Branched Chain Amino Acid, Meat Intake and Risk of Type 2 Diabetes in the Women's Health Initiative

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List of abbreviations: BCAA (branch chain amino acid); BMI (body mass index); CT (clinical trial); DM (dietary modification); DM-C (dietary modification trial comparison); FFQ (food frequency questionnaire); NBS (Nutritional Biomarkers Study); OS (observational study); T2D (type 2 diabetes); WHI (Women's' Health Initiative Study);

Abstract

Knowledge regarding association of dietary branched chain amino acid (BCAA) and type 2 diabetes (T2D), and the contribution of BCAA from meat to the risk of T2D are scarce. We evaluated associations between dietary BCAA intake, meat intake, interaction between BCAA and meat intake and risk of T2D.Data analyses were performed for 74,155 participants aged 50−79 y at baseline from the Women's Health Initiative for up to 15 years of follow-up. We excluded from analysis participants with treated T2D, and factors potentially associated with T2D or missing covariate data. The BCAA and total meat intake was estimated from food frequency questionnaire (FFQ). Using Cox proportional hazards models assessed the relationship between BCAA intake, meat intake, and T2D, adjusting for confounders. A 20% increment in total BCAA intake (g/day and %energy) was associated with a 7% higher risk for T2D (HR: 1·07; 95% CI: 1·05-1·09). For total meat intake, a 20% increment was associated with a 4% higher risk of T2D (HR: 1·04; 95% CI: 1·03-1·05). The associations between BCAA intake and T2D were attenuated but remained significant after adjustment for total meat intake. These relations did not materially differ with or without adjustment for BMI. Our results suggest that dietary BCAAs and meat intake are positively associated with T2D among postmenopausal women. The association of BCAA and diabetes risk was attenuated but remained positive after adjustment for meat intake suggesting that BCAA intake in part but not in full is contributing to the association of meat with T2D risk.

INTRODUCTION

Dietary protein, comprised of amino acids, is an important modulator of glucose metabolism, insulin sensitivity, and, therefore, T2D [1]. Higher dietary protein intake has been associated with reduction in total energy intake and as a result may play a role in therapeutic care for individuals with obesity-related chronic disease, including T2D [2]. Contrary to this evidence, emerging data from epidemiological studies have suggested a positive association between higher protein and meat intake and incident T2D [2-7], despite protein's role in enhancing satiety and diet-induced thermogenesis. The association of protein intake and risk of T2D has been studied in two large populations that included thousands of incident T2D cases over 8-12 years of follow-up [6, 8]. In particular, in the Women's Health Initiative (WHI) [6] study, a ~20% increase in protein intake (corresponding to ~12 g protein and 3·4% energy from protein) was associated with a 5% higher risk of T2D. In the MALMO study [8], in 72,992 women from the Nurses' Health Study, 92,088 women from Nurses' Health Study II and 40,722 men from the Health Professionals Follow-up Study; participants in the highest quintiles of percentage of energy derived from total protein and animal protein (21·6 % of Energy) had 7% higher risks of T2D compared with those in the lowest quintiles (14·8 % of Energy).

Of note, a pooled analysis encompassing over four million person-years of follow-up and 15,580 cases of T2D suggested animal protein was associated with higher, whereas vegetable protein was associated with lower, risk of T2D [8]. These results suggest that protein source, in addition to quantity, may be related to the development of T2D. In fact, higher consumption of meat, particularly red meat, has been associated with a higher risk of T2D [9]. Overall, it is unclear whether it is the protein or other characteristics (i.e. nutrients, cooking methods) of protein-rich foods which explain the association with T2D.

One postulated explanation for the differential results is that higher animal protein intake may result in higher intake of branched chain amino acids (BCAA). BCAAs are essential amino acids that need to be obtained from diet, which can be found mostly in meat, chicken, fish, dairy products and eggs [10]. BCAAs (leucine, isoleucine and valine) have a critical role in promoting skeletal muscle mass as well as glucose uptake within the muscle [2, 11]. Circulating BCAAs are positively associated with insulin resistance, as measured by HOMA and Hemoglobin A1c (HbA1C) [12-14]. Recent data from the Nurses' Health Studies (I and II) and the Health Professionals Follow-up Study suggest total and animal protein are associated with higher risk of T2D [8]. What is less clear is whether BCAA may be

systemically elevated in response to an unfavorable and accelerated degradation to these important diet-derived compounds during a metabolically perturbed state rather than causal in insulin resistance development. The purpose of this analysis is to expand upon earlier findings in WHI relating protein intake to T2D risk by evaluating the associations of BCAA and meat intake and risk of T2D within the WHI, a large cohort of racially and ethnically diverse postmenopausal women, and the impact of jointly adjusting for BCAA and meat intake on the risk of T2D.

SUBJECTS AND METHODS

The WHI

The design and baseline descriptions of the WHI studies have been published [15-17]. Data for the present study were selected from the WHI clinical trials (CT) (Dietary Modification, Control Arm (DM-C), Hormone Therapy, and Calcium/Vitamin D), and WHI observational study (OS). Briefly, 68,132 and 93,676 generally healthy postmenopausal women aged 50–79 y were enrolled in the CT or the OS at 40 clinical centers across the United States between 1993 and 1998·

Incident T2D during follow-up was documented by self-report at each semiannual contact when participants were asked by self-administered medical history update questionnaire, "Since the date given on the front of this form, has a doctor prescribed any of the following pills or treatments?" Choices included "pills for diabetes" and "insulin shots for diabetes." Data from a WHI T2D confirmation study showed that prevalent and incident T2D were consistent (self-reported treated diabetes was concordant with the medication inventory in 79% of CT, and 77% in the OS participants) with medication inventories of oral agents or insulin. Demographic and risk exposure data, as well as data regarding family and medical history, were obtained by self-report using standardized questionnaires. WHI-certified staff took physical measurements using standardized equipment, including blood pressure, height and weight, and blood samples at the clinic visit [15].

Assessment of dietary intake

Dietary intake was estimated using the food frequency questionnaire (FFQ) designed for the WHI that was administered to all participants at baseline [18]. For participants in the dietary modification trial the baseline FFQ was used for screening eligibility in relation to fat intake and the intervention arm received support to change diet in a way that would alter meat and BCAA intake. As

such, in DM women only the control arm year 1 FFQ was used in this analysis of nutrient intake. Nutrient intake including BCAA content was derived from the USDA nutrient database [19]. To determine total BCAA intake we calculated the sum of isoleucine, leucine and valine consumption from the usual dietary intake.

Calibration of Dietary Protein Intake

As previously described [6], the WHI-Nutritional Biomarkers Study (WHI-NBS) sub-study developed biomarker-based calibration equations to reduce measurement error in self-reported intake of energy and protein by using linear regression models that predicted true intakes of energy and protein given the self-reported intake and data on study subject characteristics [6].

Baseline (as described above) FFQ energy, BCAAs, and BCAA density served as the uncalibrated baseline nutrient consumption estimates. For the calibrated energy and protein, logs of nutrient consumption were obtained directly from the biomarker measurements for the 276 DM-C women included in the WHI-NBS. For women not in the WHI-NBS, the WHI-NBS calibration equations were applied [6]. To estimate grams of calibrated BCAA, we multiplied the proportion of BCAA: total uncalibrated protein in grams by calibrated protein.

Analytic data set

We excluded from analysis participants with treated T2D, i.e., those who reported T2D at enrollment (n=6447) or during the first year of follow-up for the DM-C (n = 217) to correspond with the FFQ analysis time points. To align the participant characteristics of the DM-C and other participants for these analyses, we then applied the following DM trial exclusionary criteria to all participants in the analysis sample: breast or colorectal cancer ever (n=5,566), other cancer (except non-melanoma skin cancer) within 10 y preceding enrollment (n = 2,667), stroke or acute myocardial infarction 6 months before enrollment (n = 115), BMI <18 (n =774), hypertension (>200/>105 mm Hg) (n = 224), FFQ reported daily energy intake of <600 kcal or >5000 kcal) (n =4,706), ≥10 meals prepared away from home per week (n =4,749), special low-fiber diet (n = 568), special diet due to malabsorption (n = 510), and unintentional weight loss of >15 lb (6·8 kg) in the 6 months preceding baseline (n = 486) (**Supplemental figure 1**). Finally, 17,518 participants were excluded with missing model covariate data. After the above exclusion criteria were applied and the participants with complete data were selected, the analytic data set included 32,024 CT and 62,241 OS participants. The WHI and NBS

protocol and consent forms were approved by the Institutional Review Board for each participating institution and the Clinical Coordinating Center (Fred Hutchinson Cancer Research Center, Seattle, WA).

Statistical Analysis

We performed a secondary analysis using subsample of WHI CT and OS data. Demographic and health characteristics are reported by quintile of baseline total BCAA intake (sum of valine, leucine, and isoleucine), as estimated from the FFQ. Accompanying p-values for trend derived from either linear (continuous, ordinal demographics) or logistic (dichotomous) regression models with the demographic of interest as a function of linear trend over quintiles (quintile 1 = 1, quintile 2 = 2, etc.). Follow-up times started with the dietary modification comparison at year 1 or the OS at year 3 and continued to the earliest of treated diabetes, death, or loss to follow-up [(6)].

For analysis, BCAA intake was characterized as absolute (g/day), relative to energy intake (% energy/day), and relative to protein intake (% protein/day). Using Cox proportional hazards models, the relationship between BCAA intake (modelled continuously for a 20 percent increase and categorically by quintiles) and T2D is reported by hazard ratio (HR) and the corresponding 95% confidence intervals (CI). To be comparable with our prior analysis [(6)], the final model was adjusted for age, race/ ethnicity, BMI, education, income, history of CHD, current smoking, current alcohol use, physical activity, hypertension, family history of T2D, hormone use, glycemic load, glycemic index, and total energy intake. Models were additionally stratified within the model by the hormone therapy arms and 5-year age groups. Trend p-values across quintiles are computed from separate proportional hazards models with the outcome of interest as a function of linear trend over quintiles. Similarly, we assessed associations between meat intake and T2D, as categorized by My Pyramid Equivalents Database (MPED) categories. In sensitivity analyses, we further adjusted BCAA intake for total meat intake and omitted adjusting for BMI.

Results

Higher BCAA intake was associated with younger age, measures of socioeconomic status (white race, higher education and higher income per year), less likely to report current smoking, greater physical activity, and lower history of CHD (**Table 1**). Yet, higher BCAA intake was also associated with higher BMI and alcohol use, and higher glycemic load.

Geometric mean uncalibrated BCAA intake in our study was 10·9 g/d comprised of leucine (4·9 g/ d), isoleucine (2·8 g/ d) and valine (3·2 g/ d) (**Supplemental Table 1**). Major reported meat sources of BCAAs were red meat (1·2g/day) and poultry (0·78 g/day) in our study population (Supplemental Table 1). **Supplemental table 2** shows the quintile and median values for uncalibrated and calibrated BCAA variables, and the quintile and median values of major reported food sources for meat intake are presented in **supplemental table 3·**

A 20% increment in total BCAA intake (g/day and %energy) was associated with a 7% higher risk for T2D (HR: 1·07; 95% CI: 1·05, 1·09) (**Table 2**). Similarly, a 20% increment in intake (g/d and % of energy) for each of the BCAAs, including leucine, isoleucine and valine was associated with 7% higher risk of T2D with similar HR: 1·07 (95% CI: 1·05, 1·09). Inferences were similar when characterizing total BCAA intake as percent of protein intake, although isoleucine was more strongly associated with T2D risk than leucine or valine (**Table 2**). For uncalibrated protein, model estimates were similar with and without adjustment for BMI (**Table 2 and Supplemental table 4**), while with calibrated protein the strength of the association was slightly higher with adjustment for BMI (**supplemental table 5 and supplemental table 6**). Biomarker-calibration of energy and protein did not appreciably affect the results (**Supplemental table 5**).

Likewise, in categorical analyses (**Table 2**), women reporting intake in the highest quintile of uncalibrated BCAA (grams/day) had a 35% greater risk of T2D (HR 1·35, 95% CI 1·21, 1·50) compared to those in the lowest quintile of intake. When the highest quintiles of uncalibrated protein expressed as %energy/day (HR 1·21 95% CI 1·13, 1·29) or as a percentage of total protein intake (HR 1·08, 95% CI 1·01, 1·14) were compared to the lowest quintiles, the strength of the association was attenuated, but remained significant (**Table 2**).

For total meat intake, a 20% increment increase was associated with a 4% higher risk of T2D (HR: 1·04; 95% CI: 1·03, 1·05) (**Table 3**). Risk varied little across animal protein sources, although it was lower in relation to fish and poultry intake compared to red meat. A 20% increment increase in intake of red meat, fish, poultry and processed meat was associated with 3%, 2%, 1%, and 3% higher risk of T2D, respectively (**Table 3**). In models jointly adjusted for BCAA and total meat intake, the associations between BCAA intake (grams) and T2D were attenuated but retained significance (**Table 2, and supplemental table 7**).

Discussion

This study demonstrated that higher BCAA intake, with and without biomarker calibration of protein exposure estimates, was associated with higher risk of T2D in the WHI OS and CT population. Our results suggest that increased intake of dietary BCAAs may contribute to the risk of future T2D in postmenopausal women. In addition to the prospective association with risk of T2D, our findings showed that total meat intake was associated with increased risk of T2D in postmenopausal women. The association of meat intake with T2D risk was attenuated in models jointly adjusted for BCAA intake, but remained significant. These relations did not materially change with or without adjustment for BMI.

Absolute intakes of total BCAAs in WHI women were similar to those of previous US cohorts (medians across quintiles 1 through 5 were 10·1 -15·1 g/d in the Nurses' Health Study I, 12·0-18·0 g/day in the Nurses' Health Study II, and 12·6-18·8 for in the Health Professionals Follow-up Study ~12·6) [20]. To provide perspective on how these ranges relate to dietary intake, four ounces of ground beef contain 4·0 g BCAA and four chicken tenders contain 1·8g BCAA.

Studies that have examined the association of dietary BCAA consumption with T2D are scarce. Our results corroborate those of the recent study by Zheng et al. [20] which included three large, prospective cohorts of US men and women, and reported that long-term consumption of BCAAs, individually or in sum, was associated with increased risk of incident T2D. These associations were independent of traditional diabetes risk factors, including BMI.

However, in a Japanese cohort (n=13,525), BCAA as a proportion of total protein (17·23% and 17·32% in men and women, respectively) were inversely associated with T2D in women (HR 0·57, 95% CI 0·36 to 0·90 comparing 3rd to 1st tertile), but were not significantly associated with T2D in men [11]. This could be because of the population age (35 years and older) compared to WHI (50-79 years), the top two sources of BCAA in this population were cereals/potatoes and starches and fish/shellfish, and the sensitivity and specificity of the T2D ascertainment by self-report compared to HbA1c was 57·4% and 96·5%, respectively [2, 11].

Some studies of plasma BCAA levels have found associations with insulin resistance, which may explain the adverse associations of BCAA intake with development of T2D [21, 22]. It has been shown that circulating branched-chain and aromatic amino acid levels predict insulin resistance index

over 6 years in normoglycemic young adult individuals even when accounting for baseline insulin resistance [21]. In the Framingham Offspring Study, higher plasma BCAA levels were correlated positively with fasting insulin levels and predicted the future risk of T2D, a finding which was more pronounced in obese individuals [22]. The positive association of plasma BCAA and insulin resistance has also been found in studies across different settings [13, 23]. A review by Newgard et al. [23] concluded that BCAA and related metabolites are positively associated with insulin resistance and T2D. In a metabolomics study, plasma samples from obese and insulin-resistant versus lean and insulin sensitive subjects were analyzed [14], showing from principal components analysis that most of the variance in the data were explained by BCAA, which had the strongest association with insulin sensitivity, even more than the lipid profiles.

Several mechanisms may explain the relationship between BCAA and T2D. Amino acids are thought to play a significant role in the pathogenesis of insulin resistance, acting as gluconeogenic precursors and stimulating hexosamine biosynthesis [22]. Moreover, amino acid signaling is integrated by the mammalian target of rapamycin, a nutrient sensor that operates a negative feedback loop toward insulin receptor substrate 1 signaling, promoting insulin resistance for glucose metabolism [24]. Glucose utilization may also be impaired due to the inhibitory effect of amino acids on glucose transport and phosphorylation [24]. Furthermore, amino acids affect glucose metabolism via stimulation of insulin and glucagon secretion and by serving as substrates for gluconeogenesis [5]. Infusion of amino acids to raise plasma amino acid concentrations induced insulin resistance in skeletal muscle and stimulated endogenous glucose production in healthy men [25].

We also observed that higher meat intake increased the risk of T2D by 4% in postmenopausal women, which is supported by a meta-analysis by Feskens and colleagues [4]. The increased risk of T2D associated with higher meat consumption might be explained in part by meat's contribution to BCAA and/or possibly increasing the heme iron load. The BCAAs and tyrosine and phenylalanine are mainly present in meat and dairy products, although available in many protein-rich foods [26]. For this analysis, we focused on meat, rather than dairy, sources of BCAA's, as we were interested in whether factors other than BCAA's explained the observed positive association between BCAA with diabetes risk, and dairy has a weakly protective association with T2D. The earlier experimental elevations of plasma amino acids by infusion, resulted in impaired insulin-stimulated glucose disposal and insulin-mediated suppression of (hepatic) glucose production [27]. However, per 100 g of total meat, relative

risk of T2D increased 15% for (unprocessed) red meat, 13% for poultry, and 4% for processed meat. Furthermore, higher meat intakes may contribute to increased heme iron load, and iron overload is associated with increased T2D risk [26].

The current study has important strengths including its prospective design, large sample size, and long follow-up. Although T2D status, both treated and incident, was assessed by self-report without adjudication or confirmation by clinical measures, the WHI self-report data for T2D have been found to be highly consistent with medication use inventories provided by participants [28]owe. It is not known whether circulating BCAAs are causes/mediators of insulin resistance or by-products of the associated metabolic dysfunction. Thus, the present study explored the relation of dietary intake of BCAAs with T2D, but cannot inform on causality.

Some limitations of the study need to be addressed. Diabetes was assessed using self-report, which could result in misclassification error. However, a validation study in the WHI demonstrated high concordance between self-reported treated diabetes and medication inventories [28]. Although we controlled for several covariates, measurement error in these constructs may result in residual confounding; women with higher BCAA intake had higher meat and alcohol intake, were more educated, had higher income, and higher glycemic load. The role of other BCAA sources, such as dairy, will be considered in work examining the role of dietary protein sources on diabetes risk within WHI. The response to dietary protein content may be dependent on an individual's degree of underlying insulin resistance, determined by adiposity and BMI, but in our investigation adjusting for BMI did not materially changed the associations. Calibration using urinary nitrogen as a biomarker of total protein intake was incorporated into the analysis and did not materially change effect estimates in this analysis, but we did not have corresponding biomarkers of branched chain amino acid intake or meat intake. The nutrient database relied on estimation for 26-50% of dietary amino acids, e.g., similar foods or imputation. The BCAAs from meat were not able to be separated from total BCAAs. Because of the observational design, conclusions regarding causality cannot be drawn. Also, this study included postmenopausal women aged 50−79 years old from 40 designated clinical sites across, but not representative of, the U.S. and therefore caution should be taken while generalizing these results to other populations. Our findings indicated that higher BCAA and meat intakes were associated with higher risk of T2D. Thus, it may be important to further consider dietary protein sources in dietary recommendations to prevent T2D.

Conclusion

In a secondary analysis among a large cohort of postmenopausal women BCAA and meat intake were associated with higher risk for T2D. The elevation in risk was very modest, but helps to inform on future guidance for postmenopausal women at elevated risk for T2D.

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Conflict of Interest

Dr. Phillips declares that there is no duality of interest associated with this manuscript. With regard to potential conflicts of interest, within the past several years, Dr. Phillips has served on Scientific Advisory Boards for Boehringer Ingelheim, Janssen, and the Profil Institute for Clinical Research, and has or had research support from Merck, Amylin, Eli Lilly, Novo Nordisk, Sanofi, PhaseBio, Roche, Abbvie, Vascular Pharmaceuticals, Janssen, Glaxo SmithKline, and the Cystic Fibrosis Foundation. In the past, he was a speaker for Novartis and Merck, but not for the last five years. Dr. Phillips is also supported in part by the Veterans Health Administration (VA). This work is not intended to reflect the official opinion of the VA or the U.S. government; TBD for others**.** Masoud Isanejad, Andrea LaCroix,

Cynthia Thomson, Lesley Tinker, Joseph C Larson, Qibin Qi, Lihong Qi, Rhonda M Cooper-DeHoff, Ross L Prentice, and Jeannette M. Beasley had no potential conflict of interest to declare

Authorship

The authors' responsibilities were as follows—LFT, GES, BVH, YH, and RLP: designed the research; LFT, GES, BVH, MLN, YMR, KM, CE, LP, and RLP: conducted the research; JCL: analyzed the data; MI, JMB, AL, CT, LT,JL, QQ,LQ, RMC, LP, and RLP: wrote the manuscript; and MI and JMB: had primary responsibility for the final content.

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Table 1 Characteristics at time of protein measurement[1] by quintile of uncalibrated total branched-chain amino acid intake (g/day) *

Characteristic	n=18·971 Q1: < 7·7		n=18·629 Q2: 7·7 – <10·0		n=19·055 Q3: 10·0 - <12·3		n=18·446 Q4: 12·3 - <15·3		n=19·164 Q5: ≥ 15·3		P-trend †
	Means	SD	Means	SD	Means	SD	Means	SD	Means	SD	
Age· mean	64·3	7·3	64·1	7·2	63·9	7·1	63.8	7·1	63·4	7·1	<0·001
Ethnicity §											
White ‡	14719	77·6	15853	85·1	16832	88·3	16574	89·9	16907	88·2	0·001
Black	2165	11·4	1264	6·8	1025	5·4	520	4·4	995	5·2	
Hispanic	860	4·5	634	3·4	501	2·6	468	2·5	623	3·3	
Other / Unknown	1227	6·5	878	4·7	697	3·7	584	3·2	639	3·3	
Education §											<0·001
≤ High school / GED	4865	25·6	4086	21·9	3667	19·2	3512	19·0	3468	18·1	
School after high school	7408	39·0	7061	37·9	7036	36·9	6650	36·1	7070	36·9	
College degree or higher	6698	35·3	7482	40·2	8352	43·8	8284	44·9	8626	45·0	
Income §											<0·001
≤ $20·000	3601	19·0	2735	14·7	2497	13·1	2388	12·9	2777	14·5	
$20·000 - $49·999	8592	45·3	8311	44·6	8412	44·1	8255	44·8	8697	45·4	
≥ $50·000	6778	35·7	7583	40·7	8146	42·7	7803	42·3	7690	40·1	
Body Mass Index· kg/m^2 §											<0·001
Underweight (<18·5)	107	0·6	86	0·5	78	0·4	57	0·3	57	0·3	
Normal (18·5 - 24·9)	8293	43·7	7616	40·9	7400	38·8	6641	36·0	5600	29·2	
Overweight (25·0 – 29·9)	6422	33·9	6640	35·6	6843	35·9	6541	35·5	6582	34·3	
Obese (≥ 30·0)	4149	21·9	4287	23·0	7434	24·8	5207	28·2	692	36·1	

Current smoker §	1523	8·0	1266	6·8	1205	6·3	1124	6·1	1194	6·2	<0·001
Current alcohol use §	12550	66·2	13362	71·7	14104	74·0	13640	73·9	13753	71·8	<0·001
Hormone therapy use §											<0·001
Never	8114	42·8	7627	240·9	7771	40·8	7719	41·8	7985	41·7	
Past	2985	15·7	2935	15·8	2908	15·3	2780	15·1	2957	15·4	
Current	7872	41·5	8067	43·3	8376	44·0	7947	43·1	8222	42·9	
History of CHD §	582	3·1	523	2·8	501	2·6	427	2·3	442	2·3	<0·001
History of hypertension §	8346	44·0	7875	42·3	7995	42·0	7782	42·2	8404	43·9	0·770
Physical activity (METs/wk)	12·5	14·0	13·3	14·8	13·4	13·8	136·6	14·0	13·6	14·2	<0·001
Total energy intake (kcal)	976·1	238·1	1276·1	252·4	1515·0	282·3	1780·5	322·5	2352·4	574·0	<0·001
Glycemic Index	52·8	3·9	52·4	3·7	52·2	3·6	51·9	3·6	51·5	3·8	<0·001
Glycemic load	65·8	23·0	81·0	25·0	93·9	26·9	107·8	30·4	136·1	42·2	<0·001
Total meat (servings)	1·7	0·9	2·5	1.1	3.0	1.3	3.7	1·6	5·0	2·3	<0·001
Red meat (servings)	0·7	0·5	1·0	0·7	1·2	0·9	1·5	1·0	2·1	1.5	<0·001
Fish (servings)	0·3	0·3	0·5	0·4	0·5	0·4	0·6	0·5	0·8	0·6	<0·001
Poultry (servings)	0·4	0·4	0·6	0·5	0·8	0·6	0·9	0·6	1.2	0·8	<0·001
Processed meat (servings)	0·2	0·2	0·3	0·3	0·3	0·3	0·4	0·4	0·6	0·5	<0·001

* Baseline (or year 1 for DM trial participants)
† trend p-value from a linear (continuous and ordinal characteristics) or logistic (dichotomous characteristics) regression model with the characteristic of interest as a function of linear trend over the medians of each BCAA quintile.
‡ p-value trend is based on trend of BCAA quintiles on white ethnicity (yes/no)
§ frequency ± % (all such values)

5Geometric means and standard deviations are presented, with trend tested over log transformed data

Table 2 Hazard ratios for the risk of diabetes by quintile of uncalibrated branched-chain amino acid (BCAA) intake

	Intake (grams)				Percent caloric intake				Percent protein intake			
	Events	Ann%	HR (95% CI) *	p-value †	Events	Ann%	HR (95% CI)	P	Events	Ann%	HR (95% CI)	P-value
Total BCAA				<0·001				<0·001				0·02
Q1	2043	0·88	1·00 (ref)		2083	0·91	1·00 (ref)		2100	0·88	1·00 (ref)	
Q2 vs. Q1	2023	0·86	1·04 (0·97, 1·12)		2186	0·88	1·00 (0·94, 1·06)		2246	0·99	1·05 (0·98, 1·11)	
Q3 vs. Q1	2186	0·90	1·10 (1·02, 1·19)		2209	0·92	1·05 (0·99, 1·12)		2388	0·98	1·05 (0·99, 1·11)	
Q4 vs. Q1	2242	0·95	1·17 (1·07, 1·27)		2315	0·98	1·11 (1·04, 1·18)		2292	0·98	1·07 (1·01, 1·14)	
Q5 vs. Q1	2748	1·15	1·35 (1·21, 1·50)		2449	1·06	1·21 (1·13, 1·29)		2216	0·92	1·08 (1·01, 1·14)	
Continuous ‡			1·07 (1·05, 1·09)	<0·001			1·07 (1·05, 1·09)	<0·001			1·11 (1·01, 1·22)	0·03
Leucine				<0·001				<0·001				0·01
Q1	2016	0·88	1·00 (ref)		2124	0·90	1·00 (ref)		2086	0·88	1·00 (ref)	
Q2 vs. Q1	2097	0·87	1·05 (0·98, 1·12)		1998	0·88	1·01 (0·95, 1·07)		2379	1·00	1·06 (1·00, 1·13)	
Q3 vs. Q1	2158	0·89	1·09 (1·00, 1·17)		2167	0·92	1·06 (1·00, 1·13)		2328	0·98	1·05 (0·99, 1·12)	
Q4 vs. Q1	2317	0·96	1·16 (1·06, 1·27)		2505	0·98	1·11 (1·05, 1·18)		2251	0·95	1·06 (1·00, 1·13)	
Q5 vs. Q1	2654	1·15	1·33 (1·19, 1·48)		2448	1·06	1·23 (1·15, 1·31)		2198	0·94	1·09 (1·02, 1·16)	
Continuous ‡			1·07 (1·05, 1·09)	<0·001			1·07 (1·05, 1·09)	<0·001			1·10 (1·01, 1·20)	0·03
Isoleucine				<0·001				<0·001				<0·001
Q1	2020	0·87	1·00 (ref)		2066	0·89	1·00 (ref)		1908	0·81	1·00 (ref)	
Q2 vs. Q1	2025	0·87	1·06 (0·99, 1·14)		2175	0·88	1·02 (0·96, 1·08)		2184	0·92	1·04 (0·98, 1·11)	
Q3 vs. Q1	2183	0·90	1·12 (1·03, 1·21)		2169	0·92	1·06 (1·00, 1·13)		2293	0·97	1·06 (1·00, 1·13)	
Q4 vs. Q1	2248	0·95	1·18 (1·08, 1·29)		2286	0·98	1·12 (1·06, 1·20)		2354	0·99	1·09 (1·02, 1·16)	

Q5 vs. Q1	2766	1·16	1·38 (1·24, 1·54)		2546	1·09	1·23 (1·16, 1·31)		2503	1·06	1·18 (1·11, 1·26)	
Continuous ‡			1·07 (1·05, 1·09)	<0·001			1·07 (1·05, 1·09)	<0·001			1·27 (1·15, 1·40)	<0·001
Valine				<0·001				<0·001				0·80
Q1	2062	0·90	1·00 (ref)		2052	0·91	1·00 (ref)		2188	0·95	1·00 (ref)	
Q2 vs. Q1	2034	0·86	1·02 (0·95, 1·10)		2284	0·91	1·04 (0·98, 1·11)		2362	1·00	1·00 (0·95, 1·07)	
Q3 vs. Q1	2232	0·91	1·09 (1·01, 1·18)		2025	0·92	1·05 (0·99, 1·12)		2328	0·99	1·02 (0·96, 1·08)	
Q4 vs. Q1	2226	0·94	1·12 (1·03, 1·23)		2381	0·97	1·11 (1·05, 1·19)		2311	0·97	1·05 (0·98, 1·11)	
Q5 vs. Q1	2688	1·14	1·30 (1·17, 1·45)		2500	1·05	1·23 (1·15, 1·31)		2053	0·85	0·98 (0·92, 1·05)	
Continuous ‡			1·07 (1·05, 1·09)	<0·001			1·07 (1·05, 1·09)	<0·001			0·98 (0·90, 1·07)	0·62

* Hazard ratios and confidence intervals from proportional hazards models with incident diabetes as a function of the protein variable of interest adjusted for age, ethnicity, BMI, education, income, history of CHD, current smoking, current alcohol use, physical activity, hypertension, family history of diabetes, hormone use, glycemic load, glycemic index, and total energy intake. Models are additionally stratified within the model for WHI intervention arms and 5-year age groups

†p-values for categorical protein variables are from a separate model looking at linear trend over the medians of each quintile.

‡ Hazard ratios, confidence intervals, and p-values in the continuous models for a 20% increase of the protein value of interest

Table 3 Hazard ratios for the risk of diabetes by quintile of meat intake by MPED categories (adjusted for BMI).

	Events	Ann%	HR (95% CI) *	P-value †
Total Meat				<0·001
Q1	1707	0·72	1·00 (ref)	
Q2 vs. Q1	2045	0·87	1·12 (1·05, 1·19)	
Q3 vs. Q1	2222	0·91	1·15 (1·07, 1·22)	
Q4 vs. Q1	2321	0·99	1·16 (1·08, 1·24)	
Q5 vs. Q1	2947	1·27	1·28 (1·19, 1·38)	
Continuous ‡			1·04 (1·03, 1·05)	<0·001
Red meat				<0·001
Q1	1744	0·74	1·00 (ref)	
Q2 vs. Q1	2095	0·87	1·08 (1·01, 1·15)	
Q3 vs. Q1	2178	0·92	1·10 (1·03, 1·17)	
Q4 vs. Q1	2391	1·01	1·16 (1·08, 1·24)	
Q5 vs. Q1	2834	1·21	1·19 (1·11, 1·28)	
Continuous ‡			1·03 (1·02, 1·04)	<0·001
Fish				0·002
Q1	2181	0·97	1·00 (ref)	
Q2 vs. Q1	2184	0·92	0·97 (0·92, 1·03)	
Q3 vs. Q1	2199	0·93	1·00 (0·95, 1·07)	
Q4 vs. Q1	2306	0·92	0·99 (0·93, 1·05)	
Q5 vs. Q1	2372	1·01	1·07 (1·01, 1·14)	
Continuous ‡			1·02 (1·01, 1·03)	0·001
Poultry				0·010
Q1	1918	0·82	1·00 (ref)	
Q2 vs. Q1	2200	0·92	1·03 (0·97, 1·10)	
Q3 vs. Q1	2227	0·96	1·04 (0·98, 1·11)	
Q4 vs. Q1	2217	0·99	1·06 (1·00, 1·13)	
Q5 vs. Q1	2680	1·06	1·06 (1·00, 1·13)	
Continuous ‡			1·01 (1·00, 1·02)	0·010
Processed meat				<0·001
Q1	1624	0·72	1·00 (ref)	
Q2 vs. Q1	2224	0·85	1·08 (1·02, 1·16)	
Q3 vs. Q1	2278	0·96	1·13 (1·06, 1·21)	
Q4 vs. Q1	2436	1·07	1·15 (1·08, 1·23)	
Q5 vs. Q1	2680	1·16	1·17 (1·10, 1·25)	
Continuous ‡			1·03 (1·02, 1·04)	<0·001

* Hazard ratios and confidence intervals from proportional hazards models with incident diabetes as a function of the food group of interest adjusted for age, ethnicity, education, income, history of CHD, current smoking, current alcohol use, physical activity, hypertension, family history of diabetes, hormone use, glycemic load, glycemic index, total energy intake, and BMI. Models are additionally stratified within the model for WHI hormone therapy arms and 5-year age groups

† p-values for categorical food group variables are from a separate model looking at linear trend over the medians of each quintile.

‡ Hazard ratios, confidence intervals, and p-values in the continuous models for a 20% increase of the food group value of interest