Associations between red meat intake and biomarkers of inflammation and glucose metabolism in women[1–3]

Sylvia H Ley, Qi Sun, Walter C Willett, A Heather Eliassen, Kana Wu, An Pan, Fran Grodstein, and Frank B Hu

ABSTRACT

Background: Greater red meat intake is associated with an increased type 2 diabetes and cardiovascular disease risk. However, the relation of red meat intake to biomarkers of inflammation and glucose metabolism has not been investigated thoroughly.

Objective: We hypothesized that greater red meat intake would be associated with biomarkers of inflammation and glucose metabolism, which would be partly explained by body mass index (BMI).

Design: We analyzed cross-sectional data from diabetes-free female participants in the Nurses' Health Study (n = 3690). Multiple linear regression was conducted to assess the associations of total, unprocessed, and processed red meat intakes (quartile categories) with plasma C-reactive protein (CRP), ferritin, adiponectin, fasting insulin, and hemoglobin A_{1c} (Hb A_{1c}).

Results: Greater total, unprocessed, and processed red meat intakes were associated with higher plasma CRP, ferritin, fasting insulin, and Hb A_{1c} and lower adiponectin after adjustment for demographic information (P-trend $\leq$ 0.03 for all). Adiponectin was not associated with any type of red meat intake when further adjusted for medical and lifestyle factors. After adjustment for BMI, most of these associations with inflammatory and glucose metabolic biomarkers were substantially attenuated and no longer significant. BMI accounted for a statistically significant proportion of associations with CRP, Hb A_{1c}, and fasting insulin (P-contribution $\leq$ 0.02 for all) but not with ferritin. Substituting a serving of total red meat intake with alternative protein food in a combination of poultry, fish, legumes, and nuts was associated with significantly lower CRP ($\beta \pm$ SE: -0.106 ± 0.043), ferritin (-0.212 ± 0.075), Hb A_{1c} (-0.052 ± 0.015), and fasting insulin (-0.119 ± 0.036) (all $P \leq$ 0.02 for comparison of extreme quartiles for all).

Conclusions: Greater red meat intake is associated with unfavorable plasma concentrations of inflammatory and glucose metabolic biomarkers in diabetes-free women. BMI accounts for a significant proportion of the associations with these biomarkers, except for ferritin. Substituting red meat with another protein food is associated with a healthier biomarker profile of inflammatory and glucose metabolism. *Am J Clin Nutr* 2014;99:352–60.

INTRODUCTION

Red meat intake has been associated with the development of type 2 diabetes and cardiovascular disease (CVD)[4] (1–3). Furthermore, evidence indicates that greater red meat intake is associated with weight gain (4, 5). Although obesity-related inflammation and insulin resistance have been proposed to be involved in the observed association between red meat intake and progression of metabolic abnormalities (1, 3), the underlying mechanism is not entirely known. In a controlled feeding trial in 36 participants with elevated baseline LDL cholesterol (6), varying amounts of lean red meat interventions for 5 wk had similar beneficial effects on LDL cholesterol and similar adverse effects on HDL cholesterol. Inclusion of lean red meat in a heart-healthy diet to reduce CVD risk was suggested based on this evidence (6). However, the fat content of red meat may not be solely responsible for the adverse effects of red meat contributing to progression of metabolic abnormalities (7, 8). Other components, including heme iron, and other mediating pathways, may be involved.

Greater intake of total red meat has been associated with a higher plasma concentration of C-reactive protein (CRP)—an inflammatory biomarker (9, 10). However, the association was reported only with processed meat intake and not with total red meat intake in a Dutch study (11). The authors elaborated that BMI, not CRP, is likely the main contributor of the association between red meat intake and type 2 diabetes (11). In addition, ferritin, a biomarker of inflammation and body iron stores, has been associated with the risk of type 2 diabetes (12, 13). It has been speculated that red meat intake may influence glucose metabolism via iron metabolic pathways (14). Because red meat intake is associated with type 2 diabetes (1, 3), it is expected to be associated with biomarkers of abnormal glucose metabolism. However, previous investigations of red meat intake and glucose metabolic biomarkers have been inconsistent (9, 15).

[1] From the Departments of Nutrition (SHL, QS, WCW, KW, AP, and FBH) and Epidemiology (WCW, FG, and FBH), Harvard School of Public Health, and the Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School (QS, WCW, AHE, FG, and FBH), Boston, MA; and the Saw Swee Hock School of Public Health and Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Singapore (AP).

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[3] Address correspondence and reprint requests to SH Ley, Harvard School of Public Health, Department of Nutrition, 665 Huntington Avenue, Boston, MA 02115. E-mail: sylvia.ley@channing.harvard.edu.

[4] Abbreviations used: CRP, C-reactive protein; CVD, cardiovascular disease; FFQ, food-frequency questionnaire; Hb A_{1c}, hemoglobin A_{1c}; NHS, Nurses' Health Study.

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Thus, although the associations of red meat intake with biomarkers of inflammation and glucose metabolism have been investigated in several studies (9–11, 15), findings have been inconclusive. We therefore hypothesized that higher red meat intake (ie, total, unprocessed, and processed) would be associated with suboptimal concentrations of inflammatory and glucose metabolic biomarkers (ie, higher CRP, ferritin, fasting insulin, and hemoglobin Hb A_{1c} (Hb A_{1c}) and lower adiponectin) among diabetes-free women with detailed characterization of lifestyle and medical history available and that these associations would be in part mediated by body weight. Our secondary hypothesis was that substituting red meat with alternative protein food sources, which were previously related to reduced risk of type 2 diabetes and CVD (1, 16–18), would be associated with a healthier biomarker profile of inflammatory and glucose metabolism.

SUBJECTS AND METHODS

Study population

The Nurses' Health Study (NHS) is a prospective cohort study of 121,700 female registered nurses aged 30–55 y living across the United States at the baseline data collection in 1976. The participants have been followed biennially with questionnaires on medical history and lifestyle. The blood sample collections were conducted among 32,826 participants in 1989–1990. Among participants who provided a blood sample, several substudies have been implemented to examine the association of plasma biomarkers in relation to specific disease risk. For the current investigation, we included biomarker data from participants previously selected as controls for nested case-control analyses of type 2 diabetes, coronary heart disease, and stroke (n = 2939) and for an analysis of cognitive function (n = 930). Participants with self-reported prevalent diabetes at blood draw were excluded in additional to those with measured Hb A_{1c} $\geq$6.5%. A total of 3690 individuals with red meat intake and BMI data from the 1990 questionnaire and biomarker data from the 1989–1990 blood collection were included in the current analysis. Because different combinations of biomarkers were measured by substudies, the sample sizes for each biomarker varied: CRP (n = 2314), ferritin (n = 1115), transferrin receptor to ferritin ratio (n = 1110), Hb A_{1c} (n = 2474), fasting insulin (n = 1783), and adiponectin (n = 2562). The study protocol was approved by the Institutional Review Board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

Assessment of dietary intake

Dietary intake has been assessed by using a validated semiquantitative food-frequency questionnaire (FFQ) every 4 y as described in detail previously (19, 20). For the current analysis, we used a 131-item FFQ that was sent to participants in 1990. Participants were asked to report their usual intake of a standard portion of each food item during the past year. Nine response categories were available ranging from never or $<$1 time/mo to $\geq$6 times/d. The daily nutrient intake was estimated by using the Harvard Food Composition Database derived from the USDA nutrient data (21). Questionnaire items on unprocessed red meat consumption included "beef or lamb as main dish," "pork as main dish," "hamburger," and "beef, pork, or lamb as a sandwich or mixed dish." Items on processed red meat included "bacon," "hot dogs," and "sausage, salami, bologna, and other processed red meats." The standard serving sizes for these food items were 85 g for hamburger; 45 g for hot dogs; 27 g for sausage, salami, bologna, and other processed red meats; 13 g for bacon; 85 g for red meat served as a sandwich or mixed dish; and 140 g for red meat served as a main dish. The assessment of various red meat intakes by FFQ was correlated with intakes assessed by multiple dietary records. Pearson correlation coefficients corrected for within-person variation ranging between 0.38 for hamburgers and 0.70 for bacon (22). Other major protein food sources were assessed by using the FFQ and grouped into the following food items: *1*) poultry included chicken and turkey with and without skin; *2*) fish included dark- and light-fleshed fish and canned tuna; *3*) legumes included tofu, soybeans, string beans, peas, beans, and lentils; and *4*) nuts included peanuts, peanut butter, and other nuts.

Biochemical analysis

Blood sample collection was described in detail previously (13). Briefly, a phlebotomy kit and instructions were sent to participants willing to provide blood specimens in 1989–1990. Samples were returned by overnight mail with a frozen water bottle and were processed immediately on arrival. Aliquots were placed into cryotubes as plasma, buffy coat, and erythrocytes. All cryotubes were stored in the vapor phase of liquid nitrogen freezers at -130°C or less for up to 21 y before undergoing biochemical analysis. Quality-control samples were routinely frozen with study samples to monitor potential changes due to storage and to assess assay stability.

Plasma CRP concentrations were measured by using a high-sensitivity latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring; intraassay CV: 3.8%). Plasma ferritin and transferrin receptors were measured by using a particle-enhanced immunoturbidimetric assay by using the Hitachi 911 analyzer (Roche Diagnostics; CV: 3.8% and 8.4%, respectively). Hb A_{1c} was measured by immunoassay Hitachi 911 analyzer (Roche Diagnostics; CV: 3.8%). Plasma insulin was measured by using a radioimmunoassay specific for insulin with $<$1% cross-reactivity between insulin and its precursors (Linco Research; CV: 9.5%). Plasma adiponectin concentrations were measured by competitive radioimmunoassay (Linco Research; CV: 3.4%).

Assessment of other covariates

Information on medical history, lifestyle practices, and body weight was collected at baseline and has been updated every 2 y. BMI was calculated as weight (kg) self-reported in 1990 divided by the square of height (m) self-reported in 1976. Based on the previous validation study, self-reported weights have been correlated highly with measured weights (r = 0.97) (23).

We included other covariates derived from the questionnaires obtained closest to the time of blood collection. Information on cigarette smoking, physical activity, family history of diabetes, postmenopausal hormone use, and history of hypertension or hypercholesterolemia was assessed from these questionnaires. The validity of these assessments has been documented previously (24).

Statistical analysis

Distributions of continuous variables were assessed for normality, and natural log transformations of skewed biomarkers were used in subsequent analyses. Descriptive statistics for continuous variables were summarized as means ± SEs, and categorical variables were summarized by using proportions according to quartile categories of red meat intake from lowest to highest.

General linear models were used to evaluate associations of total, unprocessed, and processed red meat intake with plasma biomarker concentrations. Model 1 was adjusted for demographic information, including age at blood draw (continuous), ethnicity (white or nonwhite), and fasting status (≥8 h yes or no). Model 2 was additionally adjusted for medical history and lifestyle variables, including postmenopausal hormone use (yes or no), family history of diabetes (yes or no), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), smoking status (current, former, or never), physical activity [metabolic equivalent tasks (h)/wk, quartiles], Alternative Healthy Eating Index score (continuous), and total energy intake (continuous). Least-squares means of biomarkers were estimated in quartile categories of red meat intake, and linear trends were tested. The Alternative Healthy Eating Index was generated excluding red meat intake (25). Because preexisting medical histories that are risk factors for diabetes and CVD may modify the association of dietary intake with biomarkers of inflammation and glucose metabolism, interaction tests were performed by adding an interaction term of red meat intake (continuous) with a family history of diabetes (yes or no), comorbidity of hypertension (yes or no), or comorbidity of hypercholesterolemia (yes or no) with adjustment for covariates included in model 2. To assess the independent effects of unprocessed compared with processed red meat intake, both were included simultaneously in the multiple linear regression models. To account for variation in sample handing and laboratory drift between substudies, CRP, ferritin, transferrin receptor to ferritin ratio, fasting insulin, and adiponectin values were recalibrated as previously described in detail (26, 27). To evaluate the contribution of BMI (continuous) on the associations between red meat intake and biomarkers, SAS macro %MEDIATE (publicly available at http://www.hsph.harvard.edu/faculty/spiegelman/mediate.html) was applied by using 1-(βmediator model/βbase model) × 100 according to the methods described by Lin et al (28).

The effect of substituting a serving of red meat with a serving of another major protein food source, which has previously been shown to be related to a reduced risk of type 2 diabetes and CVD (1, 16–18), was estimated by including both as continuous variables in the same multiple regression model (1, 18). The differences in their β coefficients, variances, and covariance were used to estimate β coefficients ± SEs and P values of the substitution effect. Substituting protein food sources included *1*) poultry, *2*) fish, *3*) legumes, *4*) nuts, and *5*) a combination of poultry, fish, legumes, and nuts. For all statistical analyses, 2-sided $P < 0.05$ was considered to be statistically significant, except for interaction terms for which $P < 0.01$ was used to reduce the likelihood of false-positive interactions. All data analyses were performed by using SAS software, version 9.3 for UNIX (SAS Institute).

RESULTS

Characteristics of 3690 middle-aged and older diabetes-free study participants are presented according to total red meat consumption categories (**Table 1**) and according to unprocessed and processed red meat consumption categories (*see* Supplemental Table 1 under "Supplemental data" in the online issue). Age and physical activity were lower across increasing intake quartile categories of red meat intake, whereas BMI and total caloric intake were higher across categories. Intakes of total and unprocessed red meat were highly correlated with heme-iron intake (Spearman $r = 0.8$ for both), whereas processed red meat intake was moderately correlated ($r = 0.3$). Within these diabetes-free women, median daily consumptions were 54 g/d (5th, 95th percentiles: 6, 141) for total red meat, 47 g/d (6, 128) for unprocessed red meat, and 4 g/d (0, 20) for processed red meat.

Assessing associations between red meat intake and biomarkers of inflammation

Multiple regression models were constructed to assess whether red meat intake was associated with plasma biomarkers of inflammation (**Table 2**). Greater total, unprocessed, and processed red meat consumptions were associated with higher plasma CRP concentrations after adjustment for demographic, medical history, and lifestyle variables (model 2; P-trend ≤ 0.04 for all). These associations became attenuated and not significant after additional adjustment for continuous BMI (Table 2).

Greater total and unprocessed red meat intakes were significantly associated with higher plasma ferritin concentration after adjustment for demographic, medical history, and lifestyle variables (model 2; P-trend ≤ 0.01 for all). These associations remained significant with additional adjustment for BMI (Table 2). Processed red meat intake was associated with ferritin with adjustment for demographic variables (model 1; $P = 0.02$). However, the association became attenuated to nonsignificance after additional adjustment for medical history and lifestyle variables, and the null associations remained after further adjustment for BMI (Table 2). To account for potential subclinical iron deficits, the association of red meat intake with an index of transferrin receptor to ferritin ratio was assessed. We observed similar associations of red meat intake with transferrin receptor to ferritin ratio as with ferritin in this population of "healthy" women (Table 2); therefore, ferritin was used in subsequent analysis.

When model 2 was mutually adjusted for both unprocessed and processed red meat, the direction and significance of associations remained the same, except for the association between unprocessed red meat and CRP, which was attenuated to nonsignificance. No significant effect modification was observed for the associations between red meat intake and the biomarkers by a medical history of hypertension, history of hypercholesterolemia, or family history of diabetes.

Assessing associations between red meat intake and biomarkers of glucose metabolism

In **Table 3**, multiple regression models were constructed to assess whether red meat intake was associated with plasma biomarkers of glucose metabolism. Greater total, unprocessed, and processed red meat intakes were associated with higher concentrations of Hb A_{1c} and fasting plasma insulin after adjustment for

TABLE 1
Characteristics of 3690 middle-aged and older diabetes-free women in the Nurses' Health Study according to quartiles of total red meat consumption[1]

	Q1 (n = 921)	Q2 (n = 924)	Q3 (n = 922)	Q4 (n = 923)
Age at blood draw (y)	60.4 ± 0.2[2]	59.5 ± 0.2	58.7 ± 0.2	58.1 ± 0.2
BMI (kg/m^2)	24.7 ± 0.2	25.4 ± 0.2	26.2 ± 0.2	26.7 ± 0.2
Hypertension (%)	29.2	31.9	30.4	31.3
Hypercholesterolemia (%)	47.6	42.8	37.0	37.6
White (%)	97.5	98.4	98.2	99.2
Family history of diabetes (%)	25.7	23.4	25.9	27.6
Fasting status (%)	79.9	77.9	75.9	78.0
Postmenopausal hormone use (%)	48.2	46.7	43.8	40.1
Smoking status (%)				
Never	44.8	45.5	43.2	46.9
Former	43.5	40.7	41.4	35.6
Current	11.6	13.9	15.4	17.4
Physical activity (MET-h/wk)	18.3 ± 0.7	16.6 ± 0.7	15.3 ± 0.7	12.7 ± 0.7
Total energy intake (kcal/d)	1512 ± 15	1646 ± 15	1804 ± 15	2088 ± 15
Alternative Healthy Eating Index	53.1 ± 0.3	49.8 ± 0.3	47.6 ± 0.3	45.9 ± 0.3

[1] MET, metabolic equivalent task; Q, quartile.
[2] Mean ± SE (all such values).

demographic variables (model 1; P-trend $\leq$ 0.003 for all). When these models were further adjusted for medical history and lifestyle variables, the associations of unprocessed red meat with Hb A_{1c} became attenuated and not significant. With further adjustment for BMI, the associations of all types of red meat consumptions with Hb A_{1c} and fasting insulin were not significant (Table 3).

Plasma adiponectin was inversely associated with total, unprocessed, and processed red meat intake with adjustment for demographic variables (model 1; P-trend $\leq$ 0.01 for all), but these associations became attenuated to nonsignificance after additional adjustment for medical and lifestyle variables (Table 3). The null associations with adiponectin remained after additional adjustment for BMI (Table 3).

When model 2 was mutually adjusted for both unprocessed and processed red meat, the significant association remained between processed red meat and fasting insulin (P = 0.005), whereas the association between processed red meat and Hb A_{1c} became attenuated and borderline significant (P = 0.06). The associations of unprocessed and processed red meat with adiponectin remained nonsignificant with mutual adjustment. Because the association between red meat and adiponectin was null, adiponectin was not included in the subsequent analysis that assessed potential explanations for observed associations or estimating substitution effects of red meat with another protein source.

Assessing associations between red meat intake and biomarkers explained by BMI

The proportions of the associations between red meat intake and biomarkers explained by BMI were assessed (**Table 4**). BMI accounted for much of the associations of total, unprocessed, and processed red meat intake with CRP, Hb A_{1c}, and fasting insulin (P-contribution $\leq$ 0.02 for all). BMI did not account for the association between red meat intake and ferritin (Table 4).

Substitution effects of red meat with other major protein food groups

The substitution effects were estimated according to exchanging a serving of daily red meat intake with another major protein food source (**Table 5**). Substituting a serving of total red meat with a combination of alternative protein food source (ie, poultry, fish, legumes, or nuts) was associated with lower CRP, ferritin, Hb A_{1c}, and fasting insulin concentrations ($P \leq 0.02$ for all). Substitution with nuts was independently associated with these 4 biomarkers, and substitution with fish was also associated with lower ferritin (Table 5).

Substitution of unprocessed red meat with a combination of alternative protein food source was associated with lower ferritin, Hb A_{1c}, and fasting insulin concentrations ($P \leq 0.02$ for all). Substitution of unprocessed red meat with nuts and legumes was associated with lower ferritin and Hb A_{1c} ($P \leq 0.02$ for all), and substitution with fish was also associated with lower ferritin (P = 0.004). Substitution of processed red meat with a combination of alternative protein food or nuts independently was associated with lower CRP, Hb A_{1c}, and fasting insulin ($P \leq 0.03$ for all). After further adjustment of substitution models for BMI, these substitution effect associations with CRP and fasting insulin were attenuated and no longer significant. Substitution effects of total and unprocessed red meat with a combination of alternative protein sources remained significantly associated with ferritin and Hb A_{1c} after adjustment for BMI (*see* Supplemental Table 2 under "Supplemental data" in the online issue).

DISCUSSION

Greater total, unprocessed, and processed red meat intakes were associated with higher CRP, ferritin, fasting insulin, and Hb A_{1c} and lower adiponectin initially. However, adiponectin was no longer associated with red meat intake after adjustment for medical and lifestyle information. After further adjustment for BMI, most associations with these biomarkers

TABLE 2
Least-squares mean (95% CI) concentrations of biomarkers of inflammation according to red meat consumption (g) among middle-aged and older diabetes-free women in the Nurses' Health Study[1]

	Q1	Q2	Q3	Q4	*P*-linear trend[2]
C-reactive protein (mg/L)					
Total red meat					
n	573	576	580	585	
Median intake (g/d)[3]	19 (8, 27)	43 (38, 48)	67 (60, 75)	113 (98, 134)	
Model 1	1.98 (1.84, 2.13)	2.22 (2.07, 2.39)[4]	2.52 (2.35, 2.71)[4]	2.54 (2.37, 2.73)[4]	<0.0001
Model 2	2.10 (1.95, 2.26)	2.25 (2.10, 2.41)	2.48 (2.31, 2.66)[4]	2.42 (2.24, 2.61)[4]	0.005
Model 2 + BMI	2.19 (2.04, 2.35)	2.29 (2.14, 2.45)	2.41 (2.26, 2.57)	2.34 (2.18, 2.51)	0.16
Unprocessed red meat					
n	563	567	610	574	
Median intake (g/d)[3]	16 (6, 22)	38 (32, 44)	62 (53, 66)	104 (88, 128)	
Model 1	2.05 (1.90, 2.20)	2.24 (2.08, 2.41)	2.40 (2.24, 2.58)[4]	2.56 (2.38, 2.75)[4]	<0.0001
Model 2	2.17 (2.01, 2.34)	2.27 (2.12, 2.44)	2.37 (2.22, 2.54)	2.42 (2.24, 2.61)	0.04
Model 2 + BMI	2.24 (2.09, 2.41)	2.31 (2.16, 2.47)	2.32 (2.18, 2.48)	2.35 (2.19, 2.52)	0.38
Processed red meat					
n	542	609	590	573	
Median intake (g/d)[3]	0 (0, 0)	3 (2, 3)	6 (5, 6)	12 (9, 17)	
Model 1	2.03 (1.89, 2.19)	2.24 (2.09, 2.40)	2.38 (2.22, 2.55)[4]	2.60 (2.42, 2.80)[4]	<0.0001
Model 2	2.11 (1.96, 2.28)	2.27 (2.12, 2.43)	2.37 (2.22, 2.54)[4]	2.48 (2.30, 2.67)[4]	0.003
Model 2 + BMI	2.23 (2.08, 2.40)	2.30 (2.16, 2.45)	2.39 (2.24, 2.55)	2.30 (2.15, 2.47)	0.43
Ferritin (ng/mL)					
Total red meat					
n	264	266	292	293	
Median intake (g/d)[3]	19 (7, 27)	43 (38, 48)	67 (60, 74)	116 (99, 137)	
Model 1	36.1 (31.6, 41.3)	46.1 (40.4, 52.7)[4]	46.9 (41.4, 53.2)[4]	51.8 (45.6, 58.7)[4]	0.0002
Model 2	37.2 (32.3, 42.8)	46.1 (40.4, 52.6)[4]	46.2 (40.8, 52.4)[4]	51.2 (44.7, 58.6)[4]	0.005
Model 2 + BMI	37.4 (32.5, 43.1)	46.3 (40.6, 52.9)[4]	46.0 (40.5, 52.2)[4]	51.0 (44.6, 58.4)[4]	0.007
Unprocessed red meat					
n	265	262	301	287	
Median intake (g/d)[3]	16 (6, 26)	38 (32, 44)	62 (53, 68)	109 (92, 128)	
Model 1	37.1 (32.4, 42.4)	45.9 (40.2, 52.4)[4]	46.0 (40.6, 52.1)[4]	52.0 (45.8, 59.1)[4]	0.0007
Model 2	38.3 (33.4, 44.0)	45.9 (40.1, 52.4)	45.4 (40.2, 51.4)	51.1 (44.7, 58.6)[4]	0.01
Model 2 + BMI	38.5 (33.5, 44.2)	46.1 (40.3, 52.6)	45.2 (40.0, 51.2)	51.0 (44.5, 58.4)[4]	0.01
Processed red meat					
n	242	297	290	286	
Median intake (g/d)[3]	0 (0, 0)	2 (2, 3)	6 (5, 6)	13 (9, 17)	
Model 1	40.3 (35.0, 46.3)	40.8 (36.0, 46.2)	52.8 (46.5, 59.9)[4]	47.0 (41.3, 53.4)	0.02
Model 2	41.8 (36.2, 48.3)	41.7 (36.8, 47.2)	52.1 (45.9, 59.1)[4]	45.0 (39.5, 51.4)	0.18
Model 2 + BMI	42.2 (36.5, 48.8)	41.8 (36.9, 47.3)	52.2 (46.0, 59.2)[4]	44.5 (39.0, 50.9)	0.25
Transferrin receptor to ferritin ratio					
Total red meat					
n	264	263	290	293	
Median intake (g/d)[3]	19 (7, 27)	43 (38, 48)	67 (60, 74)	116 (99, 137)	
Model 1	69.3 (59.0, 81.4)	52.8 (45.0, 62.0)[4]	52.1 (44.7, 60.7)[4]	45.3 (38.9, 52.7)[4]	0.0003
Model 2	66.0 (55.7, 78.1)	52.8 (45.0, 61.9)	52.6 (45.2, 61.2)	47.0 (40.0, 55.3)[4]	0.01
Model 2 + BMI	66.0 (55.7, 78.2)	52.8 (45.0, 61.9)	52.5 (45.1, 61.2)	47.0 (39.9, 55.2)[4]	0.01
Unprocessed red meat					
n	264	260	299	287	
Median intake (g/d)[3]	16 (6, 26)	38 (32, 44)	62 (53, 68)	109 (92, 128)	
Model 1	67.7 (57.6, 79.5)	53.1 (45.2, 62.4)[4]	53.0 (45.6, 61.7)[4]	45.1 (38.7, 52.6)[4]	0.0008
Model 2	64.1 (54.3, 75.8)	53.2 (45.3, 62.5)	53.5 (46.1, 62.0)	46.9 (40.0, 55.1)[4]	0.02
Model 2 + BMI	64.2 (54.3, 75.8)	53.2 (45.3, 62.5)	53.4 (46.1, 62.0)	46.9 (39.8, 55.1)[4]	0.02
Processed red meat					
n	242	296	288	284	
Median intake (g/d)[3]	0 (0, 0)	3 (2, 3)	6 (5, 6)	13 (9, 18)	
Model 1	62.3 (52.6, 73.7)	58.6 (50.4, 68.2)	45.5 (39.0, 53.1)[4]	51.8 (44.4, 60.5)	0.03
Model 2	57.9 (48.7, 68.8)	57.3 (49.3, 66.5)	46.7 (40.1, 54.4)	55.0 (46.9, 64.6)	0.37
Model 2 + BMI	57.9 (48.7, 68.9)	57.3 (49.3, 66.5)	46.7 (40.1, 54.4)	55.0 (46.9, 64.6)	0.38

[1] General linear models were used. Model 1 was adjusted for age at blood draw (continuous), ethnicity (white or nonwhite), and fasting status (yes or no). Model 2 was adjusted for model 1 variables in addition to postmenopausal hormone use (yes or no), family history of diabetes (yes or no), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), smoking status (current, former, or never), physical activity (quartiles), Alternative Healthy Eating Index (continuous), and total energy intake (continuous). A continuous variable was used for BMI. Biomarker sample sizes vary: C-reactive protein (n = 2314), ferritin (n = 1115), and transferrin receptor to ferritin ratio (n = 1110). Q, quartile.

[2] *P*-linear trend was calculated by assigning median values to each quartile and treated as a continuous variable.

[3] Values are medians; IQRs in parentheses.

[4] Significantly different from Q1, $P < 0.05$.

TABLE 3
Least-squares mean (95% CI) concentrations of biomarkers of glucose metabolism according to red meat consumption (g) among middle-aged and older diabetes-free women in the Nurses' Health Study[1]

	Q1	Q2	Q3	Q4	*P*-linear trend[2]
Hemoglobin A_{1c} (%)					
Total red meat					
n	609	620	603	642	
Median intake (g/d)[3]	19 (8, 26)	43 (38, 48)	67 (60, 75)	113 (97, 134)	
Model 1	5.38 (5.36, 5.41)	5.42 (5.39, 5.44)	5.43 (5.40, 5.45)[4]	5.47 (5.44, 5.50)[4]	<0.0001
Model 2	5.40 (5.38, 5.43)	5.42 (5.40, 5.45)	5.42 (5.39, 5.45)	5.45 (5.42, 5.48)[4]	0.03
Model 2 + BMI	5.41 (5.38, 5.44)	5.43 (5.40, 5.45)	5.42 (5.39, 5.44)	5.44 (5.42, 5.47)	0.19
Unprocessed red meat					
n	603	599	641	631	
Median intake (g/d)[3]	16 (6, 22)	38 (32, 44)	62 (53, 66)	104 (88, 128)	
Model 1	5.39 (5.37, 5.42)	5.41 (5.38, 5.44)	5.43 (5.40, 5.45)	5.47 (5.44, 5.49)[4]	<0.0001
Model 2	5.41 (5.39, 5.44)	5.42 (5.39, 5.44)	5.42 (5.40, 5.44)	5.45 (5.42, 5.47)	0.11
Model 2 + BMI	5.42 (5.39, 5.45)	5.42 (5.39, 5.44)	5.42 (5.39, 5.44)	5.44 (5.42, 5.47)	0.30
Processed red meat					
n	577	638	624	635	
Median intake (g/d)[3]	0 (0, 0)	3 (2, 3)	6 (5, 6)	12 (9, 17)	
Model 1	5.39 (5.36, 5.41)	5.42 (5.39, 5.44)	5.42 (5.39, 5.44)	5.47 (5.45, 5.50)[4]	<0.0001
Model 2	5.41 (5.38, 5.43)	5.42 (5.40, 5.45)	5.41 (5.39, 5.44)	5.45 (5.43, 5.48)[4]	0.04
Model 2 + BMI	5.42 (5.39, 5.45)	5.43 (5.40, 5.45)	5.41 (5.39, 5.44)	5.44 (5.41, 5.46)	0.66
Fasting insulin (μU/mL)					
Total red meat					
n	459	446	451	427	
Median intake (g/d)[3]	21 (10, 27)	44 (38, 49)	67 (60, 74)	111 (97, 134)	
Model 1	4.59 (4.34, 4.87)	4.89 (4.61, 5.18)	4.88 (4.61, 5.17)	5.42 (5.11, 5.76)[4]	0.0002
Model 2	4.69 (4.41, 4.98)	4.88 (4.60, 5.16)	4.85 (4.58, 5.14)	5.36 (5.03, 5.71)[4]	0.009
Model 2 + BMI	4.89 (4.62, 5.17)	4.93 (4.67, 5.19)	4.75 (4.50, 5.00)	5.19 (4.89, 5.51)	0.31
Unprocessed red meat					
n	446	442	475	420	
Median intake (g/d)[3]	16 (6, 22)	38 (32, 44)	62 (53, 66)	103 (88, 128)	
Model 1	4.66 (4.40, 4.94)	4.93 (4.65, 5.23)	4.81 (4.55, 5.09)	5.38 (5.07, 5.72)[4]	0.003
Model 2	4.76 (4.48, 5.05)	4.92 (4.64, 5.21)	4.79 (4.53, 5.06)	5.31 (4.98, 5.66)[4]	0.046
Model 2 + BMI	4.91 (4.64, 5.19)	4.93 (4.68, 5.20)	4.75 (4.52, 5.00)	5.16 (4.86, 5.47)	0.44
Processed red meat					
n	429	475	437	442	
Median intake (g/d)[3]	0 (0, 0)	3 (2, 3)	6 (5, 6)	12 (9, 17)	
Model 1	4.58 (4.32, 4.86)	4.68 (4.42, 4.95)	5.25 (4.95, 5.57)[4]	5.26 (4.96, 5.58)[4]	<0.0001
Model 2	4.64 (4.37, 4.93)	4.70 (4.45, 4.97)	5.28 (4.98, 5.59)[4]	5.15 (4.85, 5.47)[4]	0.003
Model 2 + BMI	4.90 (4.64, 5.19)	4.74 (4.50, 4.98)	5.23 (4.96, 5.52)	4.88 (4.62, 5.16)	0.52
Adiponectin (μg/mL)					
Total red meat					
n	629	626	644	663	
Median intake (g/d)[3]	19 (9, 26)	43 (38, 48)	67 (60, 75)	113 (97, 136)	
Model 1	15.0 (14.4, 15.6)	14.1 (13.5, 14.6)[4]	14.1 (13.5, 14.6)[4]	13.7 (13.1, 14.3)[4]	0.003
Model 2	14.7 (14.0, 15.3)	14.0 (13.4, 14.5)	14.2 (13.6, 14.7)	14.0 (13.4, 14.6)	0.25
Model 2 + BMI	14.5 (13.9, 15.1)	13.9 (13.3, 14.5)	14.2 (13.7, 14.8)	14.2 (13.5, 14.8)	0.63
Unprocessed red meat					
n	620	621	672	649	
Median intake (g/d)[3]	16 (6, 24)	38 (32, 44)	62 (53, 68)	104 (88, 128)	
Model 1	15.0 (14.4, 15.6)	14.0 (13.4, 14.6)[4]	13.8 (13.3, 14.4)[4]	14.0 (13.4, 14.5)[4]	0.01
Model 2	14.7 (14.1, 15.3)	13.9 (13.3, 14.5)	13.9 (13.4, 14.5)	14.3 (13.6, 14.9)	0.37
Model 2 + BMI	14.6 (14.0, 15.2)	13.9 (13.3, 14.5)	14.0 (13.4, 14.5)	14.4 (13.7, 15.0)	0.69
Processed red meat					
n	604	666	639	653	
Median intake (g/d)[3]	0 (0, 0)	3 (2, 3)	6 (5, 6)	12 (9, 18)	
Model 1	15.0 (14.4, 15.6)	14.2 (13.6, 14.7)	13.8 (13.2, 14.4)[4]	13.9 (13.3, 14.5)[4]	0.007
Model 2	14.6 (14.0, 15.2)	14.1 (13.5, 14.7)	13.8 (13.3, 14.4)[4]	14.3 (13.7, 14.8)	0.35
Model 2 + BMI	14.4 (13.8, 15.0)	14.0 (13.5, 14.6)	13.9 (13.3, 14.4)	14.5 (13.9, 15.1)	0.97

[1] General linear models were used. Model 1 was adjusted for age at blood draw (continuous), ethnicity (white or nonwhite), and fasting status (yes or no). Model 2 was adjusted for model 1 variables in addition to postmenopausal hormone use (yes or no), family history of diabetes (yes or no), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), smoking status (current, former, or never), physical activity (quartiles), Alternative Healthy Eating Index (continuous), and total energy intake (continuous). A continuous variable was used for BMI. Biomarker sample sizes vary: hemoglobin A_{1c} (n = 2474), fasting insulin (n = 1783), adiponectin (n = 2562). Q, quartile.

[2] *P*-linear trend was calculated by assigning median values to each quartile and treated as a continuous variable.

[3] Values are medians; IQRs in parentheses.

[4] Significantly different from Q1, $P < 0.05$.

TABLE 4
Proportion of the association between red meat intake and biomarkers explained by BMI among women in the Nurses' Health Study[1]

	Exposure coefficient unadjusted for BMI (95% CI)	Exposure coefficient adjusted for BMI (95% CI)	Proportion explained by biomarker (95% CI)	*P*-contribution
C-reactive protein				
Total red meat	0.171 (0.061, 0.280)	0.079 (−0.025, 0.183)	54 (19, 88)	0.003
Unprocessed red meat	0.140 (0.031, 0.249)	0.056 (−0.048, 0.160)	60 (14, 106)	0.01
Processed red meat	0.198 (0.093, 0.302)	0.044 (−0.057, 0.145)	78 (37, 118)	0.0002
Ferritin				
Total red meat	0.328 (0.118, 0.537)	0.318 (0.107, 0.529)	3 (−3, 9)	0.30
Unprocessed red meat	0.300 (0.094, 0.506)	0.290 (0.083, 0.497)	3 (−3, 10)	0.29
Processed red meat	0.086 (−0.110, 0.282)	0.061 (−0.135, 0.257)	30 (−46, 105)	0.45
Hemoglobin A_{1c} (%)				
Total red meat	0.064 (0.026, 0.103)	0.044 (0.006, 0.082)	31 (10, 52)	0.004
Unprocessed red meat	0.049 (0.010, 0.087)	0.032 (−0.005, 0.070)	34 (4, 63)	0.02
Processed red meat	0.064 (0.026, 0.101)	0.027 (−0.011, 0.064)	58 (22, 94)	0.002
Fasting insulin				
Total red meat	0.149 (0.051, 0.247)	0.074 (−0.017, 0.165)	50 (18, 83)	0.003
Unprocessed red meat	0.125 (0.031, 0.218)	0.059 (−0.028, 0.146)	53 (13, 92)	0.009
Processed red meat	0.119 (0.026, 0.213)	0.001 (−0.087, 0.090)	99 (26, 173)	0.008

[1] SAS macro %MEDIATE was applied according to the methods described by Lin et al (28) for the comparison of extreme quartiles of red meat intake. The base model was adjusted for age at blood draw (continuous), ethnicity (white or nonwhite), fasting status (yes or no), postmenopausal hormone use (yes or no), family history of diabetes (yes or no), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), ever smoking (yes or no), physical activity (high or low), Alternative Healthy Eating Index (high or low), and total energy intake (continuous).

were substantially attenuated. BMI accounted for a statistically significant proportion of associations with these biomarkers, but not with ferritin. Substitution of a serving of total red meat intake with alternative protein food consumed in a combination of poultry, fish, legumes, and nuts was associated with a healthier biomarker profile of inflammation and glucose metabolism.

In a randomized crossover controlled feeding trial in 36 participants aged 39–97 y with a BMI of 19 to 36 and elevated baseline LDL cholesterol (6), 5-wk dietary treatment with 28, 113, or 153 g lean red meat/d had similar beneficial effects on LDL cholesterol and similar adverse effects on HDL cholesterol. The authors suggested including lean red meat in a heart-healthy diet to reduce CVD risk (6). However, the fat content of red meat may not be solely responsible for the adverse effects of red meat contributing to progression of metabolic abnormalities (7, 8, 29). In our isocaloric substitution analysis, exchanging a serving of red meat intake with alternative protein food consumed in a combination of poultry, fish, legumes, and nuts was

TABLE 5
Effect estimates for changes in biomarkers corresponding to substitution of 1 serving of red meat (total, unprocessed, or processed) with alternative protein foods[1]

	C-reactive protein		Ferritin		Hemoglobin A_{1c}		Fasting insulin	
	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*
Total red meat with								
Poultry, fish, legumes, or nuts	−0.106 ± 0.043	0.02	−0.212 ± 0.075	0.007	−0.052 ± 0.015	0.001	−0.119 ± 0.036	0.002
Nuts	−0.117 ± 0.044	0.01	−0.209 ± 0.077	0.01	−0.052 ± 0.016	0.001	−0.141 ± 0.037	0.0003
Legumes	−0.043 ± 0.070	0.33	−0.253 ± 0.126	0.05	−0.055 ± 0.024	0.03	−0.054 ± 0.059	0.25
Fish	−0.052 ± 0.080	0.32	−0.406 ± 0.154	0.01	−0.032 ± 0.027	0.20	−0.026 ± 0.062	0.36
Poultry	0.087 ± 0.144	0.33	0.054 ± 0.260	0.39	−0.042 ± 0.050	0.28	0.087 ± 0.123	0.31
Unprocessed red meat with								
Poultry, fish, legumes, or nuts	−0.109 ± 0.057	0.07	−0.299 ± 0.099	0.004	−0.064 ± 0.020	0.002	−0.110 ± 0.046	0.02
Nuts	−0.117 ± 0.058	0.05	−0.298 ± 0.101	0.005	−0.064 ± 0.020	0.003	−0.129 ± 0.047	0.009
Legumes	−0.050 ± 0.080	0.33	−0.354 ± 0.143	0.02	−0.069 ± 0.028	0.02	−0.056 ± 0.065	0.28
Fish	−0.070 ± 0.087	0.29	−0.494 ± 0.165	0.004	−0.049 ± 0.030	0.10	−0.036 ± 0.067	0.35
Poultry	0.104 ± 0.149	0.31	−0.030 ± 0.268	0.40	−0.047 ± 0.052	0.26	0.111 ± 0.126	0.27
Processed red meat with								
Poultry, fish, legumes, or nuts	−0.166 ± 0.074	0.03	−0.164 ± 0.141	0.20	−0.061 ± 0.027	0.03	−0.222 ± 0.069	0.002
Nuts	−0.178 ± 0.075	0.02	−0.168 ± 0.142	0.20	−0.062 ± 0.027	0.03	−0.245 ± 0.070	0.0009
Legumes	−0.107 ± 0.091	0.20	−0.215 ± 0.170	0.18	−0.067 ± 0.033	0.049	−0.164 ± 0.082	0.05
Fish	−0.129 ± 0.098	0.17	−0.398 ± 0.191	0.045	−0.051 ± 0.034	0.13	−0.147 ± 0.083	0.08
Poultry	0.040 ± 0.158	0.39	0.138 ± 0.290	0.36	−0.045 ± 0.056	0.29	−0.005 ± 0.139	0.40

[1] General linear models were adjusted for covariates, including age at blood draw (continuous), ethnicity (white or nonwhite), fasting status (yes or no), postmenopausal hormone use (yes or no), family history of diabetes (yes or no), history of hypertension (yes or no), history of hypercholesterolemia (yes or no), smoking status (current, former, or never), physical activity (quartiles), and total energy intake (continuous).

associated with a favorable biomarker profile of inflammatory and glucose metabolism. The current evidence among diabetes-free women adds to previous reports indicating that greater consumption of protein food sources alternative to red meat may reduce the risk of progression to type 2 diabetes and CVD (1, 18). This further supports the needs to consider other components in red meat and potential mediating pathways.

Because unprocessed and processed red meat differ in many ways (8), and processed meats have been more consistently associated with risk of type 2 diabetes (1, 30–34), the associations of specific types of red meat intake with inflammatory biomarkers are of interest. Similar to our results, greater total red meat intake has been associated with a higher plasma CRP concentration (9, 10). However, only processed meat intake was associated with higher CRP, whereas total red meat intake was not in a Dutch study (11). The authors suggested that increased body adiposity might be the main contributor of the association between red meat and diabetes and that CRP was indirectly related (11). In a parallel-designed trial, partially replacing energy from carbohydrate with protein from unprocessed lean red meat for 8 wk did not increase inflammatory marker concentrations (35). Although a longer duration of intervention is needed to confirm this evidence, it further suggests that the association between red meat and inflammatory biomarkers is likely to be indirect. Our data also support this notion because BMI explained a statistically significant proportion of the associations of red meat intake with CRP. In an investigation from 3 US cohort studies including the NHS, increased daily intake of unprocessed and processed red meat intakes were associated with 4-y weight gain (4). Excessive body adiposity is known as the single most important risk factor for type 2 diabetes (36). Therefore, a diet high in red meat may promote weight gain and subsequently contribute to developing type 2 diabetes. In our study, BMI was also a significant contributor to the association between red meat intake and biomarkers of glucose metabolism, which supports the involvement of adiposity in the association between red meat and diabetes. However, BMI did not account for the association between red meat intake and ferritin in the current study.

Ferritin, a biomarker of body iron stores, was previously associated with type 2 diabetes (12, 13). Several potential mechanisms may explain the associations of total and unprocessed red meat intake with ferritin and progression to diabetes. A bidirectional relation between iron and glucose metabolism has been proposed whereby iron affects glucose metabolism and glucose metabolism impinges on iron metabolic pathways (14). Insulin stimulates iron uptake by fat cells through the redistribution of transferrin receptors from intracellular membrane to the cell surface (37, 38) and is responsible for the increased ferritin synthesis in cultured rat glioma cells (39). Furthermore, iron accumulation in the liver interferes with insulin's action of inhibiting glucose production in the liver (40). Increased iron deposition may induce insulin resistance by inhibiting glucose uptake in fat and muscle tissues (14). However, red meat intake was not significantly associated with adiponectin in our study, which suggests that the association between red meat intake and progression to diabetes might not involve worsening of insulin sensitivity via the adiponectin-related insulin signaling pathway (41). Also, iron is a prooxidant that has been associated with increased oxidative stress, which may influence progression to diabetes (42). Our null association observed between processed red meat and ferritin might be explained by a lower quantity of processed red meat consumed per serving compared with unprocessed red meat.

The strength of the current investigation is an array of plasma and red blood cell biomarkers measured from a large number of diabetes-free women with detailed characterization of lifestyle and medical information available. The current study, however, had limitations. First, our study participants were female nurses of primarily European ancestry. The observed associations may not be generalizable in other populations with various cultural practices. Because variation in animal farming and meat preparation may introduce additional confounding (43–45), our results need to be replicated in other populations. Second, our plasma samples were stored for up to 21 y at −130°C, which might have introduced molecular degradation. However, the stability of these biomarkers after long-term storage has been documented (46, 47). Ferritin and transferrin concentrations changed minimally after 25 y of storage at −25°C (46). Although all blood sample processing and biochemical assays were performed under the internal NHS study protocol, project batch effects might have been introduced as a result of variation in sample handing and laboratory drift. We recalibrated biomarker concentrations with the use of previously published methods known to account for this (26), except for Hb A_{1c}, which was reported to maintain high reproducibility after long-term storage (Pearson $r = 0.97$) (47). Third, this was a cross-sectional observational investigation; therefore, we could not conclude a causal relation. In previous controlled feeding trials (6, 35), greater lean red meat intakes for 5 to 8 wk did not alter plasma concentrations of CRP, glucose, and/or insulin. Although it is unknown whether a longer duration of intervention accounting for mediating pathways will alter these results, the results of the current investigation should be interpreted with caution. It would be valuable for future studies to investigate longer-term changes in these biomarkers. Furthermore, we cannot confirm whether BMI is a true biological mediator or confounder. Additional research using longer-term randomized controlled trials accounting for potential confounders and mediators, including changes in body adiposity, are needed to further explore reported associations.

In conclusion, greater red meat intake is associated with unfavorable plasma concentrations of inflammatory and glucose metabolic biomarkers in diabetes-free women. Body adiposity statistically accounted for a significant proportion of the associations between red meat and these biomarkers, except for ferritin. Substitution of a serving of red meat with another protein food consumed in combination of poultry, fish, legumes, and nuts is associated with a healthier biomarker profile of inflammatory and glucose metabolism. Long-term controlled feeding studies are warranted to confirm the causality of these associations and potential mediating pathways to determine optimal preventative dietary strategies for progression to type 2 diabetes and CVD.

The authors' responsibilities were as follows—SHL, WCW, and FBH: designed the analysis; SHL and FBH: had primary responsibility for the final content; and SHL: analyzed and interpreted the data and wrote the manuscript. All authors read and approved the final manuscript and contributed to the revision of the manuscript critically for important intellectual content. None of the authors had a conflict of interest to disclose.

REFERENCES

1. Pan A, Bernstein AM, Schulze MB, Manson JE, Willett WC, Hu FB. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. Am J Clin Nutr 2011;94:1088–96.
2. The InterAct Consortium. Association between dietary meat consumption and incident type 2 diabetes: the EPIC-InterAct study. Diabetologia 2013;56:47–59.
3. Feskens EJ, Sluik D, Woudenbergh G. Meat consumption, diabetes, and its complications. Curr Diab Rep 2013;13:298–306.
4. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. N Engl J Med 2011;364:2392–404.
5. Vergnaud A-C, Norat T, Romaguera D, et al. Meat consumption and prospective weight change in participants of the EPIC-PANACEA study. Am J Clin Nutr 2010;92:398–407.
6. Roussell MA, Hill AM, Gaugler TL, West SG, Vanden Heuvel JP, Alaupovic P, Gillies PJ, Kris-Etherton PM. Beef in an Optimal Lean Diet study: effects on lipids, lipoproteins, and apolipoproteins. Am J Clin Nutr 2012;95:9–16.
7. Bernstein AM, Willett WC. Conclusions of the Optimal Lean Diet Study go beyond the data. Am J Clin Nutr 2012;95:1294–5.
8. 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;14:515–24.
9. Montonen J, Boeing H, Fritsche A, Schleicher E, Joost H-G, Schulze M, Steffen A, Pischon T. Consumption of red meat and whole-grain bread in relation to biomarkers of obesity, inflammation, glucose metabolism and oxidative stress. Eur J Nutr 2013;52:337–45.
10. Azadbakht L, Esmaillzadeh A. Red meat intake is associated with metabolic syndrome and the plasma C-reactive protein concentration in women. J Nutr 2009;139:335–9.
11. van Woudenbergh GJ, Kuijsten A, Tigcheler B, Sijbrands EJG, van Rooij FJA, Hofman A, Witteman JCM, Feskens EJM. Meat consumption and its association with C-reactive protein and incident type 2 diabetes: the Rotterdam Study. Diabetes Care 2012;35:1499–505.
12. Zhao Z, Li S, Li S, Liu G, Yan F, Ma X, Huang Z, Tian H. Body iron stores and heme-iron intake in relation to risk of type 2 diabetes: a systematic review and meta-analysis. PLoS ONE 2012;7:e41641.
13. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 2004;291:711–7.
14. Fernández-Real JM, López-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002;51:2348–54.
15. Babio N, Sorlí M, Bulló M, Basora J, Ibarrola-Jurado N, Fernández-Ballart J, Martínez-González MA, Serra-Majem L, González-Pérez R, Salas-Salvadó J. Association between red meat consumption and metabolic syndrome in a Mediterranean population at high cardiovascular risk: cross-sectional and 1-year follow-up assessment. Nutr Metab Cardiovasc Dis 2012;22(3):200-7.
16. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH, Willett WC. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr 1999;70:1001–8.
17. Jiang R, Manson JE, Stampfer MJ, Liu S, Willett WC, Hu FB. Nut and peanut butter consumption and risk of type 2 diabetes in women. JAMA 2002;288:2554–60.
18. 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;122:876–83.
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 W. Nutritional epidemiology. 2nd ed. New York, NY: Oxford University Press, 1998.
21. US Department of Agriculture. Composition of foods—raw, processed, and prepared, 1963-1992. Agricultural handbook no. 8 Series. Washington, DC: Government Printing Office, 1993.
22. 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.
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. Colditz GA, Martin P, Stampfer MJ, Willett WC, Sampson L, Rosner B, Hennekens CH, Speizer FE. Validation of questionnaire information on risk factors and disease outcomes in a prospective cohort study of women. Am J Epidemiol 1986;123:894–900.
25. McCullough ML, Feskanich D, Stampfer MJ, Giovannucci EL, Rimm EB, Hu FB, Spiegelman D, Hunter DJ, Colditz GA, Willett WC. Diet quality and major chronic disease risk in men and women: moving toward improved dietary guidance. Am J Clin Nutr 2002;76:1261–71.
26. Rosner B, Cook N, Portman R, Daniels S, Falkner B. Determination of blood pressure percentiles in normal-weight children: some methodological issues. Am J Epidemiol 2008;167:653–66.
27. Rice MS, Tworoger S, Rosner B, Pollak M, Hankinson S, Tamimi R. Insulin-like growth factor-1, insulin-like growth factor-binding protein-3, growth hormone, and mammographic density in the Nurses' Health Studies. Breast Cancer Res Treat 2012;136:805–12.
28. Lin DY, Fleming TR, De Gruttola V. Estimating the proportion of treatment effect explained by a surrogate marker. Stat Med 1997;16:1515–27.
29. Micha R, Michas G, Lajous M, Mozaffarian D. Processing of meats and cardiovascular risk: time to focus on preservatives. BMC Med 2013;11:136.
30. Montonen J, Jarvinen R, Heliovaara M, Reunanen A, Aromaa A, Knekt P. Food consumption and the incidence of type II diabetes mellitus. Eur J Clin Nutr 2005;59:441–8.
31. Villegas R, Shu XO, Gao YT, Yang G, Cai H, Li H, Zheng W. The association of meat intake and the risk of type 2 diabetes may be modified by body weight. Int J Med Sci 2006;3:152–9.
32. Fretts AM, Howard BV, McKnight B, Duncan GE, Beresford SA, Mete M, Eilat-Adar S, Zhang Y, Siscovick DS. Associations of processed meat and unprocessed red meat intake with incident diabetes: the Strong Heart Family Study. Am J Clin Nutr 2012;95:752–82.
33. Männistö S, Kontto J, Kataja-Tuomola M, Albanes D, Virtamo J. High processed meat consumption is a risk factor of type 2 diabetes in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study. Br J Nutr 2010;103:1817–22.
34. Steinbrecher A, Erber E, Grandinetti A, Kolonel L, Maskarinec G. Meat consumption and risk of type 2 diabetes: the Multiethnic Cohort. Public Health Nutr 2011;14:568–74.
35. Hodgson JM, Ward NC, Burke V, Beilin LJ, Puddey IB. Increased lean red meat intake does not elevate markers of oxidative stress and inflammation in humans. J Nutr 2007;137:363–7.
36. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001;345:790–7.
37. Davis RJ, Corvera S, Czech MP. Insulin stimulates cellular iron uptake and causes the redistribution of intracellular transferrin receptors to the plasma membrane. J Biol Chem 1986;261:8708–11.
38. Tanner LI, Lienhard GE. Localization of transferrin receptors and insulin-like growth factor II receptors in vesicles from 3T3-L1 adipocytes that contain intracellular glucose transporters. J Cell Biol 1989;108:1537–45.
39. Yokomori N, Iwasa Y, Aida K, Inoue M, Tawata M, Onaya T. Transcriptional regulation of ferritin messenger ribonucleic acid levels by insulin in cultured rat glioma cells. Endocrinology 1991;128:1474–80.
40. Niederau C, Berger M, Stremmel W, Starke A, Strohmeyer G, Ebert R, Siegel E, Creutzfeldt W. Hyperinsulinaemia in non-cirrhotic haemochromatosis: impaired hepatic insulin degradation? Diabetologia 1984;26:441–4.
41. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784–92.
42. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB. The role of iron in type 2 diabetes in humans. Biochim Biophys Acta 2009;1790:671–81.
43. Bogen KT, Keating GA. U.S. dietary exposures to heterocyclic amines. J Expo Anal Environ Epidemiol 2001;11:155–68.
44. Keating GA, Bogen KT. Estimates of heterocyclic amine intake in the US population. Journal of Chromatography B 2004;802:127–33.
45. Pourkhalili A, Mirlohi M, Rahimi E. Heme iron content in lamb meat is differentially altered upon boiling, grilling, or frying as assessed by four distinct analytical methods. ScientificWorldJournal 2013;2013:5.
46. Gislefoss RE, Grimsrud TK, Mørkrid L. Stability of selected serum proteins after long-term storage in the Janus Serum Bank. Clin Chem Lab Med 2009;47:596–603.
47. Selvin E, Coresh J, Jordahl J, Boland L, Steffes MW. Stability of haemoglobin A1c (HbA1c) measurements from frozen whole blood samples stored for over a decade. Diabet Med 2005;22:1726–30.