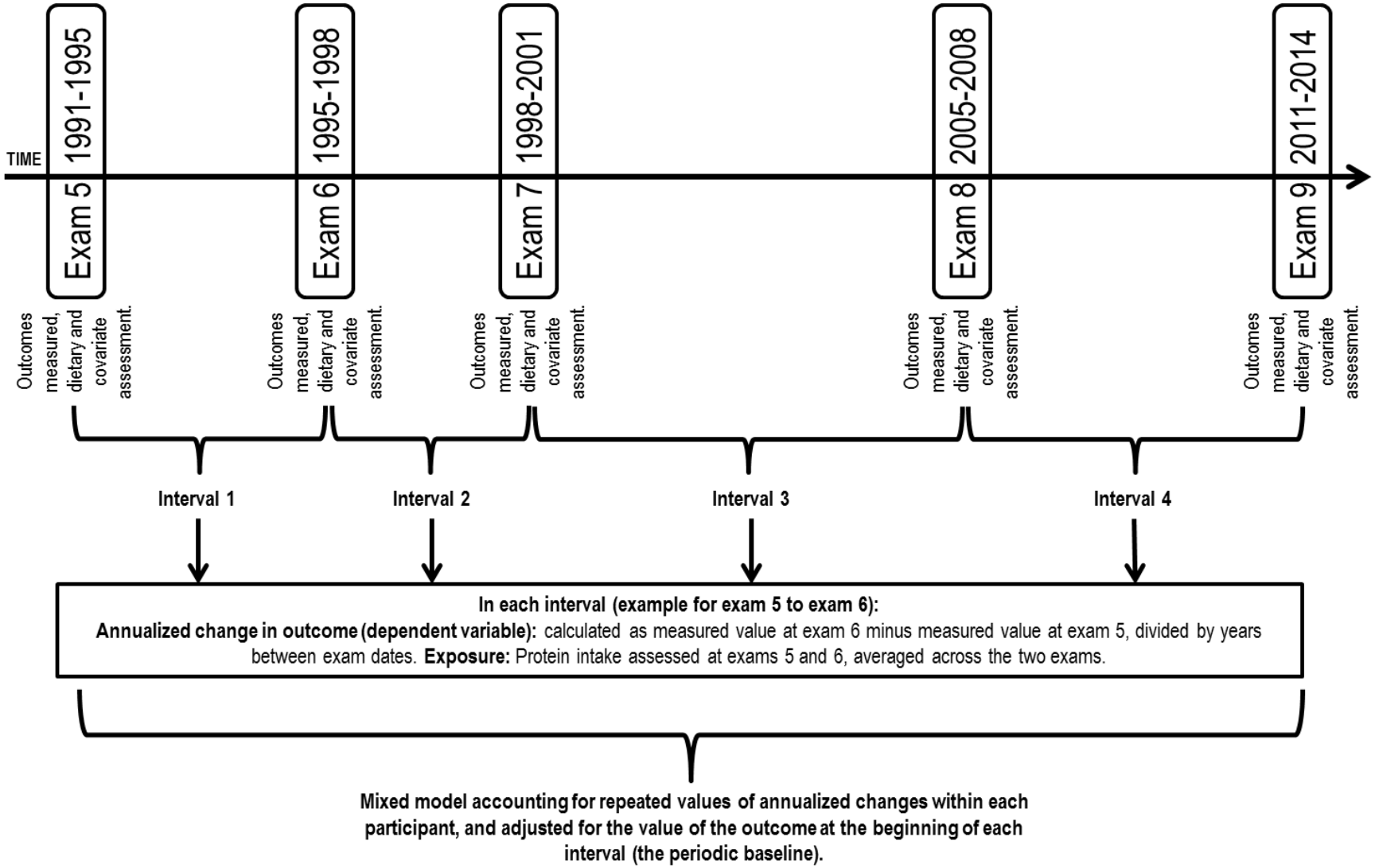
Supplemental Material

"Dietary Protein and Changes in Markers of Cardiometabolic Health across 20 Years of Follow-Up in Middle-Age Americans."

Contents

Supplemental Figure 1. Diagram of mixed model approach of analyzing annualized changes in cardiometabolic outcomes.



Supplemental Table 1. Mean protein intake and status at baseline by body mass index category in 3,066 participants of the Framingham Heart Study Offspring cohort.

		Body Mass Index Category		
	Total sample	**<25 kg/m^2**	**25–<30 kg/m^2**	**≥30 kg/m^2**
N	3066	1056	1281	729
Protein, g/d	77.5 (15.8)	76.6 (15.3)	77.4 (15.6)	78.8 (16.5)
Protein, g/kg BW/d	1.04 (0.30)	1.24 (0.30)	1.00 (0.25)	0.83 (0.21)
Protein, % energy	16.8 (3.3)	16.7 (3.3)	16.8 (3.4)	17.1 (3.4)
Not meeting RDA, %	21.5	6.2	20.6	45.4

BW, body weight; RDA, US Recommended Dietary Allowance.

Supplemental Table 2. Averaged protein intake expressed in g/d, and mean annualized changes in outcomes by strata of age, sex, BMI, eGFR, and type 2 diabetes status.

Change in outcome	β (SE) per 10 g/d averaged protein intake	*P* value	β (SE) per 10 g/d averaged protein intake	*P* value	*P* interaction*
	eGFR <60 mL/min/1.73 m²		*eGFR ≥60 mL/min/1.73 m²*		
DBP, mmHg	-0.011 (0.014)	0.44	-0.083 (0.040)	0.04	0.64
eGFR, mL/min/1.73 m²	0.018 (0.020)	0.36	-0.063 (0.045)	0.16	0.62
FG, mmol/L	0.0035 (0.0016)	0.03	0.0042 (0.0053)	0.42	0.23
HDL cholesterol, mmol/L	0.0004 (0.0004)	0.28	-0.0009 (0.0012)	0.45	0.28
LDL cholesterol, mmol/L	-0.0004 (0.0011)	0.69	-0.0031 (0.0030)	0.30	0.76
SBP, mmHg	-0.076 (0.024)	0.002	-0.259 (0.079)	0.001	0.19
Cholesterol, mmol/L	0.0005 (0.0013)	0.69	-0.0034 (0.0033)	0.31	0.48
Triglycerides, mmol/L	-0.0010 (0.0010)	0.33	-0.0010 (0.0028)	0.71	0.96
WC, cm	0.010 (0.007)	0.16	-0.012 (0.018)	0.49	0.01
Weight, kg	0.007 (0.010)	0.46	0.010 (0.026)	0.72	0.41
	Age <58 years		*Age ≥58 years*		
DBP, mmHg	0.0001 (0.019)	1.00	-0.035 (0.018)	0.05	0.81
eGFR, mL/min/1.73 m²	0.015 (0.033)	0.65	0.063 (0.027)	0.02	0.82
FG, mmol/L	0.0066 (0.0023)	0.004	0.0023 (0.0022)	0.31	0.86
HDL cholesterol, mmol/L	0.0012 (0.0006)	0.04	-0.0006 (0.0005)	0.22	0.03
LDL cholesterol, mmol/L	-0.0023 (0.0015)	0.13	-0.0002 (0.0014)	0.88	0.04
SBP, mmHg	-0.043 (0.030)	0.16	-0.127 (0.034)	0.0002	0.41
Cholesterol, mmol/L	-0.0016 (0.0019)	0.40	0.0002 (0.0017)	0.88	0.01
Triglycerides, mmol/L	-0.0021 (0.0016)	0.19	-0.0005 (0.0012)	0.68	0.09
WC, cm	0.003 (0.010)	0.80	0.004 (0.009)	0.67	0.20
Weight, kg	0.017 (0.015)	0.27	-0.002 (0.012)	0.90	0.77

	BMI <25 kg/m²		*BMI ≥25 kg/m²*		
DBP, mmHg	-0.035 (0.023)	0.13	-0.013 (0.016)	0.41	0.17
eGFR, mL/min/1.73 m²	0.003 (0.036)	0.92	0.065 (0.026)	0.01	0.15
FG, mmol/L	-0.0008 (0.0017)	0.66	0.0039 (0.0021)	0.06	0.31
HDL cholesterol, mmol/L	0.0009 (0.0008)	0.24	0.0005 (0.0004)	0.19	0.77
LDL cholesterol, mmol/L	-0.0017 (0.0017)	0.32	-0.0008 (0.0013)	0.53	0.16
SBP, mmHg	-0.124 (0.042)	0.004	-0.082 (0.028)	0.004	0.01
Cholesterol, mmol/L	0.0004 (0.0022)	0.86	-0.0001 (0.0015)	0.93	0.12
Triglycerides, mmol/L	0.0004 (0.0016)	0.79	-0.0028 (0.0012)	0.02	0.61
WC, cm	-0.024 (0.011)	0.03	0.012 (0.008)	0.13	0.87
Weight, kg	0.011 (0.011)	0.31	0.003 (0.012)	0.81	0.88
	Male		*Female*		
DBP, mmHg	-0.001 (0.019)	0.97	-0.035 (0.018)	0.05	0.16
eGFR, mL/min/1.73 m²	0.047 (0.030)	0.12	0.053 (0.032)	0.09	0.69
FG, mmol/L	0.0020 (0.0025)	0.43	0.0052 (0.0019)	0.01	0.24
HDL cholesterol, mmol/L	-0.0001 (0.0004)	0.84	0.0005 (0.0006)	0.43	0.86
LDL cholesterol, mmol/L	-0.0004 (0.0014)	0.75	-0.0009 (0.0014)	0.51	0.73
SBP, mmHg	-0.054 (0.034)	0.11	-0.115 (0.032)	0.0003	0.04
Cholesterol, mmol/L	0.0003 (0.0017)	0.85	-0.0002 (0.0017)	0.91	0.93
Triglycerides, mmol/L	-0.0002 (0.0015)	0.87	-0.0015 (0.0012)	0.20	0.77
WC, cm	0.014 (0.006)	0.02	-0.004 (0.010)	0.72	0.16
Weight, kg	0.012 (0.013)	0.35	0.002 (0.013)	0.86	0.98
	Type 2 diabetes absent		*Type 2 diabetes present*		
DBP, mmHg	-0.021 (0.014)	0.12	0.017 (0.050)	0.74	0.21
eGFR, mL/min/1.73 m²	0.046 (0.022)	0.03	-0.007 (0.092)	0.94	0.79
FG, mmol/L	-0.0014 (0.0009)	0.11	0.0306 (0.0138)	0.03	<0.001†

HDL cholesterol, mmol/L	0.0004 (0.0004)	0.32	-0.0009 (0.0012)	0.47	0.35
LDL cholesterol, mmol/L	-0.0002 (0.0010)	0.83	-0.0026 (0.0040)	0.52	0.27
SBP, mmHg	-0.093 (0.024)	<0.001	-0.025 (0.091)	0.78	0.20
Cholesterol, mmol/L	0.0004 (0.0013)	0.74	-0.0007 (0.0045)	0.88	0.85
Triglycerides, mmol/L	-0.0016 (0.0009)	0.08	0.0009 (0.0050)	0.87	0.003†
WC, cm	0.005 (0.006)	0.45	0.011 (0.025)	0.68	0.93
Weight, kg	0.009 (0.009)	0.35	-0.021 (0.040)	0.60	0.21

DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FG, fasting glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglyceride; WC, waist circumference.

*Interactions were modeled using continuous cross-product terms and corresponding first-order terms. The model was otherwise adjusted for age; sex; energy intake; the baseline measure of the outcome (i.e., the value at the beginning of the exam interval); smoking status; alcohol intake; pharmacological treatment for dyslipidemia, cardiovascular disease, hypertension, or diabetes; history of cancer; change in weight, except for when the outcome was change in weight; and the Dietary Guidelines for Americans 2010 Index Score.

†Interactions significant at the corrected *P* value. Given the 5 hypothesis-free interaction tests for each outcome, a Bonferroni-corrected *P* value was used, with a significance cut point of <0.01 (0.05/5).

Supplemental Table 3. Adjusted least square means of fasting insulin and insulin resistance after 7 years of follow-up by averaged total, animal, and plant protein intake in participants of the Framingham Heart Study Offspring cohort.

			Adjusted least square mean (SE) per quartile category of averaged intake (g/d)				
			Total protein				
Outcome at exam 7	**Model***	**N**	**64.4**	**74.6**	**82.5**	**93.3**	**P trend**
Fasting insulin, mU/mL†	1	2422	2.57 (0.04)	2.59 (0.03)	2.61 (0.04)	2.58 (0.03)	0.31
	2	2422	2.65 (0.04)	2.65 (0.04)	2.66 (0.04)	2.62 (0.04)	0.33
	3	2410	2.64 (0.04)	2.65 (0.04)	2.66 (0.04)	2.62 (0.04)	0.34
HOMA-IR†	1	2422	1.22 (0.04)	1.26 (0.04)	1.28 (0.04)	1.27 (0.04)	0.046
	2	2422	1.37 (0.04)	1.38 (0.04)	1.39 (0.04)	1.36 (0.04)	0.84
	3	2410	1.37 (0.05)	1.39 (0.04)	1.40 (0.04)	1.37 (0.04)	0.96
			Animal protein				
			39.9	**50.0**	**58.0**	**69.6**	***P* trend**
Fasting insulin, mU/mL†	1	2422	2.57 (0.04)	2.58 (0.03)	2.62 (0.03)	2.59 (0.03)	0.24
	2	2422	2.64 (0.04)	2.64 (0.04)	2.66 (0.04)	2.63 (0.04)	0.82
	3	2410	2.64 (0.04)	2.64 (0.04)	2.66 (0.04)	2.63 (0.04)	0.73
HOMA-IR†	1	2422	1.23 (0.04)	1.24 (0.04)	1.29 (0.04)	1.27 (0.04)	0.04
	2	2422	1.37 (0.05)	1.37 (0.04)	1.40 (0.04)	1.37 (0.04)	0.68
	3	2410	1.38 (0.05)	1.37 (0.04)	1.41 (0.04)	1.38 (0.04)	0.72
			Plant protein				
			64.4	**74.6**	**82.5**	**93.3**	***P* trend**
Fasting insulin, mU/mL†	1	2422	2.58 (0.04)	2.60 (0.03)	2.61 (0.03)	2.55 (0.04)	0.25
	2	2422	2.65 (0.04)	2.65 (0.04)	2.66 (0.04)	2.61 (0.04)	0.18
	3	2410	2.65 (0.04)	2.65 (0.04)	2.66 (0.04)	2.61 (0.04)	0.25
HOMA-IR†	1	2422	1.26 (0.04)	1.27 (0.04)	1.27 (0.04)	1.21 (0.04)	0.15
	2	2422	1.39 (0.04)	1.38 (0.04)	1.39 (0.04)	1.34 (0.05)	0.09
	3	2410	1.39 (0.05)	1.39 (0.04)	1.39 (0.04)	1.34 (0.05)	0.16

HOMA-IR, homeostatic model assessment of insulin resistance.

*Model 1 was adjusted for age; sex; energy intake; and the baseline measure of the outcome (i.e., the value at exam 5). Model 2 was adjusted as for model 1, plus smoking status; alcohol intake; pharmacological treatment for dyslipidemia, cardiovascular disease, hypertension, or diabetes; and history of cancer. Model 2 also included change in weight between exams 5 and 7. Model 3 was additionally adjusted for the Dietary Guidelines for Americans 2010 Index Score.
†Means are expressed on the natural log scale.

Supplemental Table 4. Mean annualized changes in outcomes by categories of averaged protein intake, expressed in g/kg body weight/d, in participants of the Framingham Heart Study Offspring cohort.

Annualized change in…	Model	Adjusted mean annualized change (SE) per quartile category of averaged protein intake (g/kg/d)				*P* trend	β (SE) per 1 g/kg/d averaged protein intake	*P* continuous
		0.7	0.9	1.1	1.4			
DBP, mmHg	1	-0.23 (0.04)	-0.16 (0.03)	-0.11 (0.03)	-0.25 (0.04)	0.59	-0.083 (0.069)	0.23
	2	-0.21 (0.04)	-0.14 (0.03)	-0.10 (0.03)	-0.26 (0.03)	0.28	-0.108 (0.065)	0.10
	3	-0.23 (0.04)	-0.14 (0.03)	-0.09 (0.03)	-0.25 (0.03)	0.45	-0.090 (0.068)	0.19
eGFR, mL/min/1.73 m^2	1	-1.18 (0.06)	-1.07 (0.05)	-0.88 (0.05)	-0.75 (0.06)	<.0001	0.482 (0.115)	<.0001
	2	-1.10 (0.06)	-1.01 (0.05)	-0.87 (0.05)	-0.81 (0.06)	0.001	0.310 (0.113)	0.01
	3	-1.09 (0.06)	-1.01 (0.05)	-0.87 (0.05)	-0.81 (0.06)	0.002	0.300 (0.116)	0.01
FG, mmol/L	1	0.039 (0.004)	0.025 (0.004)	0.016 (0.004)	-0.004 (0.004)	<.0001	-0.054 (0.007)	<.0001
	2	0.039 (0.004)	0.026 (0.004)	0.017 (0.004)	0.000 (0.004)	<.0001	-0.050 (0.008)	<.0001
	3	0.040 (0.004)	0.026 (0.004)	0.017 (0.004)	-0.001 (0.004)	<.0001	-0.053 (0.008)	<.0001
HDL cholesterol, mmol/L	1	0.010 (0.001)	0.012 (0.001)	0.016 (0.001)	0.019 (0.001)	<.0001	0.014 (0.002)	<.0001
	2	0.009 (0.001)	0.012 (0.001)	0.016 (0.001)	0.019 (0.001)	<.0001	0.014 (0.002)	<.0001
	3	0.010 (0.001)	0.012 (0.001)	0.015 (0.001)	0.018 (0.001)	<.0001	0.013 (0.002)	<.0001
LDL cholesterol, mmol/L	1	-0.048 (0.003)	-0.045 (0.003)	-0.041 (0.003)	-0.040 (0.003)	0.06	0.005 (0.005)	0.30
	2	-0.041 (0.003)	-0.038 (0.002)	-0.037 (0.002)	-0.044 (0.003)	0.44	-0.009 (0.005)	0.06
	3	-0.042 (0.003)	-0.038 (0.002)	-0.037 (0.002)	-0.043 (0.003)	0.59	-0.009 (0.005)	0.09
SBP, mmHg	1	0.12 (0.06)	0.18 (0.06)	0.33 (0.06)	0.00 (0.06)	0.20	-0.268 (0.114)	0.02
	2	0.10 (0.06)	0.23 (0.06)	0.38 (0.06)	0.03 (0.06)	0.39	-0.213 (0.113)	0.06
	3	0.09 (0.06)	0.22 (0.06)	0.39 (0.06)	0.02 (0.06)	0.34	-0.241 (0.119)	0.04
Cholesterol, mmol/L	1	-0.049 (0.003)	-0.043 (0.003)	-0.030 (0.003)	-0.023 (0.003)	<.0001	0.032 (0.006)	<.0001
	2	-0.044 (0.003)	-0.035 (0.003)	-0.026 (0.003)	-0.027 (0.003)	0.0004	0.019 (0.006)	0.001
	3	-0.045 (0.003)	-0.035 (0.003)	-0.026 (0.003)	-0.026 (0.003)	0.0001	0.021 (0.006)	0.001
Triglycerides, mmol/L	1	-0.011 (0.003)	-0.018 (0.002)	-0.021 (0.002)	-0.035 (0.002)	<.0001	-0.030 (0.005)	<.0001

	2	-0.013 (0.003)	-0.018 (0.002)	-0.020 (0.002)	-0.032 (0.002)	<.0001	-0.024 (0.005)	<.0001
	3	-0.015 (0.003)	-0.019 (0.002)	-0.020 (0.002)	-0.032 (0.002)	<.0001	-0.021 (0.005)	<.0001
Waist circumference, cm	1	1.02 (0.03)	0.71 (0.03)	0.45 (0.03)	0.02 (0.03)	<.0001	-1.454 (0.065)	<.0001
	2	0.65 (0.02)	0.60 (0.02)	0.52 (0.02)	0.36 (0.02)	<.0001	-0.467 (0.038)	<.0001
	3	0.65 (0.02)	0.59 (0.02)	0.52 (0.02)	0.37 (0.02)	<.0001	-0.469 (0.040)	<.0001
Weight, kg	1	0.49 (0.03)	0.19 (0.02)	-0.03 (0.02)	-0.29 (0.03)	<.0001	-1.049 (0.059)	<.0001
	2	0.50 (0.03)	0.18 (0.02)	-0.04 (0.02)	-0.30 (0.03)	<.0001	-1.074 (0.061)	<.0001
	3	0.54 (0.03)	0.19 (0.02)	-0.05 (0.02)	-0.33 (0.03)	<.0001	-1.181 (0.064)	<.0001

DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FG, fasting glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure.

*Model 1 was adjusted for age; sex; energy intake; and the baseline measure of the outcome (i.e., the value at the beginning of the exam interval). Model 2 was adjusted as for model 1, plus smoking status; alcohol intake; pharmacological treatment for dyslipidemia, cardiovascular disease, hypertension, or diabetes; and history of cancer. Model 2 also included change in weight, except for when the outcome was change in weight. In model 3, we additionally adjusted for the Dietary Guidelines for Americans 2010 Index Score.

Supplemental Table 5. Mean annualized changes in outcomes by categories of averaged protein intake, expressed in g/kg body weight/d, stratified by body mass index category, in participants of the Framingham Heart Study Offspring cohort.*

Annualized change in…	BMI Cat. (kg/m^2)	β (SE) per g/kg BW/d averaged protein intake	*P* continuous
Diastolic BP, mmHg	<25	-0.232 (0.117)	0.05
	25-<30	-0.164 (0.138)	0.24
	≥30	0.304 (0.199)	0.13
eGFR, mL/min/1.73 m^2	<25	0.201 (0.182)	0.27
	25-<30	0.370 (0.218)	0.09
	≥30	0.375 (0.328)	0.25
Fasting plasma glucose, mmol/L	<25	-0.014 (0.009)	0.12
	25-<30	-0.005 (0.015)	0.72
	≥30	-0.059 (0.030)	0.05
HDL cholesterol, mmol/L	<25	0.011 (0.004)	0.01
	25-<30	0.005 (0.004)	0.19
	≥30	0.007 (0.005)	0.12
LDL cholesterol, mmol/L	<25	-0.017 (0.009)	0.06
	25-<30	-0.003 (0.011)	0.78
	≥30	0.020 (0.015)	0.18
Systolic BP, mmHg	<25	-0.511 (0.217)	0.02
	25-<30	-0.145 (0.242)	0.55
	≥30	0.154 (0.356)	0.66
Cholesterol, mmol/L	<25	0.006 (0.011)	0.57
	25-<30	0.009 (0.013)	0.47
	≥30	0.034 (0.017)	0.04
Triglycerides, mmol/L	<25	-0.007 (0.008)	0.40
	25-<30	-0.015 (0.011)	0.15

	≥30	-0.020 (0.013)	0.13
Waist circumference, cm	<25	-0.511 (0.065)	<.0001
	25-<30	-0.271 (0.068)	<.0001
	≥30	-0.775 (0.118)	<.0001
Weight, kg	<25	-0.586 (0.068)	<.0001
	25-<30	-1.133 (0.092)	<.0001
	≥30	-2.396 (0.212)	<.0001

BMI, body mass index; BP, blood pressure; BW, body weight; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*The model was adjusted for age; sex; energy intake; the baseline measure of the outcome (i.e., the value at the beginning of the exam interval); smoking status; alcohol intake; pharmacological treatment for dyslipidemia, cardiovascular disease, hypertension, or diabetes; history of cancer; change in weight, except for when the outcome was change in weight; and the Dietary Guidelines for Americans 2010 Index Score.