.** LDL subclass phenotypes and the risk factors of the insulin resistance syndrome. *Int J Obes Relat Metab Disord*. 1995;19 Suppl 1:S22–6.
9. **Austin MA, Mykkanen L, Kuusisto J et al.** Prospective study of small LDLs as a risk factor for non-insulin dependent diabetes mellitus in elderly men and women. *Circulation*. 1995;92(7):1770–1778.
10. **Rizzo M, Berneis K.** Low-density lipoprotein size and cardiovascular risk assessment. *QJM*. 2006;99(1):1–14.
11. **Qiu C, Rudra C, Austin MA, Williams MA.** Association of gestational diabetes mellitus and low-density lipoprotein (LDL) particle size. *Physiol Res*. 2007;56(5):571–578.
12. **Rizzo M, Berneis K, Altinova AE et al.** Atherogenic lipoprotein phenotype and LDL size and subclasses in women with gestational diabetes. *Diabet Med*. 2008;25(12):1406–1411.
13. **Sattar N, Greer IA, Louden J et al.** Lipoprotein subfraction changes in normal pregnancy: threshold effect of plasma triglyceride on appearance of small, dense low density lipoprotein. *J Clin Endocrinol Metab*. 1997;82(8):2483–2491.
14. **Krauss RM.** All low-density lipoprotein particles are not created equal. *Arterioscler Thromb Vasc Biol*. 2014;34(5):959–961.
15. **Gordon NP.** Similarity of the Adult Kaiser Permanente Membership in Northern California to the Insured and General Population in Northern California: Statistics from the 2009 California Health Interview Survey. 1–24–2012. Oakland, CA, Kaiser Permanente Division of Research.
16. **Collen MF.** Multiphasic health testing services. New York: John Wiley, Sons, 1978.
17. **Ferrara A, Kahn HS, Quesenberry C, Riley C, Hedderson MM.** An increase in the incidence of gestational diabetes mellitus: Northern California, 1991–2000. *Obstet Gynecol*. 2004;103(3):526–533.
18. **Selby JV, Ray GT, Zhang D, Colby CJ.** Excess costs of medical care for patients with diabetes in a managed care population. *Diabetes Care*. 1997;20:1396–1402.
19. **Committee opinion no. 504: screening and diagnosis of gestational diabetes mellitus.** *Obstet Gynecol* 2011;118(3):751–753.
20. **Caulfield MP, Li S, Lee G et al.** Direct Determination of Lipoprotein Particle Sizes and Concentrations by Ion Mobility Analysis. Clin Chem 2008.
21. **Musunuru K, Orho-Melander M, Caulfield MP et al.** Ion mobility analysis of lipoprotein subfractions identifies three independent axes of cardiovascular risk. *Arterioscler Thromb Vasc Biol*. 2009; 29(11):1975–1980.
22. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412–419.
23. **Haffner SM, Mykkanen L, Robbins D et al.** A preponderance of small dense LDL is associated with specific insulin, proinsulin and

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.

the components of the insulin resistance syndrome in non-diabetic subjects. *Diabetologia*. 1995;38(11):1328–1336.
24. **Krayenbuehl PA, Wiesli P, Schmid C, Lehmann R, Spinas GA, Berneis K.** Insulin sensitivity in type 2 diabetes is closely associated with LDL particle size. *Swiss Med Wkly*. 2008;138(19–20):275–280.
25. **Suh S, Park HD, Kim SW et al.** Smaller Mean LDL Particle Size and Higher Proportion of Small Dense LDL in Korean Type 2 Diabetic Patients. *Diabetes Metab J*. 2011;35(5):536–542.
26. **Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE, Ridker PM.** Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. *Diabetes*. 2010; 59(5):1153–1160.
27. **Gunderson EP, Quesenberry CP, Jr., Jacobs DR, Jr., Feng J, Lewis CE, Sidney S.** Longitudinal Study of Prepregnancy Cardiometabolic Risk Factors and Subsequent Risk of Gestational Diabetes Mellitus: The CARDIA Study. *Am J Epidemiol*. 2010;172(10):1131–1143.
28. **Buchanan TA, Xiang AH.** Gestational diabetes mellitus. *J Clin Invest*. 2005;115(3):485–491.
29. **Berneis KK, Krauss RM.** Metabolic origins and clinical significance of LDL heterogeneity. *J Lipid Res*. 2002;43(9):1363–1379.
30. **Oberley LW.** Free radicals and diabetes. *Free Radic Biol Med*. 1988; 5(2):113–124.
31. **Ceriello A, Motz E.** Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arterioscler Thromb Vasc Biol*. 2004;24(5):816–823.
32. **Karpe F, Tornvall P, Olivecrona T, Steiner G, Carlson LA, Hamsten A.** Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. *Atherosclerosis*. 1993; 98(1):33–49.
33. **Lemieux I, Couillard C, Pascot A et al.** The small, dense LDL phenotype as a correlate of postprandial lipemia in men. *Atherosclerosis*. 2000;153(2):423–432.
34. **Mora S, Rifai N, Buring JE, Ridker PM.** Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. *Circulation*. 2008;118(10):993–1001.
35. **Tsai MY, Steffen BT, Guan W et al.** New automated assay of small dense low-density lipoprotein cholesterol identifies risk of coronary heart disease: the Multi-ethnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2014;34(1):196–201.

The Endocrine Society. Downloaded from press.endocrine.org by [${individualUser.displayName}] on 18 April 2016. at 18:04 For personal use only. No other uses without permission. . All rights reserved.