Prepregnancy low-carbohydrate dietary pattern and risk of gestational diabetes mellitus: a prospective cohort study[1–4]

Wei Bao, Katherine Bowers, Deirdre K Tobias, Sjurdur F Olsen, Jorge Chavarro, Allan Vaag, Michele Kiely, and Cuilin Zhang

ABSTRACT

Background: Low-carbohydrate diets (LCDs) have been vastly popular for weight loss. The association between a low-carbohydrate dietary pattern and risk of gestational diabetes mellitus (GDM) remains unknown.

Objective: We aimed to prospectively examine the association of 3 prepregnancy low-carbohydrate dietary patterns with risk of GDM.

Design: We included 21,411 singleton pregnancies in the Nurses' Health Study II. Prepregnancy LCD scores were calculated from validated food-frequency questionnaires, including an overall LCD score on the basis of intakes of carbohydrate, total protein, and total fat; an animal LCD score on the basis of intakes of carbohydrate, animal protein, and animal fat; and a vegetable LCD score on the basis of intakes of carbohydrate, vegetable protein, and vegetable fat. A higher score reflected a higher intake of fat and protein and a lower intake of carbohydrate, and it indicated closer adherence to a low-carbohydrate dietary pattern. RRs and 95% CIs were estimated by using generalized estimating equations with log-binomial models.

Results: We documented 867 incident GDM pregnancies during 10 y follow-up. Multivariable-adjusted RRs (95% CIs) of GDM for comparisons of highest with lowest quartiles were 1.27 (1.06, 1.51) for the overall LCD score (P-trend = 0.03), 1.36 (1.13, 1.64) for the animal LCD score (P-trend = 0.003), and 0.84 (0.69, 1.03) for the vegetable LCD score (P-trend = 0.08). Associations between LCD scores and GDM risk were not significantly modified by age, parity, family history of diabetes, physical activity, or overweight status.

Conclusions: A prepregnancy low-carbohydrate dietary pattern with high protein and fat from animal-food sources is positively associated with GDM risk, whereas a prepregnancy low-carbohydrate dietary pattern with high protein and fat from vegetable food sources is not associated with the risk. Women of reproductive age who follow a low-carbohydrate dietary pattern may consider consuming vegetable rather than animal sources of protein and fat to minimize their risk of GDM. *Am J Clin Nutr* 2014;99:1378–84.

INTRODUCTION

Carbohydrate-restricted diets or low-carbohydrate diets (LCDs)[5] were first introduced ~150 y ago (1). These diets remain very popular for weight loss because they result in a rapid reduction in body weight without having to count calories or compromise the consumption of many palatable foods (2). However, debates and concerns continue with regard to the long-term safety and efficacy of these diets (2, 3), and it has been shown that the weight loss by LCDs may dissipate after 1 y (4, 5). Moreover, associations between adherence to low-carbohydrate dietary patterns and cardiometabolic outcomes, such as type 2 diabetes (T2D) (6, 7) and cardiovascular disease (8, 9), remain controversial.

Gestational diabetes mellitus (GDM), which is a common pregnancy complication defined as glucose intolerance with onset or first recognition during pregnancy (10), is a growing health concern (11). GDM is not only associated with short-term adverse perinatal outcomes (12) but also related to elevated long-term metabolic risk in both mothers and their offspring (10, 11, 13). For instance, 35–60% of women who have had GDM will develop T2D in the next 10–20 y (14). Thus, it is crucial to identify modifiable risk factors that may contribute to the prevention of GDM. Low-carbohydrate dietary patterns represent combinations of a lower content of carbohydrate and higher contents of fat and protein from the diet. Increased intakes of fat and protein are naturally needed to compensate energy requirements. In previous studies, dietary intakes of animal fat and animal protein were

[1] From the Epidemiology Branch, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, Rockville, MD (WB, MK, and CZ); the Division of Biostatistics and Epidemiology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, Cincinnati, OH (KB); the Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA (DKT and JC); the Centre for Fetal Programming, Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark (SFO); the Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (JC); and the Department of Endocrinology, Rigshospitalet, Copenhagen, Denmark (AV).

[2] Presented in abstract form at the 46th Society for Epidemiologic Research Annual Meeting, in Boston, MA, 18–21 June 2013.

[3] Supported by the Intramural Research Program of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH (contract HHSN275201000020C). The Nurses' Health Study II was funded by the NIH (research grants DK58845, CA50385, P30 DK46200, and UM1 CA176726).

[4] Address correspondence to C Zhang, Epidemiology Branch, Division of Epidemiology, Statistics and Prevention Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, 6100 Executive Boulevard, Rockville, MD 20852. E-mail: zhangcu@mail.nih.gov.

[5] Abbreviations used: FFQ, food-frequency questionnaire; GDM, gestational diabetes mellitus; LCD, low-carbohydrate diet; NHS, Nurses' Health Study; T2D, type 2 diabetes.

Received December 31, 2013. Accepted for publication March 19, 2014.

First published online April 9, 2014; doi: 10.3945/ajcn.113.082966.

positively associated with GDM risk, whereas intake of vegetable protein was inversely associated with risk (15, 16). Theoretically, long-term adherence to low-carbohydrate dietary patterns, particularly those that are mainly based on animal foods, may have detrimental effects on GDM risk because they result in an increase in animal fat intakes and a decrease in the consumption of whole grains, dietary fiber, fruits, and vegetables. However, the effect of low-carbohydrate dietary patterns on the development of GDM remains unknown. With the use of data from a large cohort study, we aimed to prospectively examine the association between 3 prepregnancy low-carbohydrate dietary patterns and risk of GDM.

SUBJECTS AND METHODS

Study population

The Nurses' Health Study II (NHS II) is an ongoing, prospective cohort study of 116,671 female nurses aged 25–44 y at study inception in 1989 (17). Participants receive biennial questionnaires regarding disease outcomes and lifestyle behaviors, such as smoking status and medication use. The follow-up for each questionnaire cycle is>90%. This study was approved by the Partners Human Research Committee (Boston, MA) with participant consent implied by the return of questionnaires.

We included NHS II participants in this analysis if they reported at least one singleton pregnancy that lasted >6 mo between 1991 and 2001. The 1991 questionnaire was the first time dietary information was administered. Thus, we set this year as the baseline for this analysis, and we only included pregnancies after the return of the 1991 questionnaire. The 2001 questionnaire was the last time GDM incidence was ascertained because the majority of NHS II participants had passed reproductive age by then; thus, the follow-up was through the return of the 2001 questionnaire. Pregnancies became eligible if there was no GDM reported in a previous pregnancy or a previous diagnosis of T2D, cardiovascular disease, or cancer. We excluded pregnancies if the participant did not return a prepregnancy food-frequency questionnaire (FFQ), left >70 FFQ items blank, or reported unrealistic total energy intake (<600 or >3500 kcal/d). Women with GDM in a previous pregnancy were not included because they may have changed their diets and lifestyles during the next pregnancy to prevent recurrent GDM.

Exposure assessment

Beginning in 1991 and every 4 y thereafter, we asked participants to report their food intakes by using a semiquantitative FFQ. We computed intake of individual nutrients including protein by multiplying the frequency of consumption of each food by the nutrient content of the specified portion on the basis of food-composition data from USDA (18). The reproducibility and validity of the FFQ has been extensively documented (19–21). In a previous validation study that compared energy-adjusted macronutrient intake assessed by using a FFQ with four 1-wk diet records, Pearson's correlation coefficients were 0.61 for total carbohydrate, 0.52 for total protein, and 0.54 for total fat (20). Missing exposure data were carried forward from the most recent FFQ for which data were captured. Overall, missing exposure existed in ~6% of pregnancies.

To represent the adherence to various low-carbohydrate dietary patterns, we calculated 3 LCD scores (ie, overall LCD, animal LCD, and vegetable LCD scores) for each participant as previously described (8). Briefly, we divided study participants into 11 strata according to each of fat, protein, and carbohydrate intakes expressed as percentages of energy. We assigned the participants 0–10 points for increasing intake of total fat, 0–10 points for increasing intake of total protein, and, inversely, 10–0 points for increasing intake of carbohydrate. We summed points for the 3 macronutrients to create the overall LCD score, which ranged from 0 to 30. Similarly, we also created an animal LCD score on the basis of the percentage of energy of carbohydrate, animal protein, and animal fat and a vegetable LCD score on the basis of the percentage of energy of carbohydrate, vegetable protein, and vegetable fat. A higher score reflected higher intake of fat and protein and lower intake of carbohydrate, and it indicated closer adherence to a low-carbohydrate dietary pattern. LCD scores have been used in previous studies in association with risk of T2D (6, 7), cardiovascular disease (8), and mortality (22).

Covariate assessment

Participants reported their heights and weights in 1989 and updated their weights on each biennial questionnaire. The self-reported weight was highly correlated with the measured weight (r = 0.97) in a previous validation study (23). BMI (in kg/m^2) was computed as weight divided by the square of height. Total physical activity was ascertained by the frequency that participants engaged in common recreational activities from which metabolic equivalent task hours per week were derived. Questionnaire-based estimates correlated well with detailed activity diaries in a previous validation study (r = 0.56) (24).

Outcome ascertainment

Incident GDM was ascertained by a self-report on each biennial questionnaire through 2001. In the case of more than one pregnancy that lasted >6 mo and reported within a 2-y questionnaire period, GDM status was attributed to the first pregnancy. In a previous validation study in a subgroup of the NHS II cohort, 94% of GDM self-reports were confirmed by medical records (17). In a random sample of parous women without GDM, 83% of subjects reported a glucose screening test during pregnancy, and 100% of subjects reported frequent prenatal urine screenings, which suggested a high level of GDM surveillance in this cohort (17).

Statistical analysis

We divided women into quartiles according to their prepregnancy LCD scores. To represent the long-term habitual diet and reduce measurement error (25), we calculated a cumulative average LCD score on the basis of the information from 1991, 1995, and 1999 FFQs. Generalized estimating equations, which allowed us to account for correlations in repeated observations (pregnancies) contributed by a single participant (26), with log-binomials models (27) were used to estimate RRs and 95% CIs. In a few instances, models did not converge, and log-Poisson models (28), which provide consistent but not fully efficient risk estimates, were used.

Covariates in multivariable models included age (mo), parity (0, 1, 2, or ≥3), race-ethnicity (white, African American, Hispanic, Asian, and others), family history of diabetes (yes or no), cigarette smoking (never, past, or current), alcohol intake (0.0, 0.1–5.0, 5.1–10.0, or >10.0 g/d), physical activity (quartiles), total energy intake (quartiles), and BMI (9 categories as follows: <21.0, 21.0–22.9, 23.0–24.9, 25.0–26.9, 27.0–28.9, 29.0–30.9, 31.0–32.9, 33.0–34.9, and ≥35.0). We updated all these covariates, except race-ethnicity and family history of diabetes that were reported in 1989. We conducted tests of linear trend across quartiles of the LCD score by assigning the median value for each quartile and fitting this as a continuous variable in models.

To evaluate a potential effect modification, we performed stratified analyses according to age (<35 compared with ≥35), parity (nulliparous compared with parous), family history of diabetes (yes compared with no), physical activity (higher than median compared with lower than median), and overweight (BMI <25 compared with ≥25). We also conducted interaction tests via multiplicative interaction terms in multivariable models. To explore potential dietary contributors for the association, we additionally adjusted for each nutrient component of LCD scores (eg, animal fat, animal protein, vegetable fat, and vegetable protein), other nutrients (eg, saturated fat, dietary cholesterol, heme iron, dietary fiber, and glycemic load), and foods or food groups (eg, red meat, poultry, fish, eggs, dairy food, fruits, vegetables, whole grains, nuts, and legumes), as previously described (7). To minimize the potential effects of changes in diet during pregnancy, we also conducted a sensitivity analysis by excluding current pregnancies at the time of each FFQ. To further address the possibility of residual confounding, we additionally adjusted for a propensity score that reflected associations of LCD scores with the other variables, as previously mentioned, in the multivariate-adjusted model (29). All statistical analyses were performed with SAS software (version 9.2; SAS Institute Inc.). $P < 0.05$ was considered statistically significant.

RESULTS

We documented 867 incident GDM pregnancies in 21,411 singleton pregnancies in 15,265 women during 10 y of follow-up. At baseline, women with higher LCD scores were more likely to be current smokers, reported less physical activity, had higher BMI, and consumed more heme iron, red meat, poultry, and high-fat dairy but less total calories, dietary fiber, magnesium, vitamin C, vitamin E, low-fat dairy, fruit, vegetables, whole grains, and sugar-sweetened beverages (**Table 1**). We observed similar results for the animal LCD score. For the vegetable LCD score, participants with higher scores consumed more nuts, legumes, fruit, and whole grains but less calcium than did women with a lower score. Each of these 3 LCD scores was inversely associated with the dietary glycemic index and glycemic load.

Overall and animal LCD scores were positively associated with GDM risk, whereas the vegetable LCD score was not associated with the risk. Multivariable-adjusted RRs (95% CIs) of GDM for comparisons of highest with lowest quartiles were 1.53 (1.28, 1.82) for the overall LCD score (P-trend < 0.001), 1.63 (1.36, 1.96) for the animal LCD score (P-trend < 0.001), and 0.91 (0.74, 1.11) for the vegetable LCD score (P-trend = 0.39) (**Table 2**). The significant association of overall and animal LCD scores with GDM risk remained after additional adjustment for BMI, with corresponding RRs (95% CIs) of 1.27 (1.06, 1.51) (P-trend = 0.03) and 1.36 (1.13, 1.64) (P-trend = 0.003), respectively. When LCD scores were modeled as a continuous variable, we showed 6% higher (RR: 1.06; 95% CI: 1.02, 1.11) risk of GDM associated with each 5-unit increment of the overall LCD score and 8% higher (RR 1.08; 95% CI 1.03, 1.12) risk of GDM associated with each 5-unit increment of the animal LCD score. Associations between LCD scores and GD risk were not significantly differentiated by overweight status (*see* Supplementary Figures 1–3 under "Supplemental data" in the online issue.). In addition, associations were not significantly modified by other risk factors of GDM such as age, parity, family history of diabetes, or physical activity.

Associations between LCD scores and GDM risk were robust in multiple sensitivity analyses. First, similar results were observed in a propensity score analysis; adjusted RRs (95% CIs) of GDM for comparisons of highest with lowest quartiles were 1.24 (1.04, 1.49) for the overall LCD score, 1.33 (1.10, 1.60) for the animal LCD score, and 0.85 (0.69, 1.03) for the vegetable LCD score. Second, a sensitivity analysis in which missing exposure data were not carried forward also yielded similar results compared with those in our main analysis; adjusted RRs (95% CIs) of GDM risk for comparisons of highest with lowest quartiles were 1.33 (1.10, 1.61) for the overall LCD score, 1.48 (1.21, 1.80) for the animal LCD score, and 0.83 (0.68, 1.03) for the vegetable LCD score. Third, we observed similar results in a sensitivity analysis by excluding current pregnancies at the time when women completed the FFQ; adjusted RRs (95% CIs) of GDM for comparisons of highest with the lowest quartiles were 1.17 (0.87, 1.57) for the overall LCD score, 1.38 (1.02, 1.88) for the animal LCD score, and 0.81 (0.57, 1.15) for the vegetable LCD score. In addition, we conducted a sensitivity analysis by dividing LCD scores into more refined categories (ie, deciles). Adjusted RRs (95% CIs) of GDM risk for comparison of highest with lowest deciles were 1.46 (1.08, 1.95) for the overall LCD score, 1.67 (1.25, 2.24) for the animal LCD score, and 0.76 (0.55, 1.05) for the vegetable LCD score.

To examine which dietary variable was responsible for these associations between LCD scores and GDM risk, we conducted additional adjustments for several foods, food groups, or nutrients. The association of the animal LCD score with GDM risk for comparisons of highest with lowest quartiles was no longer significant after additional adjustment for quartiles of red meat (servings/d) (RR: 1.08; 95% CI: 0.88, 1.33), animal fat (percentage of energy) (RR: 1.03; 95% CI: 0.76, 1.40), or heme iron (mg/d) (RR: 1.06; 95% CI: 0.83, 1.36), which indicated that red meat, animal fat, and heme iron may be the main contributors to the observed association between the animal LCD score and GDM risk. We performed similar analyses for the vegetable LCD score by adjusting for dietary sources of vegetable protein and vegetable fat; however, these adjustments did not substantially alter the association.

DISCUSSION

In this prospective cohort study, we observed that a prepregnancy dietary score that represented a low-carbohydrate, high animal protein and animal fat dietary pattern was significantly and positively associated with GDM risk. Conversely, a prepregnancy dietary score that represented a dietary pattern low in

TABLE 1
Age-adjusted characteristics of the study population in 1991 according to quartile of prepregnancy LCD scores in 15,265 women[1]

	Overall LCD score				Animal LCD score				Vegetable LCD score			
Characteristic	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Participants (*n*)	4404	4205	3268	3388	3976	4315	3582	3391	5044	4004	3484	2732
Age in 1991 (y)	31.9 ± 3.3[2]	32.1 ± 3.3	32.0 ± 3.3	31.9 ± 3.2	32.0 ± 3.3	32.1 ± 3.3	31.9 ± 3.3	31.9 ± 3.1	31.7 ± 3.2	32.1 ± 3.3	32.1 ± 3.3	32.3 ± 3.4
White (%)	92	94	94	93	92	93	94	93	92	93	94	94
Family history of diabetes (%)	10	11	11	13	10	11	10	13	11	11	10	12
Nulliparous (%)	40	35	34	34	43	35	32	34	33	36	38	41
Current smoking (%)	7	8	10	12	7	9	10	11	8	8	10	11
Alcohol (g/d)	2.8 ± 4.8	3.1 ± 5.6	3.3 ± 5.5	3.0 ± 4.9	2.8 ± 4.5	3.1 ± 5.3	3.2 ± 5.7	3.1 ± 5.3	2.5 ± 4.9	3.1 ± 5.4	3.3 ± 5.2	3.6 ± 5.2
BMI (kg/m^2)	22.7 ± 3.8	23.2 ± 4.0	23.7 ± 4.3	24.5 ± 5.0	22.6 ± 3.8	23.2 ± 4.1	23.7 ± 4.2	24.5 ± 5.0	23.3 ± 4.2	23.3 ± 4.1	23.5 ± 4.3	23.8 ± 4.7
Physical activity (MET-h/wk)	27.2 ± 32.7	23.6 ± 29.4	21.8 ± 26.1	19.1 ± 23.9	28.1 ± 34.7	23.2 ± 27.7	21.1 ± 24.6	19.8 ± 25.8	24.2 ± 30.4	23.4 ± 28.1	23.2 ± 29.0	21.3 ± 26.0
Total calories (kcal/d)	1906 ± 567	1866 ± 538	1813 ± 543	1714 ± 534	1886 ± 575	1879 ± 540	1815 ± 540	1731 ± 531	1900 ± 551	1832 ± 544	1813 ± 552	1732 ± 540
Carbohydrate (% of energy)	58.9 ± 4.6	51.8 ± 2.3	47.3 ± 2.2	41.6 ± 3.8	58.8 ± 5.2	52.1 ± 3.4	47.8 ± 3.2	42.1 ± 4.2	54.6 ± 6.8	50.6 ± 6.8	48.5 ± 6.6	46.0 ± 5.4
Protein (% of energy)	16.6 ± 2.4	19.1 ± 2.7	20.1 ± 2.9	21.9 ± 2.8	16.3 ± 2.4	18.7 ± 2.4	20.2 ± 2.6	22.2 ± 2.7	18.9 ± 3.5	19.7 ± 3.3	19.3 ± 3.2	18.9 ± 3.0
Animal protein (% of energy)	11.3 ± 2.6	14.0 ± 2.7	15.2 ± 3.0	17.4 ± 2.9	10.7 ± 2.4	13.7 ± 2.2	15.4 ± 2.4	18.0 ± 2.8	14.6 ± 3.5	14.7 ± 3.6	14.1 ± 3.6	13.3 ± 3.3
Vegetable protein (% of energy)	5.3 ± 1.4	5.1 ± 1.0	4.8 ± 0.8	4.4 ± 0.8	5.6 ± 1.4	5.0 ± 0.9	4.7 ± 0.8	4.3 ± 0.7	4.4 ± 0.9	5.0 ± 1.1	5.2 ± 1.1	5.6 ± 1.0
Total fat (% of energy)	26.0 ± 3.8	30.0 ± 3.7	33.0 ± 3.9	36.6 ± 3.8	26.5 ± 4.6	30.0 ± 4.3	32.5 ± 4.3	35.6 ± 4.3	27.6 ± 4.6	30.4 ± 4.7	32.7 ± 4.6	35.6 ± 4.4
Animal fat (% of energy)	13.4 ± 3.2	16.6 ± 2.8	18.7 ± 3.0	22.0 ± 3.7	12.5 ± 2.7	16.3 ± 2.3	18.8 ± 2.4	22.6 ± 3.3	17.1 ± 4.4	17.6 ± 4.7	17.5 ± 4.7	17.0 ± 4.0
Vegetable fat (% of energy)	12.6 ± 3.4	13.4 ± 3.7	14.3 ± 4.2	14.5 ± 3.9	14.0 ± 4.0	13.8 ± 4.0	13.6 ± 3.8	13.0 ± 3.4	10.4 ± 2.3	12.8 ± 2.2	15.2 ± 2.3	18.7 ± 3.4
Saturated fat (% of energy)	9.2 ± 1.8	10.8 ± 1.7	12.0 ± 1.9	13.4 ± 2.0	9.1 ± 1.8	10.8 ± 1.7	11.9 ± 1.9	13.3 ± 2.0	10.5 ± 2.3	11.1 ± 2.4	11.6 ± 2.3	12.1 ± 2.2
Monounsaturated fat (% of energy)	9.7 ± 1.7	11.2 ± 1.7	12.5 ± 1.8	13.9 ± 1.8	10.0 ± 2.0	11.3 ± 2.0	12.2 ± 2.0	13.4 ± 1.9	10.2 ± 1.9	11.4 ± 2.0	12.4 ± 2.0	13.7 ± 2.0
Polyunsaturated fat (% of energy)	4.8 ± 1.1	5.3 ± 1.1	5.8 ± 1.3	6.1 ± 1.3	5.1 ± 1.3	5.4 ± 1.3	5.6 ± 1.3	5.7 ± 1.2	4.5 ± 0.8	5.3 ± 0.8	5.9 ± 0.9	6.9 ± 1.4
trans Fat (% of energy)	1.3 ± 0.5	1.5 ± 0.5	1.7 ± 0.6	1.9 ± 0.6	1.4 ± 0.6	1.6 ± 0.6	1.7 ± 0.6	1.8 ± 0.6	1.3 ± 0.4	1.5 ± 0.5	1.7 ± 0.6	2.0 ± 0.7
Cholesterol (mg/d)[3]	187 ± 49	231 ± 47	255 ± 50	293 ± 61	179 ± 45	226 ± 42	256 ± 46	299 ± 58	232 ± 62	243 ± 67	242 ± 67	233 ± 60
Glycemic index[3]	55.1 ± 3.2	54.0 ± 3.1	53.5 ± 3.0	53.1 ± 3.3	55.1 ± 3.1	54.1 ± 3.1	53.6 ± 3.1	53.0 ± 3.3	54.2 ± 3.6	53.9 ± 3.1	53.8 ± 3.1	53.8 ± 2.9
Glycemic load[3]	146 ± 16	126 ± 10	114 ± 9	100 ± 11	146 ± 17	127 ± 12	116 ± 11	101 ± 12	133 ± 21	123 ± 19	118 ± 19	112 ± 15
Total fiber (g/d)[3]	19.9 ± 6.8	18.6 ± 5.1	17.3 ± 4.1	15.7 ± 3.7	20.6 ± 6.8	18.5 ± 5.0	17.2 ± 4.1	15.5 ± 3.7	16.9 ± 5.5	18.5 ± 5.8	18.7 ± 5.2	18.7 ± 4.6
Magnesium (mg/d)[3]	326 ± 84	326 ± 73	315 ± 66	303 ± 64	329 ± 85	321 ± 72	316 ± 67	305 ± 65	317 ± 78	324 ± 74	319 ± 70	313 ± 68
Heme iron (mg/d)[3]	0.8 ± 0.3	1.0 ± 0.3	1.2 ± 0.3	1.4 ± 0.4	0.7 ± 0.3	1.0 ± 0.3	1.2 ± 0.3	1.5 ± 0.4	1.0 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.4
Potassium (mg/d)[3]	2915 ± 579	2932 ± 505	2862 ± 460	2802 ± 435	2898 ± 583	2905 ± 507	2881 ± 474	2839 ± 436	2929 ± 549	2930 ± 499	2865 ± 471	2757 ± 462
Calcium (mg/d)[3]	1048 ± 419	1117 ± 432	1077 ± 418	1037 ± 414	1012 ± 409	1082 ± 417	1107 ± 431	1087 ± 428	1185 ± 471	1086 ± 402	1003 ± 361	928 ± 368
Vitamin C (mg/d)[3]	299 ± 302	253 ± 267	222 ± 248	198 ± 254	301 ± 312	248 ± 261	227 ± 253	204 ± 250	266 ± 267	255 ± 287	233 ± 259	220 ± 284
Vitamin E (mg/d)[3]	22.4 ± 48.1	20.2 ± 44.0	21.0 ± 47.6	17.4 ± 34.4	23.4 ± 50.1	20.2 ± 44.6	19.8 ± 43.4	17.8 ± 36.5	19.9 ± 43.2	20.6 ± 43.8	20.1 ± 42.0	21.6 ± 49.5
Red meat (servings/d)	0.5 ± 0.4	0.7 ± 0.5	0.8 ± 0.5	1.0 ± 0.6	0.5 ± 0.4	0.7 ± 0.5	0.8 ± 0.5	1.0 ± 0.6	0.7 ± 0.5	0.8 ± 0.6	0.8 ± 0.6	0.7 ± 0.5
Poultry (servings/d)	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.3 ± 0.2	0.5 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.3
Fish (servings/d)	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Eggs (servings/d)	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2
Low-fat dairy (servings/d)	1.6 ± 1.3	1.7 ± 1.3	1.5 ± 1.2	1.3 ± 1.2	1.4 ± 1.1	1.7 ± 1.3	1.6 ± 1.3	1.5 ± 1.3	2.0 ± 1.4	1.6 ± 1.2	1.3 ± 1.0	0.9 ± 0.8
High-fat dairy (servings/d)	0.8 ± 0.7	1.0 ± 0.9	1.1 ± 1.0	1.2 ± 1.1	0.8 ± 0.6	1.0 ± 0.9	1.1 ± 1.0	1.2 ± 1.1	1.0 ± 1.0	1.0 ± 0.9	1.0 ± 0.9	1.0 ± 0.9

(Continued)

TABLE 1 (*Continued*)

Characteristic	Overall LCD score				Animal LCD score				Vegetable LCD score			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Nuts (servings/d)	0.2 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.2 ± 0.4	0.3 ± 0.4	0.3 ± 0.4	0.2 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.3	0.4 ± 0.6
Legumes (servings/d)	0.4 ± 0.4	0.4 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.4 ± 0.4	0.4 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.3
Vegetables (servings/d)	3.4 ± 2.2	3.3 ± 2.0	3.1 ± 1.8	2.9 ± 1.7	3.5 ± 2.3	3.3 ± 2.0	3.1 ± 1.8	2.9 ± 1.6	2.9 ± 1.8	3.3 ± 2.1	3.4 ± 2.1	3.3 ± 2.0
Fruit (servings/d)	1.5 ± 1.2	1.3 ± 0.9	1.1 ± 0.7	0.8 ± 0.6	1.5 ± 1.2	1.3 ± 0.9	1.1 ± 0.8	0.9 ± 0.7	1.4 ± 1.1	1.3 ± 0.9	1.2 ± 0.9	0.9 ± 0.7
Whole grains (servings/d)	1.3 ± 1.2	1.2 ± 1.1	1.0 ± 0.9	0.9 ± 0.9	1.4 ± 1.2	1.2 ± 1.0	1.0 ± 0.9	0.8 ± 0.8	0.9 ± 0.9	1.1 ± 1.0	1.2 ± 1.1	1.3 ± 1.2
SSBs (servings/d)	1.0 ± 1.3	0.5 ± 0.7	0.3 ± 0.5	0.2 ± 0.3	0.9 ± 1.2	0.5 ± 0.8	0.4 ± 0.6	0.2 ± 0.4	1.0 ± 1.2	0.4 ± 0.6	0.3 ± 0.4	0.2 ± 0.3

[1] Values were standardized to the age distribution of the study population. All comparisons were significant in trend tests across categories except for the following: nuts for the overall LCD score and vitamin E and high-fat dairy for the vegetable LCD score. LCD, low-carbohydrate diet; MET-h, metabolic equivalent task hours; Q, quartile; SSB, sugar-sweetened beverage.

[2] Mean ± SD (all such values).

[3] Values were energy adjusted.

carbohydrate and high in vegetable protein and vegetable fat was not significantly associated with GDM risk. To our knowledge, the current study is the first attempt to examine the association between a low-carbohydrate dietary pattern and risk of GDM incidence in a large prospective cohort. Although we are unaware of previous studies that specifically evaluated a prepregnancy low-carbohydrate dietary pattern and risk of GDM, our results were largely consistent with previous findings of a low-carbohydrate dietary pattern in association with T2D risk in the Health Professionals Follow-Up Study (7).

To interpret associations between a low-carbohydrate dietary pattern and risk of GDM, each of the macronutrients and their major food sources should be considered, because an individual with a low-carbohydrate dietary pattern tends to have a relatively higher intake of fat and protein to compensate energy requirements. Observed divergent associations of animal compared with vegetable LCD scores with GDM risk indicated that associations may not have been the result of a lower quantity of carbohydrate intake. A previous study (30) has shown a null association of total carbohydrate intake but significant association of the quality of carbohydrate with GDM risk. The positive association of GDM risk with the LCD score, in particular the animal LCD score, could have been attributable to detrimental effects of animal fat and animal protein. The relation between dietary fat, especially animal fat, and impaired glucose metabolism has been well documented (31). For dietary protein, an animal protein–rich meal compared with a vegetable protein-rich meal resulted in higher plasma concentrations of branched-chain amino acids (32), which have been positively linked to the development of insulin resistance and incident diabetes in recent metabolomics studies (33–35). Higher intakes of animal fat (15) and animal protein (16) were previously associated with increased risk of GDM, whereas higher intake of vegetable protein was associated with lower risk (16). Red meat, which is a major dietary source of animal protein and animal fat that was associated with GDM risk (16, 36), was shown in the current study to be responsible for the association between the animal LCD score and GDM risk. Besides animal fat, we also showed that heme iron was a contributor to the association, which was consistent with previous findings (37, 38). Other aspects of red meat, such as advanced glycation end products formed during grilling red meat (39) and nitrite and nitrate preservatives in processed red meat (40), may also contribute to the association. However, we were unable to assess their roles in our current analysis because of the lack of such data.

Our study has several strengths, including the prospective design that established the temporal direction of associations, large sample size, long-term follow-up, high response rates (>90%) of each questionnaire cycle, and detailed prospective dietary assessments with extensively validated FFQs (19–21). We acknowledge that there were several limitations. First, the misclassification of dietary intakes of carbohydrate, fat, and protein was possible. However, the random ,within-person error would have been nondifferential because the prepregnancy dietary information was captured prospectively; therefore, our observed associations may have underestimated the true RRs. Furthermore, the use of cumulative averages of dietary intakes for participants with more than one prepregnancy FFQ reduced the random error. Second, our study population consisted mostly of white American women in whom we showed a high correlation

TABLE 2
Risk of GDM according to quartile of prepregnancy LCD scores[1]

	LCD scores				
	Q1[2]	Q2	Q3	Q4	*P*-trend
Overall LCD score					—
Median score	6	12	18	24	—
GDM/pregnancies	227/6273	223/5973	164/4574	253/4591	—
Model 1	1.00	1.05 (0.88, 1.26)[3]	1.03 (0.85, 1.26)	1.60 (1.34, 1.90)	<0.001
Model 2	1.00	1.06 (0.89, 1.27)	1.04 (0.85, 1.27)	1.53 (1.28, 1.82)	<0.001
Model 3	1.00	1.00 (0.84, 1.20)	0.92 (0.76, 1.13)	1.27 (1.06, 1.51)	0.03
Animal LCD score					
Median score	5	12	18	25	—
GDM/pregnancies	196/5659	230/6050	186/5060	255/4642	—
Model 1	1.00	1.14 (0.95, 1.38)	1.14 (0.93, 1.38)	1.70 (1.42, 2.04)	<0.001
Model 2	1.00	1.15 (0.96, 1.39)	1.13 (0.93, 1.38)	1.63 (1.36, 1.96)	<0.001
Model 3	1.00	1.09 (0.90, 1.31)	1.01 (0.82, 1.23)	1.36 (1.13, 1.64)	0.003
Vegetable LCD score					
Median score	9	13	17	22	—
GDM/pregnancies	290/7175	234/5692	201/4859	142/3685	—
Model 1	1.00	0.99 (0.83, 1.17)	0.97 (0.81, 1.15)	0.88 (0.72, 1.07)	0.22
Model 2	1.00	1.03 (0.87, 1.22)	1.02 (0.85, 1.21)	0.91 (0.74, 1.11)	0.39
Model 3	1.00	1.02 (0.86, 1.21)	0.95 (0.80, 1.14)	0.84 (0.69, 1.03)	0.08

[1] Model 1 was adjusted for updated age (mo) and parity (0, 1, 2, or ≥3). Model 2 was adjusted as for model 1 and for race-ethnicity (white, African American, Hispanic, Asian, or other), family history of diabetes (yes or no), cigarette smoking (never, past, or current), alcohol intake (0.0, 0.1–5.0, 5.1–10.0, or >10.0 g/d), physical activity (Qs), and total energy intake (Qs). Model 3 was adjusted as for model 2 and for BMI (9 categories as follows: <21.0, 21.0–22.9, 23.0–24.9, 25.0–26.9, 27.0–28.9, 29.0–30.9, 31.0–32.9, 33.0–34.9, and ≥35.0 kg/m^2). RRs (95% CIs) were estimated using generalized estimating equations with log-binomial models. Trend tests across Qs of LCD scores were performed by assigning the median value for each Q and fitting this as a continuous variable in the models. GDM, gestational diabetes mellitus; LCD, low-carbohydrate diet; Q, quartile.

[2] Reference.

[3] RR; 95% CI in parentheses (all such values).

between the overall LCD score and animal LCD score ($R = 0.94$, $P < 0.001$), which indicated that most of the women who had a low-carbohydrate dietary pattern consumed animal rather than plant foods as their major sources of protein and fat. Thus, the direct generalization of our findings to other populations whose major food sources of macronutrients are different (41) may be limited. Indeed, inconsistent associations of long-term effects of LCDs on adverse health outcomes, such as cardiovascular disease (8, 9) and mortality (42), have been reported in European and US populations. The association between LCD scores and risk of GDM across different race-ethnic groups warrants additional evaluations. Third, the entire population in this study was aged ≥25 y. Because advanced maternal age is a known risk factor for GDM (43), future studies are needed to examine associations between LCD scores and GDM risk in women <25 y of age.

In conclusion, our findings indicate that a prepregnancy dietary pattern relatively low in carbohydrate and high in protein and fat from animal-food sources is positively associated with GDM risk, whereas a prepregnancy dietary pattern relatively low in carbohydrate and high in protein and fat from vegetable-food sources was not associated with the risk. Women of reproductive age who follow a low-carbohydrate dietary pattern may consider consuming vegetable rather than animal sources of protein and fat (in particular red meat) to minimize their risk of GDM. Because of the observational study design, our study cannot confirm the causation between adherence to a low-carbohydrate dietary pattern and risk of GDM. Future studies with a randomized controlled trial design are warranted.

We thank Thomas L Halton (Departments of Nutrition, Harvard School of Public Health) for devising LCD scores.

The authors' responsibilities were as follows—WB: contributed to the design and analysis of the study and wrote the manuscript; KB: conducted a technique review and reviewed and edited the manuscript; DKT: contributed to the data analysis and reviewed and edited the manuscript; SFO, JC, AV, and MK: interpreted results and reviewed and edited the manuscript; CZ: contributed to the design and analysis of the study and reviewed and edited the manuscript; and WB and CZ: are the guarantors of this work and, as such, had full access to all study data and took responsibility for the integrity of data and the accuracy of the data analysis. None of the authors had a personal or financial conflict of interest.

REFERENCES

1. Kennedy RL, Chokkalingam K, Farshchi HR. Nutrition in patients with type 2 diabetes: are low-carbohydrate diets effective, safe or desirable? Diabet Med 2005;22:821–32.
2. Malik VS, Hu FB. Popular weight-loss diets: from evidence to practice. Nat Clin Pract Cardiovasc Med 2007;4:34–41.
3. Bravata DM, Sanders L, Huang J, Krumholz HM, Olkin I, Gardner CD. Efficacy and safety of low-carbohydrate diets: a systematic review. JAMA 2003;289:1837–50.
4. Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, Szapary PO, Rader DJ, Edman JS, Klein S. A randomized trial of a low-carbohydrate diet for obesity. N Engl J Med 2003;348:2082–90.
5. Davis NJ, Tomuta N, Schechter C, Isasi CR, Segal-Isaacson CJ, Stein D, Zonszein J, Wylie-Rosett J. Comparative study of the effects of a 1-year dietary intervention of a low-carbohydrate diet versus a low-fat diet on weight and glycemic control in type 2 diabetes. Diabetes Care 2009;32:1147–52.
6. Halton TL, Liu S, Manson JE, Hu FB. Low-carbohydrate-diet score and risk of type 2 diabetes in women. Am J Clin Nutr 2008;87:339–46.

7. de Koning L, Fung TT, Liao X, Chiuve SE, Rimm EB, Willett WC, Spiegelman D, Hu FB. Low-carbohydrate diet scores and risk of type 2 diabetes in men. Am J Clin Nutr 2011;93:844–50.
8. Halton TL, Willett WC, Liu S, Manson JE, Albert CM, Rexrode K, Hu FB. Low-carbohydrate-diet score and the risk of coronary heart disease in women. N Engl J Med 2006;355:1991–2002.
9. Lagiou P, Sandin S, Lof M, Trichopoulos D, Adami HO, Weiderpass E. Low carbohydrate-high protein diet and incidence of cardiovascular diseases in Swedish women: prospective cohort study. BMJ 2012;344: e4026.
10. Reece EA, Leguizamon G, Wiznitzer A. Gestational diabetes: the need for a common ground. Lancet 2009;373:1789–97.
11. American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 2004;27(suppl 1):S88–90.
12. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, et al. Hyperglycemia and adverse pregnancy outcomes. N Engl J Med 2008;358: 1991–2002.
13. Bellamy L, Casas JP, Hingorani AD, Williams D. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. Lancet 2009;373:1773–9.
14. Centers for Disease Control and Prevention. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, 2011.
15. Bowers K, Tobias DK, Yeung E, Hu FB, Zhang C. A prospective study of prepregnancy dietary fat intake and risk of gestational diabetes. Am J Clin Nutr 2012;95:446–53.
16. Bao W, Bowers K, Tobias DK, Hu FB, Zhang C. Prepregnancy dietary protein intake, major dietary protein sources, and the risk of gestational diabetes mellitus: a prospective cohort study. Diabetes Care 2013;36: 2001–8.
17. Solomon CG, Willett WC, Carey VJ, Rich-Edwards J, Hunter DJ, Colditz GA, Stampfer MJ, Speizer FE, Spiegelman D, Manson JE. A prospective study of pregravid determinants of gestational diabetes mellitus. JAMA 1997;278:1078–83.
18. Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr 1999;70:221–7.
19. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
20. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol 1988;127:188–99.
21. Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B, Willett WC. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. Int J Epidemiol 1989;18:858–67.
22. Fung TT, van Dam RM, Hankinson SE, Stampfer M, Willett WC, Hu FB. Low-carbohydrate diets and all-cause and cause-specific mortality: two cohort studies. Ann Intern Med 2010;153:289–98.
23. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. Epidemiology 1990;1:466–73.
24. Wolf AM, Hunter DJ, Colditz GA, Manson JE, Stampfer MJ, Corsano KA, Rosner B, Kriska A, Willett WC. Reproducibility and validity of a self-administered physical activity questionnaire. Int J Epidemiol 1994;23: 991–9.
25. Hu FB, Stampfer MJ, Rimm E, Ascherio A, Rosner BA, Spiegelman D, Willett WC. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. Am J Epidemiol 1999;149:531–40.
26. Hanley JA, Negassa A, Edwardes MD, Forrester JE. Statistical analysis of correlated data using generalized estimating equations: an orientation. Am J Epidemiol 2003;157:364–75.
27. Wacholder S. Binomial regression in GLIM: estimating risk ratios and risk differences. Am J Epidemiol 1986;123:174–84.
28. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol 2004;159:702–6.
29. D'Agostino RB Jr. Propensity score methods for bias reduction in the comparison of a treatment to a non-randomized control group. Stat Med 1998;17:2265–81.
30. Zhang C, Liu S, Solomon CG, Hu FB. Dietary fiber intake, dietary glycemic load, and the risk for gestational diabetes mellitus. Diabetes Care 2006;29:2223–30.
31. Lichtenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. Atherosclerosis 2000;150:227–43.
32. Brandsch C, Shukla A, Hirche F, Stangl GI, Eder K. Effect of proteins from beef, pork, and turkey meat on plasma and liver lipids of rats compared with casein and soy protein. Nutrition 2006;22:1162–70.
33. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab 2009;9: 311–26.
34. Tai ES, Tan ML, Stevens RD, Low YL, Muehlbauer MJ, Goh DL, Ilkayeva OR, Wenner BR, Bain JR, Lee JJ, et al. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. Diabetologia 2010;53:757–67.
35. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, Lewis GD, Fox CS, Jacques PF, Fernandez C, et al. Metabolite profiles and the risk of developing diabetes. Nat Med 2011;17:448–53.
36. Zhang C, Schulze MB, Solomon CG, Hu FB. A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus. Diabetologia 2006;49:2604–13.
37. Bowers K, Yeung E, Williams MA, Qi L, Tobias DK, Hu FB, Zhang C. A prospective study of prepregnancy dietary iron intake and risk for gestational diabetes mellitus. Diabetes Care 2011;34:1557–63.
38. Qiu C, Zhang C, Gelaye B, Enquobahrie DA, Frederick IO, Williams MA. Gestational diabetes mellitus in relation to maternal dietary heme iron and nonheme iron intake. Diabetes Care 2011;34:1564–9.
39. Cai W, Ramdas M, Zhu L, Chen X, Striker GE, Vlassara H. Oral advanced glycation endproducts (AGEs) promote insulin resistance and diabetes by depleting the antioxidant defenses AGE receptor-1 and sirtuin 1. Proc Natl Acad Sci USA 2012;109:15888–93.
40. Virtanen SM, Jaakkola L, Rasanen L, Ylonen K, Aro A, Lounamaa R, Akerblom HK, Tuomilehto J. Nitrate and nitrite intake and the risk for type 1 diabetes in Finnish children. Childhood Diabetes in Finland Study Group. Diabet Med 1994;11:656–62.
41. Willett WC. Low-carbohydrate diets: a place in health promotion? J Intern Med 2007;261:363–5.
42. Noto H, Goto A, Tsujimoto T, Noda M. Low-carbohydrate diets and all-cause mortality: a systematic review and meta-analysis of observational studies. PLoS ONE 2013;8:e55030.
43. Makgoba M, Savvidou MD, Steer PJ. An analysis of the interrelationship between maternal age, body mass index and racial origin in the development of gestational diabetes mellitus. BJOG 2012; 119:276–82.