Low-carbohydrate diet scores and risk of type 2 diabetes in men[1–3]

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ABSTRACT

Background: Fat and protein sources may influence whether low-carbohydrate diets are associated with type 2 diabetes (T2D).

Objective: The objective was to compare the associations of 3 low-carbohydrate diet scores with incident T2D.

Design: A prospective cohort study was conducted in participants from the Health Professionals Follow-Up Study who were free of T2D, cardiovascular disease, or cancer at baseline (n = 40,475) for up to 20 y. Cumulative averages of 3 low-carbohydrate diet scores (high total protein and fat, high animal protein and fat, and high vegetable protein and fat) were calculated every 4 y from food-frequency questionnaires and were associated with incident T2D by using Cox models.

Results: We documented 2689 cases of T2D during follow-up. After adjustments for age, smoking, physical activity, coffee intake, alcohol intake, family history of T2D, total energy intake, and body mass index, the score for high animal protein and fat was associated with an increased risk of T2D [top compared with bottom quintile; hazard ratio (HR): 1.37; 95% CI: 1.20, 1.58; P for trend < 0.01]. Adjustment for red and processed meat attenuated this association (HR: 1.11; 95% CI: 0.95, 1.30; P for trend = 0.20). A high score for vegetable protein and fat was not significantly associated with the risk of T2D overall but was inversely associated with T2D in men aged <65 y (HR: 0.78; 95% CI: 0.66, 0.92; P for trend = 0.01, P for interaction = 0.01).

Conclusions: A score representing a low-carbohydrate diet high in animal protein and fat was positively associated with the risk of T2D in men. Low-carbohydrate diets should obtain protein and fat from foods other than red and processed meat. *Am J Clin Nutr* 2011;93:844–50.

INTRODUCTION

Low-carbohydrate diets have been popularized for weight loss by several books, some of which advocate a high protein intake to induce mild ketoacidosis and catabolism of body fat (1). Current literature indicates that low-carbohydrate ad libitum dietary patterns are more effective for short-term weight loss (ie, 6 mo), but little is known about their long-term influence on chronic disease risk (2).

Most carbohydrate-restricted diets tend to encourage consumption of animal products, which may increase the risk of chronic conditions such as type 2 diabetes (T2D). In the Nurses' Health Study, we found that diets lower in carbohydrates and higher in protein and fat were not associated with long-term risk of diabetes in women (3). However, when vegetable sources of fat and protein were chosen, these diets were associated with a moderately lower risk of T2D (3).

The objective of this study was to examine the associations of several low-carbohydrate diet scores with risk of T2D in large well-characterized cohort of men and to identify the roles of foods and nutrients that are correlated with the scores.

SUBJECTS AND METHODS

Subjects

The Health Professionals Follow-Up Study is a prospective cohort study of 51,529 middle-aged (age 40–75 y at baseline) male health professionals. Participants were recruited in 1986 and were mailed questionnaires every second year to assess health status and lifestyle. Follow-up response rates range from 90% to 94% on each biennial questionnaire. The Health Professionals Follow-Up Study was approved by the Harvard Institutional Review board (Dietary Etiologies of Heart Disease and Cancer Protocol no. 10446). The procedures followed were in accordance with the ethical standards of Harvard University and were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Dietary assessment

Diet was assessed with a 131-item semiquantitative food-frequency questionnaire (FFQ) every 4 y. Participants were asked to select their usual intake (never to >6 times/d) of a standard portion of each item. Daily nutrient and energy intakes were calculated by multiplying the frequency of intake by the nutrient

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and energy content and summing across all items. The FFQ was validated in a sample of 127 participants against two 7-d diet records (administered 6 mo apart) for macronutrients (Pearson correlations between measures: total fat, ≈0.6; carbohydrate, ≈0.7; protein, ≈0.4) (4). Reliability was assessed by repeat administration after 1 y, and most correlations were ≈0.5 (4).

The 3 low-carbohydrate diet scores were developed previously (5). Briefly, the percentages of energy from carbohydrate, protein, and fat were divided into 11 categories with equal sample sizes. The carbohydrate categories were scored from 10 (lowest intake) to 0 (highest intake), whereas protein and fat categories were scored from 0 (lowest intake) to 10 (highest intake). Ranks were added to create a total score with a maximum value of 30, which represented the highest intake of total protein and total fat and the lowest intake of carbohydrate. Separate scores were created for animal protein and fat and vegetable protein and fat.

Ascertainment of T2D endpoints

A supplementary questionnaire was mailed to participants reporting a T2D diagnosis. The diagnostic criteria for T2D cases occurring before 1998 were from the National Diabetes Data Group (6), and American Diabetes Association criteria were used for cases occurring after 1998 (7). Cases of type 1 diabetes were excluded. In a validation study, a medical record review confirmed 97% (57 of 59) of self-reported T2D cases (8).

Statistical analysis

Participants with implausible energy intakes (<800 or >4200 kcal/d), history of T2D, cardiovascular disease (heart attack, stroke, angina, and coronary artery bypass graft), or cancer at baseline (1986) were excluded. This left 40,475 participants for analysis.

Age-standardized mean characteristics of the participant were calculated by using general linear models, and trends across quintiles of diet scores were evaluated by using a Wald test of the median value in each quintile. Scatter plots of the 3 scores were prepared, and Pearson correlations were calculated if distributions were roughly linear.

Person-time was measured from the return of the 1986 questionnaire until 31 January 2006, death, loss to follow-up, development of T2D, or whichever occurred first. Hazard ratios representing the associations between quintiles of diet scores and risk of T2D were modeled by using age-stratified (in mo) Cox proportional hazard models with time-varying covariates. Cumulative averages of the low-carbohydrate diet scores and dietary covariates (macronutrients and alcohol) were calculated at each time point but were not updated if participants reported a chronic disease diagnosis (eg, cancer and cardiovascular disease) to control recall bias. Other covariates were updated at each time point and included smoking [never, previous, current (1–14 or 14 cigarettes/d), or missing], physical activity [quintiles; metabolic equivalents (METS, in h/wk), missing], coffee intake (quintiles; cups/d), alcohol intake (abstainers, 0–10 g/d, 10–20 g/d, or >20 g/d), family history of T2D in 1986, total energy intake (quintiles), and body mass index (BMI; in kg/m^2; <23, 23–23.9, 24–24.9, 25–26.9, 27–28.9, 29–30.9, 31–32.9, 33–34.9, ≥35, or missing). Values for smoking, physical activity, and BMI were carried forward from previous years if missing and were coded as "missing" if absent at baseline. Linear trends were evaluated by the Wald test of the median diet score in each quintile, and 5-knot splines were used to assess nonlinearity.

To quantify the effect of foods (red and processed meat, chicken, fish, dairy, eggs, whole grains, refined grains, fruit, vegetables, nuts, and legumes) and nutrient components of the scores (eg, animal protein and animal fat), we adjusted for them. We also examined the role of other nutrients (glycemic load, heme iron, and saturated fat) by using an identical approach. If a low-carbohydrate diet score was positively associated with a harmful food or nutrient as well as T2D, then controlling for it would attenuate its association with T2D. Conversely, if a low-carbohydrate diet was positively associated with a protective food or nutrient as well as T2D, adjustment for it would strengthen the association. These findings would be reversed if foods or nutrients were inversely correlated with the scores. Foods or nutrients that partially explained the relation between a score and T2D were mutually adjusted in a separate model to determine which, if any, were independently related to risk.

Analyses were repeated by using baseline dietary data to assess the predictive power of a single measure of dietary intake. The effect of abdominal obesity was assessed by adjusting for waist-to-hip ratio (WHR), and continuous covariates were adjusted to assess residual confounding. Stratified analyses and interaction tests were performed according to age (<65 compared with ≥65 y), which is equivalent to a test for proportional hazards, alcohol intake (drinker compared with abstainer), family history, physical activity (low [quintile 1–quintile 2], medium [quintiles 3–quintile 4], or high [quintile 5]), and BMI (in kg/m^2; <25, 25–30, or ≥30). Cross-product terms (eg, median low-carbohydrate score × median BMI) were used for interaction testing.

Finally, we examined the effect of measurement error in the low-carbohydrate scores by using a novel risk set regression calibration method, which corrects for within-person variation assessed in a validation study (4) in a time-dependent manner across the entire follow-up (9). This method adjusts both the point and interval estimates of relative risk of measurement error in the cumulative average exposure variable. We could only perform this analysis on the high total protein and fat score because animal protein, animal fat, vegetable protein, and vegetable fat were not measured in the validation study.

SAS version 9.1 (SAS Institute, Cary, NC) was used for all analyses, and a P value <0.05 was considered statistically significant. A FORTRAN program was used for measurement error correction (9).

RESULTS

Baseline characteristics

Each low-carbohydrate score had a mean of 15 points; however, the high animal protein and fat score had the most variation (SD = 7.8), followed by the high total (SD = 7.1) and high vegetable protein and fat scores (SD = 5.3). The high total and high animal protein and fat scores were highly correlated. ($R_{\text{total, animal}}$ = 0.92, $R_{\text{total, vegetable}}$ = 0.39, $R_{\text{animal, vegetable}}$ = 0.07).

An increase in the high total or high animal protein and fat score was significantly associated with higher intakes of red and processed meat, chicken, fish, dairy, and eggs, but lower intakes of whole grains, refined grains, fruit, vegetables, and legumes (**Table 1**). The high total protein and fat score was associated

with a higher intake of nuts, whereas the high animal protein and fat score was associated with a lower intake of nuts. The high vegetable protein and fat score was associated with a lower intake of all animal-based foods and fruit and higher intakes of whole grains, refined grains, vegetables, nuts, and legumes.

The high total and high animal protein and fat scores were significantly associated with several adverse health behaviors, including a higher intake of *trans* fat and prevalence of current smoking and a lower intake of cereal fiber and physical activity. They were also associated with a higher BMI and greater family history, but a lower glycemic load and energy intake. (Table 1) The high animal protein and fat score was significantly associated with greater alcohol intake. Like the high total and high animal protein and fat scores, a higher vegetable protein and fat score was significantly associated with a higher *trans* fat intake, a lower glycemic load, and a lower physical activity; however, the high vegetable protein and fat score was positively associated with cereal fiber and total energy and was inversely associated with current smoking. This score was not significantly associated with alcohol intake, BMI, or family history of T2D.

Regression analysis

During 20 y of follow-up (371,138 observations and 712,103 person-years), 2689 cases of T2D were confirmed. An increase in the low-carbohydrate, high total and animal protein and fat scores was significantly associated with greater risk of T2D after adjustment for age in a comparison of the top with the bottom quintile: total protein and fat score (HR: 2.12; 95% CI: 1.87, 2.41; P for trend < 0.01) and animal protein and fat score (HR: 2.29; 95% CI: 2.01, 2.62; P for trend < 0.01) (**Table 2**). Associations were strongly attenuated after adjustment for other variables, including total energy and especially BMI. The high vegetable protein and fat score was not significantly associated with T2D in a comparison of the top with the bottom quintile: total protein and fat score (HR: 1.31; 95% CI: 1.14, 1.49; P for trend < 0.01), animal protein and fat score (HR: 1.37; 95% CI: 1.20, 1.58; P for trend < 0.01), and vegetable protein and fat score (HR: 0.95; 95% CI: 0.84, 1.07; P for trend = 0.64). No evidence of nonlinearity was found in the spline analysis (P values for curvature >0.32; graphs not shown).

TABLE 1
Age-adjusted characteristics of participants at baseline (1986) by quintile (Q) of low-carbohydrate diet scores[1]

	Low-carbohydrate, high total protein and fat score		Low-carbohydrate, high animal protein and fat score		Low-carbohydrate, high vegetable protein and fat score	
	Q1 (0–8)	Q5 (22–30)	Q1 (0–7)	Q5 (23–30)	Q1 (0–10)	Q5 (20–30)
n	8059	8139	7804	7952	8330	8252
Median score	5	25	4	26	9	22
Red and processed meat (servings/d)	0.6 ± 0.5[2]	1.4 ± 0.9	0.5 ± 0.4	1.5 ± 0.9	1.0 ± 0.8	1.0 ± 0.7
Red meat (servings/d)	0.4 ± 0.3	0.9 ± 0.6	0.3 ± 0.3	1.0 ± 0.6	0.6 ± 0.5	0.6 ± 0.4
Processed meat (servings/d)	0.2 ± 0.3	0.5 ± 0.5	0.2 ± 0.3	0.5 ± 0.6	0.4 ± 0.5	0.4 ± 0.4
Chicken (servings/d)	0.3 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	0.4 ± 0.4	0.4 ± 0.3	0.3 ± 0.2
Fish (servings/d)	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3	0.3 ± 0.3
Dairy products (servings/d)	1.7 ± 1.2	2.0 ± 1.5	1.6 ± 1.1	2.1 ± 1.6	2.4 ± 1.6	1.6 ± 1.2
Eggs (servings/d)	0.2 ± 0.3	0.5 ± 0.6	0.2 ± 0.3	0.5 ± 0.6	0.3 ± 0.4	0.3 ± 0.4
Whole grains (servings/d)	1.8 ± 1.7	1.0 ± 1.1	2.0 ± 1.8	0.9 ± 1.0	1.1 ± 1.2	1.6 ± 1.5
Refined grains (servings/d)	1.6 ± 1.4	1.3 ± 1.1	1.6 ± 1.4	1.3 ± 1.1	1.4 ± 1.2	1.5 ± 1.3
Fruit (servings/d)	3.4 ± 2.1	1.6 ± 1.0	3.3 ± 2.1	1.6 ± 1.1	2.8 ± 2.0	1.9 ± 1.3
Vegetables (servings/d)	3.3 ± 1.9	2.8 ± 1.6	3.4 ± 2.0	2.8 ± 1.5	2.7 ± 1.6	3.2 ± 1.7
Nuts (servings/d)	0.4 ± 0.5	0.6 ± 0.9	0.5 ± 0.7	0.3 ± 0.5	0.2 ± 0.2	1.1 ± 1.1
Legumes (servings/d)	0.3 ± 0.3	0.2 ± 0.2	0.3 ± 0.4	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.3
Carbohydrate (% of energy)	57.7 ± 6.0	37.3 ± 4.9	57.4 ± 6.2	37.4 ± 5.2	51.2 ± 8.9	43.0 ± 6.4
Protein (% of energy)	15.7 ± 2.3	21.5 ± 2.9	15.7 ± 2.4	21.6 ± 3.0	18.2 ± 3.7	18.4 ± 3.0[3]
Total fat (% of energy)	25.6 ± 4.6	38.6 ± 4.3	26.5 ± 5.5	37.5 ± 4.8	28.3 ± 6.1	36.4 ± 5.1
Animal protein (% of energy)	10.2 ± 2.4	17.1 ± 3.2	9.8 ± 2.2	17.6 ± 3.0	14.0 ± 3.8	12.7 ± 3.3
Animal fat (% of energy)	13.1 ± 3.9	24.6 ± 5.1	12.1 ± 3.2	25.6 ± 4.3	19.1 ± 6.0	17.4 ± 4.9
Vegetable protein (% of energy)	5.5 ± 1.5	4.4 ± 1.1	5.9 ± 1.5	4.1 ± 0.8	4.2 ± 1.0	5.8 ± 1.2
Vegetable fat (% of energy)	12.5 ± 3.8	14.0 ± 5.0	14.4 ± 4.8	11.9 ± 3.7	9.2 ± 2.4	19.0 ± 4.3
Alcohol intake (g/d)	9.6 ± 14.3	9.6 ± 11.5[3]	8.2 ± 12.1	11.3 ± 13.8	10.6 ± 16.6	11.3 ± 13.6[3]
trans Fat intake (g/d)	2.4 ± 1.1	3.2 ± 1.0	2.5 ± 1.2	3.1 ± 1.0	2.4 ± 0.9	3.1 ± 1.2
Glycemic load	157 ± 54	93.6 ± 34.3	157 ± 55	93.4 ± 35.0	137 ± 53	113 ± 42
Cereal fiber (g/d)	7.4 ± 4.7	4.3 ± 2.7	7.9 ± 5.3	4.1 ± 2.6	5.4 ± 4.0	5.8 ± 3.3
Total energy intake (kcal/d)	2 018 ± 632	1 933 ± 622	2 023 ± 639	1 919 ± 617	1 999 ± 632	2 008 ± 632
BMI (kg/m^2)	24.7 ± 3.0	26.2 ± 3.5	24.6 ± 3.0	26.3 ± 3.4	25.4 ± 3.3	25.5 ± 3.2[3]
Physical activity (METs/wk)	25.9 ± 34.2	17.6 ± 25.6	26.5 ± 35.8	17.5 ± 26.1	21.6 ± 30.1	21.0 ± 29.3
Current smoking [*n* (%)]	516 (6.4)	1058 (13.0)	437 (5.6)	1105 (13.9)	841 (10.1)	784 (9.5)
Family history of T2D [*n* (%)]	886 (11.0)	1058 (13.0)	858 (11.0)	1002 (12.6)	925 (11.1)	949 (11.5)[3]

[1] T2D, type 2 diabetes; METs, metabolic equivalents. Linear trend tests for participant characteristics across quintiles were performed by using general linear models. All trends were significant by the Wald test ($P < 0.05$) unless noted otherwise.
[2] Mean ± SD (all such values).
[3] NS.

TABLE 2
Risk of type 2 diabetes (T2D) according to quintiles (Q) of cumulatively averaged low-carbohydrate diet scores

	Q1	Q2	Q3	Q4	Q5	*P* for linear trend
Low-carbohydrate, high total protein and fat score						
Quintile range	0–8	9–12	13–17	18–21	22–30	
Median	5	11	15	19	25	
Person-years	140,266	140,130	152,646	146,225	132,836	
T2D cases	359	422	555	627	726	
Age adjusted	1.00	1.15 (1.00, 1.33)[1]	1.42 (1.24, 1.62)	1.61 (1.41, 1.84)	2.12 (1.87, 2.41)	<0.01
Multivariate[2]	1.00	1.13 (0.98, 1.30)	1.37 (1.20, 1.57)	1.55 (1.36, 1.77)	1.96 (1.72, 2.24)	<0.01
Multivariate + BMI[2]	1.00	1.03 (0.89, 1.19)	1.14 (0.99, 1.31)	1.17 (1.02, 1.33)	1.31 (1.14, 1.49)	<0.01
Low-carbohydrate, high animal protein and fat score						
Quintile range	0–7	8–12	13–17	18–22	23–30	
Median	4	10	15	20	26	
Person-years	137,104	148,384	150,648	141,900	134,066	
T2D cases	324	462	559	618	726	
Age adjusted	1.00	1.32 (1.14, 1.52)	1.56 (1.36, 1.79)	1.82 (1.59, 2.09)	2.29 (2.01, 2.62)	<0.01
Multivariate[2]	1.00	1.29 (1.11, 1.48)	1.51 (1.31, 1.74)	1.76 (1.53, 2.02)	2.15 (1.88, 2.46)	<0.01
Multivariate + BMI[2]	1.00	1.13 (0.97, 1.30)	1.22 (1.08, 1.40)	1.26 (1.09, 1.44)	1.37 (1.20, 1.58)	<0.01
Low-carbohydrate, high vegetable protein and fat score						
Quintile range	0–10	11–13	14–16	17–19	20–30	
Median	9	12	15	18	22	
Person-years	136,487	145,673	152,762	139,337	137,843	
T2D cases	539	512	586	519	533	
Age adjusted	1.00	0.89 (0.78, 1.00)	0.97 (0.86, 1.10)	0.93 (0.82, 1.05)	0.96 (0.85, 1.09)	0.81
Multivariate[2]	1.00	0.91 (0.80, 1.02)	0.99 (0.88, 1.12)	0.95 (0.84, 1.08)	0.98 (0.87, 1.11)	0.96
Multivariate + BMI[2]	1.00	0.90 (0.79, 1.02)	0.96 (0.85, 1.08)	0.94 (0.83, 1.06)	0.95 (0.84, 1.07)	0.64

[1] Hazard ratio (95% CI) from Cox proportional hazards models (all such values).

[2] Multivariate models were adjusted for age, smoking [never, past, current (1–15 or >15 cigarettes/d), or missing], physical activity (quintiles of metabolic equivalents/wk or missing), coffee intake (quintiles), alcohol intake (abstainers or 0–10, 10–20, or >20 g/d), family history of T2D, total energy intake (quintiles of kcal/d), and BMI (in kg/m^2; <23, 23–23.9, 24–24.9, 25–26.9, 27–28.9, 29–30.9, 31–32.9, 33–34.9, ≥35, or missing]; the percentages of observations assigned to missing were as follows (baseline/follow-up): 3.9%/3.6% for smoking, 0.5%/0.1% for physical activity, and 2.2%/0.5% for BMI.

After adjustment for red and processed meat, the associations between the high total (data not shown) and high animal protein and fat scores with T2D were no longer significant in a comparison of the top with the bottom quintile (HR: 1.11; 95% CI: 0.95, 1.30; *P* for trend = 0.20) (**Table 3**). Because the association between the high total protein and fat score and T2D was clearly being driven by animal protein and fat, we did not perform any additional analysis on this score. The high animal protein and fat score adjusted for red and processed meat can be interpreted as a low-carbohydrate diet high in animal protein and fat but containing no red and processed meat. Adjustment for other sources of animal protein and fat yielded only small changes.

TABLE 3
Risk of type 2 diabetes according to quintile of low-carbohydrate, high animal protein and fat score after adjustment for foods and nutrients[1]

	Quintile (range)					
	1 (0–7)	2 (8–12)	3 (13–17)	4 (18–22)	5 (23–30)	*P* for trend
Low-carbohydrate, high animal protein and fat score	1.00	1.13 (0.97, 1.30)	1.22 (1.08, 1.40)	1.26 (1.09, 1.44)	1.37 (1.20, 1.58)	<0.01
Adjusted for red meat	1.00	1.06 (0.92, 1.23)	1.11 (0.95, 1.28)	1.10 (0.95, 1.28)	1.17 (1.00, 1.36)	0.05
Adjusted for processed meat	1.00	1.06 (0.91, 1.23)	1.11 (0.96, 1.29)	1.12 (0.97, 1.29)	1.20 (1.03, 1.38)	0.01
Adjusted for red and processed meat	1.00	1.04 (0.89, 1.20)	1.07 (0.92, 1.24)	1.06 (0.91, 1.23)	1.11 (0.95, 1.30)	0.20
Adjusted for chicken	1.00	1.13 (0.98, 1.31)	1.22 (1.06, 1.41)	1.25 (1.08, 1.44)	1.35 (1.17, 1.56)	<0.01
Adjusted for fish	1.00	1.14 (0.98, 1.31)	1.22 (1.06, 1.41)	1.26 (1.10, 1.45)	1.38 (1.21, 1.59)	<0.01
Adjusted for dairy	1.00	1.14 (0.99, 1.32)	1.24 (1.08, 1.43)	1.29 (1.12, 1.49)	1.42 (1.23, 1.63)	<0.01
Adjusted for eggs	1.00	1.11 (0.95, 1.28)	1.18 (1.02, 1.36)	1.20 (1.04, 1.39)	1.31 (1.13, 1.50)	<0.01
Adjusted for animal protein	1.00	1.19 (1.02, 1.38)	1.30 (1.10, 1.53)	1.33 (1.12, 1.59)	1.40 (1.16, 1.70)	<0.01
Adjusted for animal fat	1.00	1.04 (0.89, 1.23)	1.09 (0.92, 1.30)	1.12 (0.92, 1.36)	1.20 (0.96, 1.49)	0.10
Adjusted for glycemic load	1.00	1.17 (1.01, 1.35)	1.30 (1.12, 1.50)	1.37 (1.18, 1.60)	1.57 (1.33, 1.85)	<0.01
Adjusted for heme iron	1.00	1.06 (0.91, 1.24)	1.10 (0.93, 1.29)	1.10 (0.93, 1.31)	1.18 (0.99, 1.42)	0.07
Adjusted for saturated fat	1.00	1.09 (0.94, 1.27)	1.17 (1.01, 1.37)	1.21 (1.03, 1.42)	1.34 (1.13, 1.57)	<0.01

[1] Values are hazard ratios (95% CIs) from Cox proportional hazards models. Models were adjusted for the indicated foods and nutrients (quintiles) and all covariates from Table 1. An increase in the hazard ratio after adjustment indicates that the intake of a food or nutrient is protective (eg, adjustment for the net benefit of decreasing glycemic load strengthens the association). A decrease in the hazard ratio after adjustment indicates that the intake of the food or nutrient is harmful (eg, adjustment for the net harm of increasing heme iron weakens the association).

For example, adjustment for dairy intake strengthened the association, whereas adjustment for eggs weakened it. These findings suggest that dairy products are slightly protective, whereas eggs are slightly harmful. However, the overall effect of these foods in the low-carbohydrate diets was very small.

The association between the high animal protein and fat score and T2D was not significant after adjustment for animal fat or heme iron (Table 3). Adjustment for glycemic load strengthened the association, whereas adjustment for saturated fat slightly attenuated it.

Red and processed meat, animal fat, and heme iron were moderately correlated with one another ($R_{\text{animal fat, heme iron}} = 0.65$, $R_{\text{animal fat, red/processed meat}} = 0.59$, and $R_{\text{heme, red/processed meat}} = 0.51$), and in a separate model animal fat and heme iron were significantly associated with T2D after adjustment for one another (data not shown). However, after the inclusion of all 3 factors in the same model, only red and processed meat was significantly associated with T2D in a comparison of the top with the bottom quintile (HR: 1.64; 95% CI: 1.37, 1.97; P for trend < 0.01).

A similar analysis was performed on the high vegetable protein and fat score by adjusting for vegetable sources of protein and fat (eg, nuts and legumes) and other plant-based foods (eg, fruit and vegetables); however, these adjustments did not substantially alter the associations (data not shown).

Trends were attenuated when only baseline scores were used in a comparison of the top with the bottom quintile: total protein and fat score (HR: 1.18; 95% CI: 1.04, 1.33; P for trend < 0.01), animal protein and fat score (HR: 1.17; 95% CI: 1.03, 1.33; P for trend < 0.01), and vegetable protein and fat score (HR: 0.95; 95% CI: 0.84, 1.07; P for trend = 0.49). Adjustment for WHR or continuous covariates did not materially alter the results of the main analysis (data not shown), and no significant interactions with alcohol intake, family history, physical activity, or BMI were found (**Table 4**). The low-carbohydrate, high vegetable protein and fat score was associated with a decreased risk of T2D in men younger than age 65 y (P for interaction = 0.01).

We corrected for measurement error in the low-carbohydrate total protein and fat score across the follow-up while adjusting for age and BMI, which are the 2 most important confounders in our analysis. Because of limitations in the program and sparseness of the validation study data, further multivariate adjustment was not possible. The association between the low-carbohydrate, high total protein and fat score and T2D was strengthened after correction for measurement error. In the age and BMI-adjusted model, a 20-unit increase (similar to a comparison of the top with the bottom quintile; Table 2) was associated with a rate ratio of 1.34 (95% CI: 1.19, 1.51; $P < 0.01$), which increased to 1.74 (95% CI: 1.25, 2.42; $P < 0.01$) after correction.

DISCUSSION

In this analysis we found that a score representing a low-carbohydrate, high animal protein and fat diet was positively associated with T2D in a cohort of male health professionals. This association was mainly due to intake of red and processed

TABLE 4
Analysis stratified by age, alcohol intake, family history, physical activity, and BMI[1]

	Low-carbohydrate, high animal protein and fat score (range)				Low-carbohydrate, high vegetable protein and fat score (range)			
	Q1 (0–7)	Q3 (13–17)	Q5 (23–30)	P for trend	Q1 (0–10)	Q3 (14–16)	Q5 (20–30)	P for trend
Age								
<65 y	1.00	1.52 (1.24, 1.85)	1.81 (1.49, 2.19)	<0.01	1.00	0.79 (0.67, 0.93)	0.78 (0.66, 0.92)	0.01
≥65 y	1.00	1.30 (1.06, 1.59)	1.64 (1.34, 2.01)	<0.01	1.00	1.14 (0.95, 1.37)	1.15 (0.95, 1.39)	0.07
P for interaction			0.51				0.01	
Alcohol intake								
Drinker	1.00	1.38 (1.17, 1.62)	1.62 (1.38, 1.90)	<0.01	1.00	0.91 (0.80, 1.05)	0.91 (0.79, 1.05)	0.33
Abstainer	1.00	1.17 (0.85, 1.60)	1.60 (1.18, 2.15)	0.02	1.00	0.98 (0.74, 1.30)	1.04 (0.77, 1.39)	0.65
P for interaction			0.56				0.43	
Family history of type 2 diabetes								
Yes	1.00	1.16 (0.84, 1.59)	1.27 (0.93, 1.74)	0.15	1.00	0.84 (0.64, 1.10)	0.75 (0.56, 0.99)	0.15
No	1.00	1.48 (1.26, 1.74)	1.82 (1.55, 2.13)	<0.01	1.00	0.96 (0.83, 1.10)	1.01 (0.87, 1.16)	0.75
P for interaction			0.06				0.17	
Physical activity								
Low (Q1, Q2)	1.00	1.42 (1.13, 1.78)	1.79 (1.44, 2.21)	<0.01	1.00	0.88 (0.74, 1.05)	0.94 (0.78, 1.12)	0.56
Medium (Q3, Q4)	1.00	1.23 (0.98, 1.55)	1.40 (1.12, 1.76)	<0.01	1.00	0.97 (0.78, 1.20)	0.93 (0.75, 1.16)	0.59
High (Q5)	1.00	1.83 (1.29, 2.58)	1.72 (1.19, 2.47)	<0.01	1.00	0.98 (0.71, 1.35)	0.88 (0.63, 1.22)	0.98
P for interaction			0.87				0.98	
BMI								
<25 kg/m^2	1.00	1.34 (0.99, 1.82)	1.44 (1.03, 2.02)	0.01	1.00	1.00 (0.74, 1.35)	1.03 (0.76, 1.40)	0.73
25–29.9 kg/m^2	1.00	1.18 (0.97, 1.44)	1.46 (1.20, 1.77)	<0.01	1.00	0.88 (0.74, 1.05)	0.83 (0.69, 0.99)	0.14
≥30 kg/m^2	1.00	1.43 (1.05, 1.96)	1.71 (1.27, 2.30)	<0.01	1.00	1.04 (0.84, 1.30)	1.00 (0.80, 1.26)	0.88
P for interaction			0.65				0.74	

[1] Values are hazard ratios (95% CIs) from Cox proportional hazards models. Q, quintile. All models were adjusted for age, smoking [never, past, or current (1–15 or >15 cigarettes/d)], physical activity (continuous), coffee intake (continuous), alcohol intake (continuous), family history of type 2 diabetes, total energy intake (quintiles of kcal/d), and BMI (continuous), except when stratified. Interaction tests were performed by using the Wald test on a multiplicative term (eg, median low-carbohydrate diet score × median BMI). At baseline, the number of participants in each category was as follows: age <65 y (35,003), age ≥65 y (5472), drinkers (31,222), abstainers (9253), family history (4707), no family history (35,768), low activity (15,810), moderate activity (16,214), high activity (8451), BMI (in kg/m^2) < 25 (19,683), 25 ≤ BMI < 30 (17,821), and BMI ≥ 30 (2971).

meat. Conversely, a score representing a diet low in carbohydrate and high in vegetable protein and fat was not significantly associated with T2D.

Low-carbohydrate diets have received attention because high-protein variants are associated with satiety. This may help with weight maintenance and loss (10). Energy requirements for protein digestion are also higher than carbohydrate and fat, which could increase metabolic rate and insulin sensitivity (10, 11). However, low-carbohydrate diets could increase the risk of T2D if they contain a high burden of unhealthy foods such as red and processed meat. In 2 recent meta-analyses (12, 13), the Health Professionals Follow-Up Study (14) and the Nurses' Health Study (15), intake of red and processed meat was positively associated with T2D.

In an earlier analysis of the Nurses' Health Study, the low-carbohydrate, high animal protein and fat score was not significantly associated with T2D (3). This difference was likely due to dietary variation between the cohorts. Compared with men in the Health Professionals Follow-Up study, women in the Nurses' Health Study had a lower median intake of energy (1507 compared with 1918 kcal), carbohydrate (39% compared with 47% of energy), and glycemic load (78 compared with 118) but a slightly higher intake of red and processed meat (1.2 compared with 0.9 servings/d). In an updated analysis of the Nurses' Health Study (26 compared with 20 y of follow-up and 7914 compared with 4670 cases of T2D), the high animal protein and fat score was significantly associated with T2D after adjustment for glycemic load in a comparison of the top with the bottom quintile (HR: 1.31; 95% CI: 1.20, 1.44; P for trend < 0.01; L de Koning, unpublished data, 2010). This indicates that a sufficiently reduced glycemic load in a low-carbohydrate diet can offset the harmful effect of red and processed meat. A simpler and potentially beneficial approach, however, would be to replace red and processed meat with a variety of other protein and fat sources, such as chicken, fish, eggs, dairy, legumes, and nuts.

Red and processed meat contains several components that may elevate T2D risk. First, heme iron can generate reactive hydroxyl radicals from less-reactive ones, such as hydrogen peroxide (16). Iron deposition in muscle, liver, and pancreas may cause focal tissue and β cell damage, which disrupts insulin signaling (17). Both the Iowa Women's Health and Nurses' Health studies report positive associations between heme iron intake and T2D (18, 19). Second, nitrites and nitrates in processed meat (20), their intestinal metabolites nitrosamines (21), and advanced glycation end products (22, 23) formed from grilling meat are risk factors for insulin resistance and diabetes in ecologic and animal studies. Third, individuals with high proportions of saturated, as opposed to *cis*-unsaturated, fatty acids in serum cholesterol esters and phospholipids have greater insulin resistance and higher T2D risk (24–26). Fourth, in small randomized trials, individuals consuming diets high in animal-derived saturated fats, as opposed to plant derived *cis*-unsaturated fats, have greater insulin resistance and glucose intolerance (27, 28). Although incompletely understood, the mechanisms responsible may involve changes in cell membrane fluidity, glucose transporter function, and gene expression (26). So far, prospective cohort studies assessing the intake of saturated fat with FFQs have not found consistent associations with incident T2D after adjustment for BMI and other lifestyle-related variables (14, 29, 30). This may be because of the variety of nutrients that saturated fat can replace in the diet (26).

In our study, intakes of animal fat, heme iron, and red and processed meat were responsible for the relation between the high animal protein and fat score and T2D. Saturated fat explained very little of this relation and, in previous publications from this cohort, was not related to T2D risk (14, 31). Interestingly, when animal fat, heme iron, and red meat were included in the same model, only red and processed meat was significantly associated with T2D. This suggests that some other aspect of red and processed meat, such as advanced glycation end products, is driving the relation. Another possibility is residual confounding by other lifestyle traits; however, we were careful to adjust for major confounders in our analysis.

In this study, the low-carbohydrate, high vegetable protein and fat score was not significantly associated with T2D overall, but was inversely associated with T2D in men younger than age 65 y. In the Nurses' Health Study, this score is inversely associated with T2D overall (3). However, in both studies, a shift from animal to vegetable sources of protein and fat would be associated with a lower risk of T2D, which is likely due in part to the protective effects of vegetable (3, 14) and polyunsaturated (30) Fat. In the Health Professionals Follow-Up Study, linoleic acid was inversely associated with risk of diabetes in men younger than 65 y or who were not overweight (14). In a recent randomized trial, a low-carbohydrate, high-protein diet rich in gluten, soy products, nuts, fruit, vegetables, cereals, and oils was no more effective at lowering blood glucose, insulin, glycated hemoglobin, and insulin resistance than was a low-fat, high-carbohydrate diet in overweight hyperlipidemic men (32). However, this diet was superior at lowering blood lipids, which suggests that other endpoints besides T2D should be considered when assessing the health benefits of low-carbohydrate diets (32).

Our study had several strengths. The first was its prospective design, which establishes the direction of the associations reported here. The second was its homogeneous population, which means that confounders will have smaller distributions and less residual confounding than other more representative studies, such as the National Health and Nutrition Examination Survey. The third was its strong methodology. Cumulative average diet scores were used and updated only in the absence of a chronic disease diagnosis. This reduces measurement error, strengthens associations by accounting for prior dietary information, and controls recall bias arising during follow-up. Confounding variables were also carefully controlled for and updated during follow-up. The fourth was its large sample size, which allowed for small differences in risk to be detected. The fifth was that we used a novel measurement error correction technique, which suggests that we may have underestimated the true associations between low-carbohydrate diets and T2D.

Our study had some limitations. The first was that most participants were highly educated white men, which means that our results may not apply to other populations. However, they were consistent in using different strata, which suggests that they may be similar elsewhere. The second major limitation was unmeasured and residual confounding. Whereas this is always a possibility in observational studies, we took care to control for known confounders and repeated the analysis using continuous diet scores and covariates. This did not alter the results. The third limitation was the lack of other studies that have used our scores; however, the Health Professionals Follow-Up Study and the Nurses' Health Study are independent cohorts. The fourth

limitation was that we did not consider other disease endpoints in our analysis, which might favor the inclusion of other foods in low-carbohydrate diets. For example, fish intake is inversely associated with the risk of sudden cardiac death (33).

In conclusion, a score representing a low-carbohydrate, high animal protein and fat diet is positively associated with the risk of T2D. Low-carbohydrate diets should obtain protein and fat from foods other than red and processed meat to minimize T2D risk.

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