Cooking Methods for Red Meats and Risk of Type 2 Diabetes: A Prospective Study of U.S. Women

Gang Liu,[1] Geng Zong,[1] Frank B. Hu,[1,2,3] Walter C. Willett,[1,2,3] David M. Eisenberg,[1] and Qi Sun[1,2]

Diabetes Care 2017;40:1041–1049 | https://doi.org/10.2337/dc17-0204

OBJECTIVE

This study examined different cooking methods for red meats in relation to type 2 diabetes (T2D) risk among U.S. women who consumed red meats regularly (≥2 servings/week).

RESEARCH DESIGN AND METHODS

We monitored 59,033 women (1986–2012) aged 30–55 years and free of diabetes, cardiovascular disease, and cancer at baseline when information on frequency of different cooking methods for red meats, including broiling, barbequing, roasting, pan-frying, and stewing/boiling, was collected.

RESULTS

During 1.24 million person-years of follow-up, we documented 6,206 incident cases of T2D. After multivariate adjustment including red meat cooking methods, total red meat and processed red meat intake were both associated with a monotonically increased T2D risk (both *P* trend <0.05). After multivariate adjustment including total red meat intake, a higher frequency of broiling, barbequing, and roasting red meats was each independently associated with a higher T2D risk. When comparing ≥2 times/week with <1 time/month, the hazard ratios (HRs) and 95% CI of T2D were 1.29 (1.19, 1.40; *P* trend <0.001) for broiling, 1.23 (1.11, 1.38; *P* trend <0.001) for barbequing, and 1.11 (1.01, 1.23; *P* trend = 0.14) for roasting. In contrast, the frequency of stewing/boiling red meats was not associated with T2D risk, and an inverse association was observed for pan-frying frequency and T2D risk. The results remained similar after cooking methods were further mutually adjusted.

CONCLUSIONS

Independent of total red meat consumption, high-temperature and/or open-flame cooking methods for red meats, especially broiling and barbequing, may further increase diabetes risk among regular meat eaters.

Type 2 diabetes (T2D) has become a global challenge with tremendous economic burden for society and public health systems (1,2). Multiple risk factors of T2D, such as obesity, sedentary lifestyle, dietary factors, and genetic predisposition (3,4), have been identified in epidemiological studies, although there is still a critical need to identify additional modifiable risk factors to facilitate diabetes prevention.

Of the many dietary risk factors for T2D, red meats, particularly processed red meats, have been consistently associated with an elevated risk of developing diabetes in prospective cohort studies (5–7). Despite the accumulating evidence demonstrating

[1]*Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA*
[2]*Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*
[3]*Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA*

Corresponding author: Qi Sun, qisun@hsph.harvard.edu.

Received 27 January 2017 and accepted 6 May 2017.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc17-0204/-/DC1.

positive associations of consuming red meats with cardiometabolic conditions, the average consumption levels of red meats in U.S. and in some other countries remain high (8).

Although high red meat consumption is related to an elevated T2D risk (5,7), whether red meat cooking methods may also play a role in diabetes risk is unclear. Cooking temperature and other conditions can influence the production of several hazardous chemicals, including heterocyclic aromatic amines, polycyclic aromatic hydrocarbons (PAHs), and advanced glycation end products (9–12), which are known carcinogens or can interfere with insulin sensitivity (12–17). Consequently, specific cooking methods might differentially modulate T2D risk linked with red meat consumption. However, evidence from human studies is scarce. Whether the common red meat cooking methods, such as broiling, barbequing, roasting, pan-frying, and stewing/boiling, are associated with T2D risk beyond the effect of red meats has not been examined.

To fill this knowledge gap, we prospectively investigated associations between different cooking methods for red meats and the risk of developing T2D in a prospective cohort of U.S. women. We hypothesized that high-temperature cooking methods could increase the risk of developing T2D, independent of the amount of red meat consumption.

RESEARCH DESIGN AND METHODS

Study Population

The Nurses' Health Study (NHS) is an ongoing prospective cohort study that began in 1976 with an enrollment of 121,700 U.S. female registered nurses aged 30–55 years (18). Information on lifestyle practice and medical history was collected through a self-administered questionnaire at baseline and then updated every 2 years using a follow-up questionnaire, with a cumulative response rate of more than 90% (18).

In the current analysis, the study baseline was 1986, when information on cooking methods for red meats was collected. Participants were excluded if they reported a diagnosis of T2D, cardiovascular disease, or cancer at baseline (n = 5,730); had missing information on cooking methods for red meats (n = 101); reported implausible daily caloric intake ($<$500 or $>$3,500 kcal/day) (n = 1,183); or completed the baseline questionnaire (1986) only (n = 690). In addition, we restricted the analysis to participants who consumed red meats regularly ($\geq$2 servings of red meat intake per week) based on a food frequency questionnaire (FFQ) at baseline. After exclusions, 59,033 women were included in the final analysis with 26 years of follow-up. The study protocol was approved by the institutional review board at Brigham and Women's Hospital (Boston, MA) and Harvard T.H. Chan School of Public Health (Boston, MA). The return of the completed self-administrated questionnaire was considered to imply informed consent.

Assessment of Diet and Red Meat Cooking Methods

In 1986 and every 4 years thereafter, a validated semiquantitative FFQ with ~130 food items was mailed to participants to evaluate and update dietary intake and alcohol consumption for the past year (19). The FFQ was validated against multiple diet records, and moderate-to-strong correlation coefficients were observed for red meat consumption in validation studies (e.g., r_s ranged from 0.38 to 0.70 for various red meat items) (6,20). The cumulative averages of foods and nutrients were calculated based on valid assessments from baseline to the end of follow-up. We stopped updating dietary information if participants reported a diagnosis of diabetes, cardiovascular disease, or cancer (19). Nutrient intake was adjusted for total energy intake using the regression-residual method (21). The 2010 Alternative Healthy Eating Index (AHEI) was calculated, with the exclusion of alcohol consumption, to evaluate overall diet quality (19).

In the 1986 questionnaire, participants were asked how often they cooked red meats (including beef, pork, or lamb) using the following methods: broiling, barbequing, roasting, pan-frying, and stewing or boiling, with six prespecified response categories (never, $<$1 time/month, 1–3 times/month, 1 time/week, 2–4 times/week, and $\geq$5 times/week). Based on the distribution of cooking frequency, we categorized the frequency of cooking methods into four groups ($<$1 time/month, 1–3 times/month, 1 time/week, and $\geq$2 times/week) to ensure an adequate number of T2D cases in each group. In the 1996 questionnaire, NHS participants were again asked the frequency of cooking beef or steak using the following methods: broiling, barbequing, and pan-frying (never, $<$1 time/month, 1 time/month, 2–3 times/month, 1 time/week, 2–3 times/week, and $\geq$4 times/week). The Spearman correlation coefficients (r_s) for the assessments between 1986 and 1996 questionnaires regarding the frequency of barbequing, roasting, and pan-frying for red meats ranged from 0.28 to 0.35 ($P < 0.001$), although the validity of the questionnaire assessments of cooking methods for red meat was not evaluated.

To account for the potential changes in red meat cooking practice during follow-up, we updated frequencies of barbequing, roasting, and pan-frying beef/steak in a sensitivity analysis, although the differences between the baseline and updated questions for cooking methods shall be noted. In the current study, participants were also asked about the type of fat (real butter, vegetable oil, lard, margarine, solid vegetable shortening, or none) used for baking, frying, or sautéing, although we did not obtain the information on specific oils used for cooking red meats.

Ascertainment of T2D

Participants who reported having diabetes on any biennial questionnaire were mailed a validated supplementary questionnaire regarding symptoms, diagnostic tests, and hypoglycemic therapy. Before the release of the American Diabetes Association criteria in 1997, we used the National Diabetes Data Group criteria to diagnose diabetes: *1*) fasting glucose levels $\geq$7.8 mmol/L, blood glucose $\geq$11.1 mmol/L during an oral glucose tolerance test, or random blood glucose $\geq$11.1 mmol/L, together with one or more diabetes-related symptoms (weight loss, polyuria, excessive thirst, or hunger); *2*) elevated glucose levels on more than one occasion in the absence of symptoms; or *3*) treatment with hypoglycemic medication (insulin or an oral hypoglycemic agent) (22,23). After 1998, on the basis of the American Diabetes Association criteria, fasting plasma glucose was lowered to 7.0 mmol/L as the cutoff point for the diagnosis of diabetes (24). In a validation study with 62 cases of T2D confirmed by supplementary questionnaires from the NHS, 61 (98%) were reconfirmed after an endocrinologist reviewed the medical records (25).

Assessment of Covariates

In the biennial follow-up questionnaires, we updated information on demographic, anthropometric, socioeconomic, and

lifestyle factors, including cigarette smoking, alcohol consumption, physical activity levels, family history of diabetes, menopausal status, and use of postmenopausal hormones and multivitamins. BMI was calculated as self-reported weight in kilograms divided by the square of height in meters (kg/m^2). Physical activity was estimated as METs per week based on average time spent on various activities, weighted by the intensity level.

Statistical Analysis

Spearman correlation coefficients were calculated between each cooking method for red meats. Factor analysis was used to explore the potential correlation patterns of cooking methods. Person-years were calculated from the return of the baseline FFQ to the date of T2D diagnosis, death, loss to follow-up, or end of follow-up in June 2012, whichever came first. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs for the association between baseline frequency of cooking methods for red meats and risk of developing T2D. To minimize sample reduction caused by missing covariates (<2%), indicator variables were used for missing categorical variables. In a sensitivity analysis, we further restricted our analyses to the participants without missing covariates to examine the effect of missing data on associations of interest.

To account for confounding by age and calendar time, we stratified the analysis jointly by age in months at the start of follow-up and the calendar year of the current questionnaire cycle. The multivariate model was adjusted for ethnicity (Caucasian, African American, Hispanic, or Asian) and BMI at age 18 (kg/m^2: <23, 23–24.9, 25–29.9, 30–34.9, ≥35, or missing), as well as time-varying covariates, including smoking status (never smoker, past smoker, or current smoker: 1–14, 15–24, or ≥25 cigarettes/day, or missing), marital status (married, not married, or missing), alcohol intake (g/day: 0, 0.1–4.9, 5.0–14.9, >15.0, or missing), multivitamin use (yes, no, or missing), family history of diabetes (yes or no), menopausal status and postmenopausal hormone use (premenopausal, postmenopausal [never, former, or current hormone use], or missing), physical activity (METs/week: 0–2.9, 3–8.9, 9–17.9, 18–26.9, ≥27.0, or missing), total energy intake (kcal/day), and AHEI score. We further mutually adjusted for other cooking methods for red meats in a fully adjusted model.

The proportional hazards assumption was tested by using a likelihood ratio test comparing models with and without multiplicative interaction terms between exposure and calendar year, and the proportional hazards assumption was not violated in any analyses. To control for potential confounding of red meat consumption, we further adjusted for total red meat intake in the model above and also conducted a stratified analysis by total red meat intake (in tertiles). The *P* values for the product terms between median frequency of cooking methods and total red meat intake were used to estimate the significance of interactions. The linear trend was tested by assigning a median value to each category of the frequency of cooking methods. Additional analyses were also conducted to treat the frequency of each cooking method for red meats as a continuous variable.

To examine the dose-response relationship between red meat intake and T2D risk, restricted cubic spline regression with five knots was used, with mutual adjustment of cooking methods (26). Tests for nonlinearity were based on the likelihood ratio test comparing two models: one with only the linear term and the other with the linear and the cubic spline terms.

Linear regression models were used to investigate the associations between each cooking method for red meats and weight change from baseline to 1994 when the mean age of the participants was ~60 years, because differential body composition changes at older age (>60 years) might influence the associations of interest (27). After obese participants (BMI ≥30 kg/m^2) were excluded at baseline, each cooking method was also examined in relation to the risk of developing obesity. The multivariate-adjusted models included the same covariates included in the analysis of cooking methods and T2D risk.

In addition, we evaluated the extent to which the associations between cooking methods and T2D risk could be explained by BMI, using a SAS macro %MEDIATE based on the work by Lin et al. (28). We also estimated the substitution effect of one cooking method for another at the frequency of once per week by including both cooking methods as continuous variables in the same multivariate model. The difference in β-coefficients and variances were used to estimate the β-coefficient and variance for the substitution effect, which was then applied for the calculation of HRs and 95% CIs for the substitution effect (6,29).

In sensitivity analyses, instead of adjusting for diet quality (as represented by AHEI), individual dietary factors (including fruits, vegetables, soda, whole grains, fiber, glycemic index, and polyunsaturated fat-to-saturated fat ratio) were adjusted for in the multivariate model. Considering that different cooking methods might be used to cook different types of red meats, we also adjusted for the intake of specific red meats (including hot dogs, hamburgers, bacon, and other processed and unprocessed meats) instead of total red meat consumption. Because cooking methods may differ across regions of the country, geographic location (north, middle, south, or unknown) was further adjusted for in the multivariate model. To exclude the possibility that participants with a very high risk of T2D or prediabetes might change their cooking practice to pursue a healthier diet, we excluded participants who reported incident T2D in the first 4 years of follow-up. In another sensitivity analysis, we considered participants with low red meat consumption (<2 servings of red meat intake per week) as the reference group.

Stratified analyses were conducted by age (<60 years, ≥60 years), BMI (<25 kg/m^2, ≥25 kg/m^2), and current smoking status (yes, no) to determine potential effect modification by these factors. Time-varying variables were used in the stratified analyses, and *P* values for the product terms between median frequency of cooking methods and stratification variables were used to estimate the significance of interactions (30). We also conducted another stratified analysis by vegetable oil use for baking, frying, or sautéing (yes, no).

All statistical analyses were performed with SAS 9.4 software (SAS Institute Inc., Cary, NC). Two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 reports the baseline characteristics of the study population according to the frequency of different cooking methods. Participants who reported higher frequency of cooking methods for red meats, including broiling, barbequing, roasting,

Table 1—Age-adjusted baseline characteristics among participants who consumed red meats regularly (≥2 servings/week) in the NHS

	Frequency of cooking methods for red meats (*N* = 59,033)									
	Broiling		Barbequing		Roasting		Pan-frying		Stewing or Boiling	
	Low <1 time/month	High 1 time/week	Low <1 time/month	High 1 time/week	Low <1 time/month	High 1 time/week	Low <1 time/month	High 1 time/week	Low <1 time/month	High 1 time/week
Age, years	52.2 ± 7.3	51.7 ± 7.0	53.5 ± 7.1	50.1 ± 6.8	52.7 ± 7.2	51.8 ± 7.0	52.3 ± 7.1	51.9 ± 7.1	51.9 ± 7.1	52.1 ± 7.1
BMI, kg/m^2	24.8 ± 4.6	25.4 ± 4.7	25.1 ± 4.7	25.3 ± 4.6	24.7 ± 4.5	25.4 ± 4.8	25.0 ± 4.5	25.4 ± 4.8	25.0 ± 4.6	25.5 ± 4.8
Physical activity, METs/week	13.3 ± 19.3	14.2 ± 20.4	13.8 ± 20.3	14.2 ± 19.3	15.2 ± 19.9	13.5 ± 19.7	15.3 ± 21.7	11.9 ± 16.3	14.2 ± 20.2	13.7 ± 21.5
Alcohol intake, g/day	5.9 ± 10.7	6.7 ± 10.6	5.2 ± 9.8	8.2 ± 12.1	6.2 ± 10.7	6.3 ± 10.5	6.5 ± 10.6	6.0 ± 10.7	6.3 ± 10.7	6.4 ± 11.0
Current smoking	19	23	20	23	18	23	20	23	21	22
White race	98	98	98	99	97	99	98	98	98	98
Family history of diabetes	26	27	26	26	25	26	26	26	26	27
Any use of postmenopausal hormone	26	27	26	28	28	26	27	26	27	26
Married	82	84	81	87	78	85	83	84	81	86
Dietary intake										
Total energy, kcal/day	1,707 ± 514	1,843 ± 518	1,730 ± 516	1,868 ± 521	1,623 ± 499	1,881 ± 518	1,703 ± 505	1,916 ± 520	1,678 ± 502	1,950 ± 524
Total red meat, servings/day	1.15 ± 0.6	1.31 ± 0.5	1.16 ± 0.5	1.36 ± 0.5	0.99 ± 0.5	1.37 ± 0.6	1.11 ± 0.5	1.44 ± 0.6	1.15 ± 0.5	1.40 ± 0.6
Processed meat, servings/day	0.32 ± 0.3	0.34 ± 0.3	0.31 ± 0.3	0.36 ± 0.3	0.27 ± 0.2	0.36 ± 0.3	0.29 ± 0.2	0.39 ± 0.3	0.31 ± 0.3	0.37 ± 0.3
Hot dog, servings/day	0.08 ± 0.08	0.08 ± 0.08	0.08 ± 0.08	0.09 ± 0.07	0.07 ± 0.08	0.08 ± 0.07	0.07 ± 0.07	0.09 ± 0.08	0.08 ± 0.08	0.09 ± 0.08
Hamburger, servings/day	0.17 ± 0.1	0.19 ± 0.1	0.17 ± 0.1	0.19 ± 0.1	0.16 ± 0.1	0.19 ± 0.1	0.16 ± 0.1	0.21 ± 0.1	0.17 ± 0.1	0.19 ± 0.1
Red meat as main dish, servings/day	0.28 ± 0.2	0.35 ± 0.2	0.29 ± 0.2	0.36 ± 0.2	0.24 ± 0.2	0.36 ± 0.2	0.29 ± 0.2	0.37 ± 0.2	0.30 ± 0.2	0.36 ± 0.2
Bacon, servings/day	0.10 ± 0.1	0.11 ± 0.1	0.09 ± 0.1	0.12 ± 0.1	0.08 ± 0.1	0.12 ± 0.1	0.09 ± 0.1	0.13 ± 0.1	0.10 ± 0.1	0.12 ± 0.1
Total vegetables, servings/day	2.9 ± 1.6	3.3 ± 1.6	3.1 ± 1.6	3.4 ± 1.6	3.1 ± 1.7	3.3 ± 1.6	3.3 ± 1.7	3.1 ± 1.5	3.0 ± 1.6	3.4 ± 1.6
Total fruits, servings/day	2.4 ± 1.5	2.6 ± 1.5	2.5 ± 1.5	2.5 ± 1.5	2.5 ± 1.6	2.5 ± 1.5	2.6 ± 1.6	2.4 ± 1.5	2.4 ± 1.5	2.7 ± 1.6
Fish, servings/day	0.28 ± 0.2	0.31 ± 0.2	0.30 ± 0.3	0.30 ± 0.2	0.33 ± 0.3	0.29 ± 0.2	0.33 ± 0.3	0.26 ± 0.2	0.31 ± 0.3	0.29 ± 0.2
Poultry, servings/day	0.31 ± 0.2	0.34 ± 0.2	0.33 ± 0.2	0.34 ± 0.2	0.36 ± 0.3	0.32 ± 0.2	0.35 ± 0.2	0.30 ± 0.2	0.33 ± 0.2	0.33 ± 0.2
Total dairy products, servings/day	2.1 ± 1.4	2.2 ± 1.4	2.2 ± 1.4	2.2 ± 1.4	2.1 ± 1.4	2.2 ± 1.4	2.1 ± 1.4	2.2 ± 1.4	2.1 ± 1.4	2.3 ± 1.4
Soda, servings/day	0.74 ± 1.0	0.85 ± 1.1	0.74 ± 1.0	0.88 ± 1.1	0.78 ± 1.1	0.82 ± 1.1	0.81 ± 1.1	0.81 ± 1.0	0.83 ± 1.1	0.78 ± 1.0
French fries, servings/day	0.05 ± 0.08	0.06 ± 0.09	0.05 ± 0.09	0.07 ± 0.1	0.05 ± 0.08	0.07 ± 0.09	0.05 ± 0.08	0.08 ± 0.09	0.06 ± 0.08	0.07 ± 0.09
Whole grain, g/day	15.1 ± 14.5	12.9 ± 11.8	15.0 ± 14.1	12.0 ± 11.0	16.9 ± 15.7	12.4 ± 11.4	15.2 ± 13.9	11.9 ± 10.7	14.5 ± 14.0	12.8 ± 11.5
Refined grain, g/day	47.9 ± 17.4	46.5 ± 15.4	47.7 ± 17.1	46.1 ± 15.4	47.7 ± 18.4	46.7 ± 15.2	46.8 ± 16.8	47.5 ± 15.3	46.9 ± 17.0	46.8 ± 15.0
Sodium, g/day	2.8 ± 1.0	2.9 ± 1.0	2.8 ± 1.0	2.9 ± 1.0	2.7 ± 1.0	2.9 ± 1.1	2.8 ± 1.0	3.0 ± 1.0	2.8 ± 1.0	3.0 ± 1.1
Carbohydrate, % energy	48.8 ± 7.8	47.5 ± 7.2	49.0 ± 7.5	46.6 ± 7.2	49.2 ± 8.1	47.7 ± 7.1	48.6 ± 7.7	47.3 ± 7.0	48.1 ± 7.9	47.8 ± 7.0
Protein, % energy	18.3 ± 3.3	18.7 ± 3.1	18.6 ± 3.3	18.6 ± 3.1	18.8 ± 3.5	18.5 ± 3.1	18.9 ± 3.4	18.1 ± 2.9	18.6 ± 3.4	18.6 ± 3.0
Trans fatty acids, % energy	1.7 ± 0.6	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.6 ± 0.6	1.8 ± 0.5	1.6 ± 0.5	1.8 ± 0.5	1.7 ± 0.5	1.7 ± 0.5
P-to-S ratio	0.56 ± 0.2	0.53 ± 0.2	0.55 ± 0.2	0.53 ± 0.2	0.59 ± 0.2	0.53 ± 0.2	0.56 ± 0.2	0.52 ± 0.2	0.55 ± 0.2	0.53 ± 0.2
AHEI*	45.6 ± 10.8	45.0 ± 9.7	45.9 ± 10.6	44.6 ± 9.5	48.7 ± 10.8	43.9 ± 9.6	47.4 ± 10.2	42.2 ± 9.1	46.1 ± 10.6	44.2 ± 9.3

Data are mean ± SD or %. P-to-S ratio, polyunsaturated fatty acids–to–saturated fatty acids ratio. *Alcohol consumption was not included in the AHEI score.

pan-frying, and stewing or boiling, tended to have a higher concurrent BMI, higher consumption of total energy, total red meats, and processed meats, less intake of whole grains, and lower AHEI score.

The partial r_s between the frequency of each cooking method for red meats are reported in Supplementary Table 1. There were modest positive associations between broiling, barbequing, and roasting (r_s ranged from 0.16 to 0.26), while in general, stewing/boiling and pan-frying were weakly to modestly correlated with other cooking methods (r_s ranged from 0.02 to 0.25). Consistently, no particular pattern of cooking methods for red meats was observed when we used the factor analysis to explore correlation patterns. We observed modest correlations between the 1986 and 1996 assessments of frequency of barbequing, roasting, and pan-frying red meats; the partial r_s ranged from 0.28 to 0.35 (all $P <$ 0.001). Lastly, a positive correlation was demonstrated between the frequency of pan-frying red meats and the consumption of monounsaturated fatty acid (MUFA) from plant sources (r_s = 0.13, $P < 0.001$) but not for the other cooking methods (r_s ranged from −0.05 to 0.02).

During 1.24 million person-years of follow-up, we documented 6,206 incident cases of T2D. Supplementary Fig. 1 shows the dose-response relationship between baseline red meat intake and risk of developing T2D. After multivariate adjustment including baseline BMI and red meat cooking methods, linear relationships ($P_{linearity} <0.001$) were demonstrated between total red meat intake, processed red meat intake, and T2D risk; each one serving/day increase of total and processed red meat consumption was associated with a 6% (95% CI 1, 12; $P <$ 0.05) and 16% (95% CI 5, 28; $P < 0.01$) increased T2D risk, respectively.

Associations between the frequency of cooking methods and risk of developing T2D are reported in Table 2. After multivariate adjustment, a higher frequency of cooking red meats by broiling, barbequing, and roasting, but not by pan-frying and stewing or boiling, was associated with an increased risk of T2D. Comparing cooking frequencies of ≥2 times/week with <1 time/month, HRs (95% CIs) of T2D were 1.32 (1.22, 1.43; *P* trend <0.001) for broiling, 1.27 (1.14, 1.41; *P* trend <0.001) for barbequing, 1.15 (1.04, 1.27; *P* trend = 0.02) for roasting, 0.89 (0.80, 0.98; *P* trend = 0.04) for pan-frying, and 0.99 (0.87, 1.12; *P* trend = 0.58) for stewing or boiling. After further

Table 2—HR (95% CIs) of T2D according to frequencies of cooking methods for red meats among participants who consumed regularly (≥2 servings/week) in the NHS

	Frequency of cooking methods for red meats				
	<1 time/month	1–3 times/month	1 time/week	≥2 times/week	*P* trend
Broiling					
Red meat intake (servings/day)	1.14 ± 0.6	1.17 ± 0.5	1.31 ± 0.5	1.46 ± 0.6	
Cases/person-years	1,779/419,537	1,858/395,712	1,612/319,030	957/166,691	
Model 1*	1.00	1.10 (1.03, 1.18)	1.19 (1.11, 1.27)	1.35 (1.25, 1.46)	<0.001
Model 2†	1.00	1.15 (1.07, 1.22)	1.21 (1.13, 1.29)	1.32 (1.22, 1.43)	<0.001
Model 3‡	1.00	1.15 (1.07, 1.22)	1.19 (1.11, 1.28)	1.29 (1.19, 1.40)	<0.001
Barbequing					
Red meat intake (servings/day)	1.14 ± 0.5	1.26 ± 0.5	1.36 ± 0.5	1.49 ± 0.6	
Cases/person-years	2,989/640,414	1,809/380,607	1,018/206,569	390/73,379	
Model 1	1.00	1.03 (0.98, 1.10)	1.09 (1.01, 1.17)	1.19 (1.07, 1.32)	<0.001
Model 2	1.00	1.11 (1.04, 1.18)	1.18 (1.09, 1.26)	1.27 (1.14, 1.41)	<0.001
Model 3	1.00	1.10 (1.03, 1.17)	1.16 (1.08, 1.25)	1.23 (1.11, 1.38)	<0.001
Roasting					
Red meat intake (servings/day)	0.98 ± 0.5	1.21 ± 0.5	1.37 ± 0.5	1.54 ± 0.6	
Cases/person-years	1,137/284,442	2,522/543,388	1,850/341,546	697/131,594	
Model 1	1.00	1.17 (1.09, 1.25)	1.36 (1.27, 1.47)	1.34 (1.22, 1.47)	<0.001
Model 2	1.00	1.11 (1.04, 1.20)	1.25 (1.15, 1.34)	1.15 (1.04, 1.27)	0.02
Model 3	1.00	1.10 (1.02, 1.18)	1.22 (1.13, 1.31)	1.11 (1.01, 1.23)	0.14
Pan-frying					
Red meat intake (servings/day)	1.10 ± 0.5	1.29 ± 0.5	1.44 ± 0.5	1.68 ± 0.6	
Cases/person-years	3,399/745,330	1,275/269,535	1,048/194,447	484/91,657	
Model 1	1.00	1.04 (0.97, 1.11)	1.18 (1.11, 1.27)	1.16 (1.06, 1.28)	<0.001
Model 2	1.00	0.96 (0.90, 1.02)	1.02 (0.95, 1.09)	0.89 (0.80, 0.98)	0.04
Model 3	1.00	0.94 (0.88, 1.01)	0.99 (0.93, 1.07)	0.85 (0.77, 0.94)	0.01
Stewing or boiling					
Red meat intake (servings/day)	1.14 ± 0.5	1.27 ± 0.5	1.40 ± 0.6	1.53 ± 0.6	
Cases/person-years	2,961/658,893	1,993/406,413	969/181,354	283/54,310	
Model 1	1.00	1.09 (1.03, 1.15)	1.19 (1.10, 1.28)	1.16 (1.02, 1.30)	0.001
Model 2	1.00	1.07 (1.01, 1.14)	1.11 (1.03, 1.20)	0.99 (0.87, 1.12)	0.58
Model 3	1.00	1.07 (1.01, 1.13)	1.09 (1.01, 1.18)	0.96 (0.85, 1.09)	0.98

*Model 1, adjusted for age. †Model 2, further adjusted for ethnicity (Caucasian, African American, Hispanic, or Asian), smoking status (never smoker, past smoker, or current smoker: 1–14, 15–24, or ≥25 cigarettes/day, or missing), BMI at age 18 (kg/m^2: <23, 23–24.9, 25–29.9, 30–34.9, ≥35, or missing), alcohol intake (g/day: 0, 0.1–4.9, 5.0–14.9, >15.0, or missing), multivitamin use (yes, no, or missing), family history of diabetes (yes or no), marital status (married, not married, or missing), menopausal status and postmenopausal hormone use (premenopause, postmenopause [never, former, or current hormone use], or missing), physical activity (METs/week: 0–2.9, 3–8.9, 9–17.9, 18–26.9, ≥27.0, or missing), total energy intake (kcal/day), and the AHEI without alcohol intake (quintiles). ‡Model 3, further adjusted for total red meat intake (quintiles).

adjusting for total red meat intake, the associations were attenuated slightly: comparing extreme cooking frequencies, the HRs (95% CIs) of T2D were 1.29 (1.19, 1.40; *P* trend <0.001) for broiling, 1.23 (1.11, 1.38; *P* trend <0.001) for barbequing, 1.11 (1.01, 1.23; *P* trend = 0.14) for roasting, 0.85 (0.77, 0.94; *P* trend = 0.01) for pan-frying, and 0.96 (0.85, 1.09; *P* trend = 0.98) for stewing or boiling (Table 2). Consistent results were observed when the frequency of each cooking method for red meats was treated as a continuous variable. For each once per week increment, the HRs (95% CIs) of T2D were 1.08 (1.05, 1.10; *P* < 0.001) for broiling, 1.07 (1.05, 1.12; *P* < 0.001) for barbequing, 1.03 (1.00, 1.06; *P* = 0.09) for roasting, 0.97 (0.92, 0.99; *P* = 0.01) for pan-frying, and 1.01 (0.97, 1.04; *P* = 0.79) for stewing or boiling. When cooking methods were further mutually adjusted, the results did not change materially: comparing the extreme groups, the HRs (95% CIs) of T2D were 1.23 (1.13, 1.33; *P* trend <0.001) for broiling, 1.18 (1.05, 1.31; *P* trend <0.001) for barbequing, 1.06 (0.98, 1.17; *P* trend = 0.22) for roasting, 0.87 (0.78, 0.96; *P* trend = 0.01) for pan-frying, and 0.92 (0.81, 1.05; *P* trend = 0.32) for stewing or boiling. No interaction was detected when analyses were stratified by total red meat intake.

Increased frequency of broiling and barbequing red meats, but not other cooking methods, was associated with greater weight gain during the follow-up period from 1986 to 1994 (Table 3). Comparing the highest frequency group to the lowest, the least squared means of weight changes ± SE were 3.46 ± 0.07 vs. 3.06 ± 0.05 kg for broiling (*P* trend <0.001), 3.44 ± 0.11 vs. 3.20 ± 0.04 kg for barbequing (*P* trend = 0.04), 3.34 ± 0.08 vs. 3.27 ± 0.06 kg for roasting (*P* trend = 0.12), 3.08 ± 0.10 vs. 3.34 ± 0.04 kg for pan-frying (*P* trend = 0.01), and 3.26 ± 0.13 vs. 3.32 ± 0.04 kg for stewing or boiling (*P* trend = 0.17) (Table 3). A similar pattern of associations was also observed

Table 3—Weight changes and risk of obesity according to frequency of cooking methods for red meats among participants who consumed regularly (≥2 servings/week) in the NHS (1986–1994)

	Frequencies of cooking methods for red meats				
	<1 time/month	1–3 times/month	1 time/week	≥2 times/week	*P* trend
Broiling					
Weight changes, kg					
Model 1*	3.06 ± 0.05	3.22 ± 0.05	3.30 ± 0.05	3.47 ± 0.07	<0.001
Model 2†	3.06 ± 0.05	3.22 ± 0.05	3.30 ± 0.05	3.46 ± 0.07	<0.001
HRs of obesity					
Model 1	1.00	1.11 (1.06, 1.17)	1.19 (1.13, 1.26)	1.38 (1.30, 1.47)	<0.001
Model 2	1.00	1.11 (1.05, 1.16)	1.18 (1.12, 1.24)	1.35 (1.27, 1.44)	<0.001
Barbequing					
Weight changes, kg					
Model 1	3.20 ± 0.04	3.20 ± 0.05	3.26 ± 0.07	3.45 ± 0.11	0.03
Model 2	3.20 ± 0.04	3.19 ± 0.05	3.26 ± 0.07	3.44 ± 0.11	0.04
HRs of obesity					
Model 1	1.00	1.03 (0.99, 1.08)	1.09 (1.03, 1.15)	1.35 (1.25, 1.46)	<0.001
Model 2	1.00	1.02 (0.98, 1.07)	1.06 (1.01, 1.13)	1.31 (1.21, 1.41)	<0.001
Roasting					
Weight changes, kg					
Model 1	3.26 ± 0.06	3.14 ± 0.04	3.27 ± 0.05	3.35 ± 0.08	0.09
Model 2	3.27 ± 0.06	3.14 ± 0.04	3.26 ± 0.05	3.34 ± 0.08	0.12
HRs of obesity					
Model 1	1.00	1.08 (1.02, 1.13)	1.13 (1.07, 1.20)	1.23 (1.14, 1.32)	<0.001
Model 2	1.00	1.06 (1.01, 1.12)	1.10 (1.04, 1.16)	1.18 (1.10, 1.27)	<0.001
Pan-frying					
Weight changes, kg					
Model 1	3.33 ± 0.04	3.16 ± 0.06	2.93 ± 0.07	3.10 ± 0.10	0.01
Model 2	3.34 ± 0.04	3.16 ± 0.06	2.92 ± 0.07	3.08 ± 0.10	0.01
HRs of obesity					
Model 1	1.00	1.00 (0.95, 1.05)	0.98 (0.93, 1.04)	1.04 (0.96, 1.12)	0.43
Model 2	1.00	0.98 (0.94, 1.03)	0.96 (0.91, 1.02)	1.00 (0.92, 1.08)	0.78
Stewing or boiling					
Weight changes, kg					
Model 1	3.32 ± 0.04	3.14 ± 0.05	3.05 ± 0.07	3.27 ± 0.13	0.20
Model 2	3.32 ± 0.04	3.14 ± 0.05	3.04 ± 0.07	3.26 ± 0.13	0.17
HRs obesity					
Model 1	1.00	0.94 (0.90, 0.98)	0.98 (0.92, 1.04)	1.11 (1.01, 1.22)	0.07
Model 2	1.00	0.93 (0.89, 0.98)	0.96 (0.91, 1.02)	1.08 (0.98, 1.19)	0.26

Data are least squared means ± SE calculated from the general linear model or HRs (95% CI) calculated from the Cox proportional hazard model. *Model 1, adjusted for baseline weight for weight change analysis or BMI at age 18 (kg/m^2: <23, 23–24.9, 25–29.9, 30–34.9, ≥35, or missing) when modeling the risk of obesity, age, ethnicity (Caucasian, African American, Hispanic, or Asian), smoking status (never smoked, past smoker, or currently smoke: 1–14, 15–24, or ≥25 cigarettes/day, or missing), alcohol intake (g/day: 0, 0.1–4.9, 5.0–14.9, >15.0, or missing), multivitamin use (yes, no, or missing), family history of diabetes (yes or no), marital status (married, not married, or missing), menopausal status and postmenopausal hormone use (premenopause, postmenopause [never, former, or current hormone use], or missing), physical activity (METs/week: 0–2.9, 3–8.9, 9–17.9, 18–26.9, ≥27.0, or missing), total energy intake (kcal/day), and the AHEI without alcohol intake (quintiles). †Model 2, further adjusted for total red meat intake (quintiles).

for the risk of developing obesity. Comparing extreme cooking frequencies, the HRs (95% CIs) of obesity were 1.35 (1.27, 1.44; *P* trend <0.001) for broiling, 1.31 (1.21, 1.41; *P* trend <0.001) for barbequing, 1.18 (1.10, 1.27; *P* trend <0.001) for roasting, 1.00 (0.92, 1.08; *P* trend = 0.78) for pan-frying, and 1.08 (0.98, 1.19; *P* trend = 0.26) for stewing or boiling.

After further adjusting for baseline BMI, the positive associations of cooking red meats using broiling and barbequing with the risk of T2D were attenuated but remained significant: comparing extreme cooking frequencies, the HRs (95% CIs) of T2D were 1.21 (1.11, 1.31; *P* trend <0.001) for broiling and 1.18 (1.06, 1.32; *P* trend <0.001) for barbequing. When further adjusting for time-varying BMI during follow-up, the associations were further attenuated: comparing extreme cooking frequencies, the HRs (95% CIs) of T2D were 1.08 (0.99, 1.17; *P* trend = 0.29) for broiling and 1.10 (0.98, 1.22; *P* trend = 0.07) for barbequing. The estimated mediation by BMI was 68.2% (95% CI 42.9, 86.0; $P < 0.001$) for broiling and 52.8% (95% CI 29.5, 74.9; $P < 0.001$) for barbequing. In substitution analysis, when substituting pan-frying and stewing/boiling for broiling or barbequing once a week, the estimated decrease in T2D risk was 9% (95% CI 7, 10) and 6% (95% CI 4, 7), respectively.

In sensitivity analyses, the results did not materially change when further adjusting for individual dietary factors (including the consumption of specific red meats) or geographic location in multivariate models (Supplementary Table 2). The results were similar when the participants with incident T2D diagnosed in the first 4 years were excluded or medication use was further adjusted (data not shown). The results were similar when the reference group was changed to the participants who were less frequent meat eaters (<2 servings/week) (Supplementary Table 3). We observed similar associations when we updated the frequencies of barbequing, roasting, and pan-frying during follow-up to account for the potential changes in cooking practices (Supplementary Table 4). In addition, after further adjusting for processed meat consumption or frequency of eating meals prepared at home, the results remained largely unchanged. For example, comparing the extreme groups with further adjustment of frequency of eating meals prepared at home, the HR (95% CI) was 1.29 (1.19, 1.40; *P* trend <0.001) for broiling, 1.24 (1.11, 1.38; *P* trend <0.001) for barbequing, 1.13 (1.02, 1.25, *P* trend = 0.07) for roasting, 0.86 (0.78, 0.95, *P* trend = 0.01) for pan-frying, and 0.98 (0.86, 1.11, *P* trend = 0.75) for stewing/boiling. Lastly, similar results were observed when we excluded the participants with missing covariates.

In analyses stratified by age (<60 years, ≥60 years), BMI (<25 kg/m^2, ≥25 kg/m^2), and current smoking status (yes, no), the results were mostly similar, although some of the associations were not statistically significant, probably as a result of diminished statistical power (Supplementary Table 5). No significant interactions of cooking methods with age, BMI, or current smoking status on T2D risk were observed, with the exception of interactions of broiling and pan-frying with age ($P_{interaction} < 0.05$). For broiling, the association was stronger among participants aged <60 years (comparing extreme quartiles, HR [95% CI]: 1.36 [1.17, 1.58] vs. 1.18 [1.07, 1.31] for participants aged ≥60 years). For pan-frying, an inverse association was primarily observed among participants aged ≥60 years (comparing extreme quartiles, HR [95% CI]: 0.79 [0.70, 0.89] vs. 1.05 [0.88, 1.24] for participants aged <60 years). In analyses stratified by vegetable oil use for baking, frying, or sautéing, the results were largely similar. For example, comparing extreme frequency of pan-frying red meats, the HR (95% CI) of T2D risk was 0.81 (0.72, 0.92; *P* trend = 0.002) for participants using vegetable oil and 0.92 (0.78, 1.08; *P* trend = 0.45) for participants not using vegetable oil ($P_{interaction}$ = 0.71). The associations for other cooking methods for red meats were largely similar between participants used or did not use vegetable oils for cooking, and no interactions were detected.

CONCLUSIONS

In this large prospective cohort study among U.S. women who consumed red meats regularly, a higher frequency of cooking red meats by broiling, barbequing, and roasting, but not by pan-frying and stewing or boiling, was associated with an increased risk of developing T2D during 26 years of follow-up. These associations were independent of red meat intake and established or potential diabetes risk factors. Moreover, a higher frequency of broiling or barbequing red meats was associated with greater weight gain and increased obesity risk. The positive associations of broiling and barbequing red meats with T2D risk appeared to be partially statistically accounted for by changes in BMI during follow-up, although the possibility of residual confounding by factors leading to both weight gain and a preference for using high-temperature cooking methods cannot be excluded.

Comparison With Other Studies

Previous studies have consistently documented that higher consumption of total red meats and processed red meats was associated with an increased risk of T2D (5,6). In line with these findings, our study also demonstrated a positive association between red meat intake and the risk of developing T2D when further adjusted by cooking methods. However, beyond red meat consumption, whether cooking methods, especially those of high-temperature or open-flame, might exert additional adverse effects on the risk of T2D has not been examined previously.

Some cross-sectional, case-control, and prospective studies have suggested that high-temperature cooking methods for red meats could increase the risk of developing certain cancers (31–33), although other studies reported null associations (10,34). Our study found that higher frequency of cooking red meats by broiling, barbequing, and roasting was linked to an increased risk of developing T2D, although the trend of the association between roasting and T2D risk did not reach statistical significance after further controlling for total red meat consumption. In contrast, pan-frying and stewing or boiling red meats were not associated with an increased risk of T2D. Interestingly, we observed an inverse association between pan-frying frequency and T2D risk, although interpreting this association must be done cautiously. The exact reason for the inverse association is unclear, but one of the possible explanations might be the use of healthy vegetable oils rich in MUFA for pan-frying red meats. Indeed, in the current study, we found that the correlation between the consumption of MUFA from plant sources and the frequency of pan-frying red meats was stronger than that for the other cooking methods, although the association for pan-frying was only slightly attenuated when further controlling for MUFA

consumption. In analyses stratified by vegetable oil use for baking, frying, or sautéing, the inverse association appeared to be stronger when participants reported using vegetable oil for cooking. Moreover, changes in fat profiles of red meats cooked by different methods could be another possible reason underlying the inverse association for pan-frying. For example, studies have suggested that pan-frying red meats with vegetable oils might lead to higher MUFA and polyunsaturated fatty acid retention than grilling (35). Nonetheless, evidence is still sparse regarding the joint effects of cooking methods and cooking oils on fatty acid profiles of cooked meats. Meanwhile, we cannot exclude the role of chance in this finding.

In addition to the findings regarding T2D risk, we also observed positive associations of high-temperature cooking methods with weight gain and obesity risk, and those changes in BMI statistically accounted for the positive associations between these cooking methods and diabetes risk. Overall, these findings suggested that high-temperature and/or open-flame cooking methods for red meats, especially broiling and barbequing, were potential independent risk factors for T2D. More prospective investigations are warranted to elucidate this novel link of red meat cooking methods with obesity and T2D.

Potential Biological Mechanisms

The exact mechanisms underlying the observed associations are unclear. Existing studies have demonstrated that some hazardous chemicals (e.g., heterocyclic aromatic amines and PAHs) are produced while cooking red meats at high temperature, such as broiling or barbequing over an open flame (36–38). Lee et al. (37) reported that the most important factor for the production of PAHs during grilling/barbequing was the smoke resulting from the incomplete combustion of fat dripping into the fire. The evidence from in vitro and in vivo studies suggested that PAHs, such as benzo[*a*]pyrene might induce proinflammatory cytokine production, reduce insulin secretion, and subsequently increase the risk of developing T2D (13,14). Several cross-sectional investigations in the National Health and Nutrition Examination Survey also demonstrated that urinary PAHs biomarkers, including 1- and 2-hydroxynapthol, 2-hydroxyphenanthrene, and summed low-molecular-weight PAH biomarkers, were associated with inflammation (e.g., serum C-reactive protein and total white blood cell count) and an increased prevalence of diabetes (15,16,39). In addition, accumulating evidence has suggested that high-temperature cooking of meats could induce the formation of advanced glycation end products, which has been linked to oxidative stress, inflammation, and insulin resistance in animal and human studies (12,17,40). Clearly, more evidence is needed to establish the mechanistic pathways linking high-temperature cooking methods with cardiometabolic health.

Strengths and Limitations

To our knowledge, this is the first study investigating the associations between cooking methods for red meats and T2D risk among women who consume red meats regularly. The strengths of our study included the prospective study design, large sample size, long follow-up period, repeated measurements of lifestyle and other dietary factors, and careful adjustments of a multitude of potential risk factors.

Several limitations should be considered as well. First, all participants in our study were female health professionals, and most of them were Caucasians. Although this homogeneity helps alleviate confounding by socioeconomic status to certain extent, it also limits the generalizability to other populations or ethnic groups. However, it is unlikely that the biological mechanisms underlying the observed associations would be different in other populations or ethnic groups.

Second, a questionnaire was used to examine red meat cooking methods at baseline, which might not represent the long-term cooking practice. Considering that the correlation of self-reported frequency of cooking methods over 10 years was modest in our study, which might be a result of changes in cooking behaviors over time and measurement errors in self-reported data, more prospective studies, ideally with repeated measurements of cooking methods for meats, are needed to confirm our findings. Given the prospective study design, changes in cooking behaviors during follow-up are unlikely related to the outcome ascertainment, and thus, the misclassification may more likely bias the associations toward the null, although we cannot rule out the possibility that measurement errors of confounders may bias the true association away from the null.

Third, although our FFQs were validated against multiple diet records and moderate-to-strong correlation coefficients were observed in validation studies (6,20), measurement errors in self-reported assessments could not be ruled out.

Fourth, we did not collect information on cooking time or doneness level for red meats. In addition, we did not obtain the information on specific oils used for cooking red meats, although we asked the participants about the type of fat used for baking, frying, or sautéing. The results did not materially change when we further controlled for these variables.

Fifth, we did not have enough sample to compare the T2D risk between vegetarians and meat eaters who used different cooking methods to cook red meats, although similar results were observed in a secondary analysis when the participants who were less frequent meat eaters were treated as the reference group.

Finally, residual or unmeasured confounding could not be entirely excluded in an observational study. For instance, it was possible that some specific foods might have been consumed together with red meats cooked in a certain way. In addition, although the observed associations were markedly attenuated after further adjustment for BMI, whether this observation reflected true mediation effects or residual confounding by other factors related to weight gain was unclear.

Implications of Findings

Our study provides the first evidence that high-temperature and/or open-flame cooking methods for red meats may be independently associated with the risk of developing T2D beyond red meat intake. These novel findings imply that both reducing the amount of red meat consumption and avoiding the use of high-temperature and/or open-flame cooking methods among meat eaters may potentially aid in T2D prevention, although further prospective studies are warranted to substantiate the relationship between meat cooking methods and metabolic risk and to elucidate the underlying mechanisms. In future studies, it would be interesting and important to explore whether high-temperature and/or open-flame cooking methods for other meats (such as fish and poultry) are also associated with T2D risk. Moreover, evidence is needed to compare the T2D risk between participants who do not consume red

meats and those who consume red meats cooked using these methods.

Conclusion

Our study suggests that beyond the risk of red meat intake, high-temperature and/or open-flame cooking methods for red meats, especially broiling and barbequing, are independently associated with a higher risk of developing T2D among regular meat eaters. Although these findings call for further replication in other prospective studies, using alternative cooking methods, as well as reducing red meat intake, is advisable for meat eaters.

Acknowledgments. The authors thank the participants for their dedication and contribution to the research.

Funding. This study was supported by National Institutes of Health grants CA-186107, CA-167552, and DK-058845. G.L. was supported by the International Postdoctoral Exchange Fellowship Program 2015 by the Office of China Postdoctoral Council. Q.S. was supported by National Institutes of Health grants HL-035464, ES-022981, and ES-021372.

Duality of Interest. D.M.E. has received consulting fees from The Culinary Institute of America, Nutrition Development Group, LLC, Infinitus Company Ltd., Barilla Center for Food and Nutrition, and Campus for Health, and has served on The Culinary Institute of America Scientific Advisory Committee and the Barilla Center for Food and Nutrition Advisory Board. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. G.L. and Q.S. participated in the study concept and design and statistical analysis and interpretation. G.L. drafted the manuscript. G.L., G.Z., F.B.H., W.C.W., D.M.E., and Q.S. participated in critical revision and approved the final version of the manuscript. G.L. and Q.S. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented in abstract form at the American Heart Association 2016 Scientific Sessions, New Orleans, LA, 12–16 November 2016.

References

1. Lam DW, LeRoith D. The worldwide diabetes epidemic. Curr Opin Endocrinol Diabetes Obes 2012;19:93–96
2. Arredondo A. Diabetes: a global challenge with high economic burden for public health systems and society. Am J Public Health 2013;103:e1–e2
3. Ardisson Korat AV, Willett WC, Hu FB. Diet, lifestyle, and genetic risk factors for type 2 diabetes: a review from the Nurses' Health Study, Nurses' Health Study 2, and Health Professionals' Follow-up Study. Curr Nutr Rep 2014;3:345–354
4. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care 2011;34:1249–1257
5. Pan A, Sun Q, Bernstein AM, Manson JE, Willett WC, Hu FB. Changes in red meat consumption and subsequent risk of type 2 diabetes mellitus: three cohorts of US men and women. JAMA Intern Med 2013;173:1328–1335
6. Pan A, Sun Q, Bernstein AM, et al. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. Am J Clin Nutr 2011;94:1088–1096
7. Micha R, Michas G, Mozaffarian D. Unprocessed red and processed meats and risk of coronary artery disease and type 2 diabetes–an updated review of the evidence. Curr Atheroscler Rep 2012;14:515–524
8. Daniel CR, Cross AJ, Koebnick C, Sinha R. Trends in meat consumption in the USA. Public Health Nutr 2011;14:575–583
9. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environ Mol Mutagen 2004;44:44–55
10. Di Maso M, Talamini R, Bosetti C, et al. Red meat and cancer risk in a network of case-control studies focusing on cooking practices. Ann Oncol 2013;24:3107–3112
11. Sinha R, Rothman N. Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer. Cancer Lett 1999;143:189–194
12. Uribarri J, Woodruff S, Goodman S, et al. Advanced glycation end products in foods and a practical guide to their reduction in the diet. J Am Diet Assoc 2010;110:911–916.e912
13. Khalil A, Villard PH, Dao MA, et al. Polycyclic aromatic hydrocarbons potentiate high-fat diet effects on intestinal inflammation. Toxicol Lett 2010;196:161–167
14. Pei XH, Nakanishi Y, Inoue H, Takayama K, Bai F, Hara N. Polycyclic aromatic hydrocarbons induce IL-8 expression through nuclear factor kappaB activation in A549 cell line. Cytokine 2002;19:236–241
15. Alshaarawy O, Zhu M, Ducatman AM, Conway B, Andrew ME. Urinary polycyclic aromatic hydrocarbon biomarkers and diabetes mellitus. Occup Environ Med 2014;71:437–441
16. Everett CJ, King DE, Player MS, Matheson EM, Post RE, Mainous AG 3rd. Association of urinary polycyclic aromatic hydrocarbons and serum C-reactive protein. Environ Res 2010;110:79–82
17. Vlassara H, Cai W, Tripp E, et al. Oral AGE restriction ameliorates insulin resistance in obese individuals with the metabolic syndrome: a randomised controlled trial. Diabetologia 2016;59:2181–2192
18. Hu FB, Manson JE, Stampfer MJ, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001;345:790–797
19. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142:1009–1018
20. Willett W. *Nutritional Epidemiology*. 2nd ed. New York, Oxford University Press, 1998
21. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65:1220S–1228S; discussion 1229S–1231S
22. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979;28:1039–1057
23. Liu G, Ding M, Chiuve SE, et al. Plasma levels of fatty acid-binding protein 4, retinol-binding protein 4, high-molecular-weight adiponectin, and cardiovascular mortality among men with type 2 diabetes: a 22-year prospective study. Arterioscler Thromb Vasc Biol 2016;36:2259–2267
24. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183–1197
25. Manson JE, Rimm EB, Stampfer MJ, et al. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet 1991;338:774–778
26. Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med 1989;8:551–561
27. Kuk JL, Saunders TJ, Davidson LE, Ross R. Age-related changes in total and regional fat distribution. Ageing Res Rev 2009;8:339–348
28. Lin DY, Fleming TR, De Gruttola V. Estimating the proportion of treatment effect explained by a surrogate marker. Stat Med 1997;16:1515–1527
29. Bernstein AM, Sun Q, Hu FB, Stampfer MJ, Manson JE, Willett WC. Major dietary protein sources and risk of coronary heart disease in women. Circulation 2010;122:876–883
30. Zong G, Eisenberg DM, Hu FB, Sun Q. Consumption of meals prepared at home and risk of type 2 diabetes: an analysis of two prospective cohort studies. PLoS Med 2016;13:e1002052
31. Sinha R, Chow WH, Kulldorff M, et al. Well-done, grilled red meat increases the risk of colorectal adenomas. Cancer Res 1999;59:4320–4324
32. John EM, Stern MC, Sinha R, Koo J. Meat consumption, cooking practices, meat mutagens, and risk of prostate cancer. Nutr Cancer 2011;63:525–537
33. Tasevska N, Sinha R, Kipnis V, et al. A prospective study of meat, cooking methods, meat mutagens, heme iron, and lung cancer risks. Am J Clin Nutr 2009;89:1884–1894
34. Tasevska N, Cross AJ, Dodd KW, Ziegler RG, Caporaso NE, Sinha R. No effect of meat, meat cooking preferences, meat mutagens or heme iron on lung cancer risk in the prostate, lung, colorectal and ovarian cancer screening trial. Int J Cancer 2011;128:402–411
35. Salcedo-Sandoval L, Cofrades S, Ruiz-Capillas C, Jiménez-Colmenero F. Effect of cooking method on the fatty acid content of reduced-fat and PUFA-enriched pork patties formulated with a konjac-based oil bulking system. Meat Sci 2014;98:795–803
36. Anderson KE, Sinha R, Kulldorff M, et al. Meat intake and cooking techniques: associations with pancreatic cancer. Mutat Res 2002;506-507:225–231
37. Lee JG, Kim SY, Moon JS, Kim SH, Kang DH, Yoon HJ. Effects of grilling procedures on levels of polycyclic aromatic hydrocarbons in grilled meats. Food Chem 2016;199:632–638
38. Friesen MD, Rothman N, Strickland PT. Concentration of 2-amino-1-methyl-6-phenylimidazo (4,5-b)pyridine (PhIP) in urine and alkali-hydrolyzed urine after consumption of charbroiled beef. Cancer Lett 2001;173:43–51
39. Alshaarawy O, Zhu M, Ducatman A, Conway B, Andrew ME. Polycyclic aromatic hydrocarbon biomarkers and serum markers of inflammation. A positive association that is more evident in men. Environ Res 2013;126:98–104
40. Vlassara H, Striker GE. AGE restriction in diabetes mellitus: a paradigm shift. Nat Rev Endocrinol 2011;7:526–539