The Journal of Nutrition
Nutritional Epidemiology

An Increase in Dietary Quality Is Associated with Favorable Plasma Biomarkers of the Brain-Adipose Axis in Apparently Healthy US Women[1–3]

Tianyi Huang,[4]* Deirdre K Tobias,[5] Adela Hruby,[6] Nader Rifai,[7] Shelley S Tworoger,[4,8] and Frank B Hu[4,8,9]

[4]Channing Division of Network Medicine, Department of Medicine, [5]Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; [6]Nutritional Epidemiology Program, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA; [7]Division of Laboratory Medicine, Boston Children's Hospital, Boston, MA; and Departments of [8]Epidemiology and [9]Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA

Abstract

Background: The associations between long-term dietary quality and biomarkers of the brain-adipose axis have not been examined.

Objective: We evaluated both cross-sectional and longitudinal associations between dietary quality and several biomarkers involved in the brain-adipose axis.

Methods: In the Nurses' Health Study II, 831 women [baseline mean age: 45 y; body mass index (BMI; in kg/m^2): 24.6] were randomly selected from women who provided 2 fasting blood samples in 1996–1999 and 2010–2011 to measure plasma concentrations of leptin, soluble leptin receptor (sOB-R), adiponectin, insulin, retinol binding protein-4 (RBP-4), interleukin-6 (IL-6), and C-reactive protein (CRP). Dietary quality was assessed by the Alternative Healthy Eating Index (AHEI-2010) with the use of semiquantitative food-frequency questionnaires administered in 1995 and 2011. We used linear mixed models to evaluate the cross-sectional associations between dietary quality and biomarker concentrations. We also examined change in dietary quality in relation to change in biomarker concentrations.

Results: In cross-sectional analyses that compared the highest with the lowest quintile of AHEI-2010, we observed significantly lower leptin (P-trend < 0.0001), insulin (P-trend < 0.0001), and CRP (P-trend = 0.02) and significantly higher sOB-R (P-trend < 0.0001) and adiponectin (P-trend = 0.0003). These associations, except for CRP, remained significant after adjustment for BMI. In longitudinal analyses, women in the highest quintile of AHEI-2010 score change (most improvement) had a 13% increase in leptin, compared with a 42% increase (P-trend < 0.0001) in the lowest quintile (least improvement). The corresponding multivariable-adjusted percentage changes for other biomarkers were 4% compared with −1% for sOB-R (P-trend = 0.04), 14% compared with 6% for adiponectin (P-trend = 0.02), and −11% compared with 16% for CRP (P-trend = 0.02). Adjustment for interim weight change attenuated these associations. No associations were observed for RBP-4 or IL-6.

Conclusion: Improvement in dietary quality was associated with favorable profiles of several biomarkers of the brain-adipose axis in women. *J Nutr* 2016;146:1101–8.

Keywords: dietary quality, brain-adipose axis, leptin, soluble leptin receptor, adiponectin

Introduction

Adherence to healthy dietary patterns is associated with maintaining a healthy body weight (1–6). This is suggested to contribute at least partly to the reduced risk of cardiovascular disease and type 2 diabetes among individuals who follow a healthy diet. However, the potential mechanistic link between healthy diets and weight gain is not fully understood. Evidence suggests that higher dietary quality may mitigate weight gain through influencing dietary quantity and reducing total caloric intake (2). This finding speaks to the potential importance of several circulating hormones and cytokines involved in the brain-adipose axis, which interact with the central nervous system (CNS)[10] and peripheral

[1] Supported by NIH grants UM1 CA176726, R01 CA67262, HL60712, and 5UM1CA17672.

[2] Author disclosures: T Huang, DK Tobias, A Hruby, N Rifai, SS Tworoger, and FB Hu, no conflicts of interest.

[3] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

*To whom correspondence should be addressed. E-mail: tih541@mail.harvard.edu.

[10] Abbreviations used: AHEI, Alternative Healthy Eating Index; CNS, central nervous system; CRP, C-reactive protein; RBP-4, retinol binding protein-4; sOB-R, soluble leptin receptor.

Manuscript received January 7, 2016. Initial review completed February 12, 2016. Revision accepted March 7, 2016.
First published online April 13, 2016; doi:10.3945/jn.115.229666.

adipose tissues to modulate feeding behaviors, energy balance, and adipogenesis (7, 8).

Although increased leptin concentrations suppress appetite to reduce energy intake, leptin is positively associated with adiposity due to leptin resistance in the hypothalamus, particularly among obese individuals (9–11). The bioavailability of leptin is also regulated by soluble leptin receptor (sOB-R), the primary leptin-binding protein in circulation. The ratio between leptin and sOB-R, denoted as free leptin index, is used to quantify unbound, biologically active leptin concentrations (12). Similarly, insulin exerts its anorexigenic effects by acting on the functional insulin receptor in the hypothalamus, in addition to its central role in glucose metabolism (13). By contrast, adiponectin, an adipocyte hormone predominantly secreted by adipose tissue, has an inverse association with obesity and acts on the CNS to induce weight loss (14, 15). Given the reciprocal effects of leptin and adiponectin, their ratio is strongly linked to insulin resistance and type 2 diabetes (16, 17). Retinol binding protein-4 (RBP-4) is another adipokine linked to insulin resistance among individuals with obesity or type 2 diabetes (18, 19), although its function in the crosstalk between adipose tissues and the CNS remains unclear. Further, several inflammatory biomarkers, such IL-6 and C-reactive protein (CRP), were also shown to interact with the brain-adipose axis to affect body weight (20, 21).

Several healthy dietary patterns, including the prudent pattern, the Mediterranean dietary pattern, and the Alternative Healthy Eating Index (AHEI), were previously correlated with more favorable profiles of inflammatory markers (22–28). Relatively few studies have specifically focused on biomarkers involved in the brain-adipose axis (22–24). No study to date has examined the associations of these biomarkers with AHEI-2010, an updated dietary quality score that shows stronger relations with major chronic diseases than previous indexes (29). Importantly, it is unknown whether changes in dietary quality are associated with change in these biomarker concentrations. In this study, we evaluate the cross-sectional and longitudinal associations between dietary quality and several biomarkers of the brain-adipose axis with the use of repeated measures of diet and plasma biomarkers. We hypothesized that higher dietary quality and improved quality are associated with favorable profiles of leptin, sOB-R, adiponectin, free leptin index, leptin-to-adiponectin ratio (leptin:adiponectin), insulin, RBP-4, IL-6, and CRP.

Methods

Study population. The Nurses' Health Study II was established in 1989 among 116,430 US female registered nurses, aged 25–42 y. All women completed a baseline questionnaire, and their health conditions and lifestyle factors were updated biennially by questionnaire. In 1996–1999, 29,611 women who had not previously reported a diagnosis of cancer participated in our blood sample collection (30). Women returned their blood samples (which was considered implied consent) with an ice pack by overnight courier to our laboratory, where the samples were processed and separated into plasma, RBC, and white blood cell components. All samples have been stored in liquid nitrogen freezers since collection. Of these, 15,982 women provided a second blood sample (with a signed consent) in 2008–2011, using the same protocol as in the first collection. Women who participated in the first and second blood collections were similar to the full cohort (when examining the responses to the 2009 questionnaire), including age (mean: 55.6, 55.9, 55.0 y, respectively), BMI (in kg/m^2; mean: 27.4, 27.1, 27.7, respectively), and alcohol consumption (mean: 6.7, 6.8, 6.4 g/d, respectively).

At the study design stage, we performed power calculations that were based on a fixed sample size of 850, α = 0.05, power = 0.80, and estimated baseline biomarker SDs from previous studies (22, 31, 32). Assuming the correlation of biomarker concentrations between baseline and follow-up was 0.6, the minimal detectable changes in biomarkers per SD change in dietary score were 1.2 ng/mL for leptin, 0.9 ng/mL for sOB-R, 0.7 μg/mL for adiponectin, 0.7 uU/mL for insulin, 1.7 μg/mL for RBP-4, and 0.25 pg/mL for IL-6. During the conduct of the study, we randomly selected 850 women from those who were free of cancer, cardiovascular disease, and type 2 diabetes at both time points and were fasting ≥8 h before blood collection. Two blood samples per woman were assayed for biomarkers described above plus CRP. A total of 831 women with dietary assessment available at the time of each blood collection was included in the current analysis. The study protocol was approved by the institutional review board of the Brigham and Women's Hospital and Harvard T.H. Chan School of Public Health.

Assessment of dietary quality. Semiquantitative FFQs were administered every 4 y, beginning in 1991, in the Nurses' Health Study II to assess dietary intake. Previous studies have shown the validity and reproducibility of the FFQs (33, 34). We used the dietary intake data captured closest to the return of the first blood sample (either 1995 or 1999). We only included women who provided their second blood sample from 2010 to 2011 to correspond with their dietary assessments in 2011.

From the dietary intake data, the AHEI-2010 scores were derived from 11 food or nutrient components that had well-established associations with chronic diseases (primarily cardiometabolic diseases) and mortality in clinical and epidemiologic studies (29). Each component was scored 0–10; higher scores were assigned to higher intakes of vegetables, fruit, whole grains, nuts/legumes, long-chain fats, and PUFAs; moderate alcohol consumption; and lower intake of sugar-sweetened beverages, red/processed meat, *trans* fat, and sodium. The total AHEI-2010 score ranged from 0 to 110, with higher scores indicating a healthier, higher-quality diet.

Measurement of plasma biomarkers. Plasma biomarker concentrations were measured in the Clinical Chemistry Laboratory at Boston Children's Hospital. Leptin, sOB-R, RBP-4, and IL-6 were measured by an ultrasensitive ELISA assay from R&D Systems. Total adiponectin was assayed with a quantitative monoclonal sandwich ELISA (ALPCO Diagnostics Inc.). Fasting insulin concentrations were determined by an electrochemiluminescence immunoassay with the use of the Roche E modular system (Roche Diagnostics), and high-sensitivity CRP was measured by an immunoturbidimetric assay (Denka Seiken). The mean interassay CVs were 4.9% for leptin, 11.1% for sOB-R, 10.1% for adiponectin, 6.4% for insulin, 10.3% for RBP-4, 1.5% for CRP, and 10.8% for IL-6.

Statistical analysis. All biomarker concentrations and the 2 ratio measures (leptin:sOB-R, as free leptin index, and leptin:adiponectin) were log-transformed to normalize the distributions. We first evaluated the age-standardized distribution of dietary quality, biomarker concentrations, and other population characteristics at each blood collection. We compared the differences in dietary quality and biomarkers between the 2 collections with the use of paired *t* tests. We fit a linear mixed-effect model to estimate the cross-sectional associations between dietary quality and biomarker concentrations. An unstructured covariance matrix was specified with a repeated statement in SAS Proc Mixed procedure to account for the within-person correlations between 2 blood samples. Biomarker concentrations from the appropriate collection were regressed on the corresponding AHEI-2010 scores (in quintiles) assessed on the repeated FFQs, and a number of time-varying covariates ascertained closest to each blood collection, including age, total caloric intake (in quintiles), physical activity (in quintiles), smoking (current, past, never), menopausal status (premenopausal, postmenopausal), and postmenopausal hormone use (current, past, never). We also included an indicator variable to designate whether the samples were from the first or second collection. Hypertension, hypercholesterolemia and blood collection characteristics, such as season and time of day of blood draw,

were also considered but not included in the final model because additionally accounting for them in the analysis did not substantively change the results. To explore whether the association was independent of obesity, we further adjusted for BMI (<20.0, 20.0–24.9, 25.0–29.9, ≥30.0) from the weight provided at each sample collection in a separate model. Multivariable-adjusted least-squares geometric means were computed for each quintile of the dietary scores. Linear trends were tested by using the median AHEI-2010 score within each quintile as a continuous variable.

For longitudinal analyses of change, we used general linear regression to evaluate within-individual changes in log-transformed biomarker concentrations as dependent variables in relation to changes in dietary quality. We calculated least-squares percentage changes in biomarkers for each quintile of AHEI-2010 score changes, adjusted for age (continuous), time intervals between blood collections (11–15 y), baseline dietary scores (in quintiles), baseline and change in total caloric intake and physical activity (both in quintiles), change in smoking status, and change in menopausal status and postmenopausal hormone use. We adjusted for concurrent weight change (in quintiles) in a separate model. Additional analyses were conducted to evaluate whether the associations may be modified by baseline dietary quality. Stratified analyses were conducted among women with baseline dietary quality ≤50, the median dietary score, compared with >50, and significance of the interaction was evaluated by testing the cross product term between change in dietary score and baseline score. All analyses were performed in SAS 9.3 (SAS Institute).

Results

At the time of the first blood collections, participants were 44.9 y of age (range: 32.8–53.4 y of age), with a mean BMI of 24.6 ± 3.5. All plasma biomarker concentrations were higher on average in the second samples collected in 2010–2011 over an average follow-up of 13.0 y (range: 10.8–15.2 y). The mean dietary quality of the study population assessed by AHEI-2010 score improved significantly from 50.9 to 64.8 ($P < 0.0001$) over time. Significant improvement was also observed for all individual components of the score ($P < 0.05$), except for red/processed meat, which remained the same between the 2 time points. In addition, participants on average had reduced total caloric intake and smoking, elevated physical activity, and higher prevalence of hypertension and hypercholesterolemia (**Table 1**).

A higher AHEI-2010 score was significantly associated with lower plasma leptin, free leptin index, leptin:adiponectin, insulin, and CRP in cross-sectional analyses (**Table 2**). After adjusting for potential confounders, women in the highest quintile of AHEI score had 25% lower leptin, 31% lower free leptin index, 32% lower leptin:adiponectin, and 23% lower insulin than women in the lowest quintile. The multivariable-adjusted geometric means that compared extreme quintiles of dietary score were 14.3 compared with 19.0 ng/mL for leptin, 0.6 compared with 0.8 for free leptin index, 1.8 compared with 2.7 for leptin:adiponectin, and 4.4 compared with 5.6 uU/mL for insulin (all P-trend < 0.0001). These associations remained significant after further adjusting for BMI. A modest inverse association was also observed for CRP, with a 6% lower concentration for every 10-point higher AHEI score (P-trend = 0.02). However, the trend for CRP was not significant after accounting for BMI (P-trend = 0.18).

Dietary quality was positively associated with plasma concentrations of sOB-R and adiponectin. In multivariable analyses, we observed 2% greater sOB-R (P-trend < 0.0001) and 3% greater adiponectin (P-trend = 0.0003)/10-point increment of AHEI score. When comparing the highest with the lowest quintile of AHEI-2010, the multivariable-adjusted geometric mean concentrations were 25.7 compared with 23.6 ng/mL for sOB-R and 8.0 compared with 7.1 μg/mL for adiponectin. Adjustment for BMI did not substantively alter these associations. IL-6 was inversely associated with AHEI only in the age-adjusted model (P-trend = 0.02); no association was observed after considering other covariates. RBP-4 was not associated with dietary quality.

The longitudinal analyses of dietary quality change with changes in biomarkers gave similar findings (**Table 3**). The

TABLE 1 Comparison of age-standardized sample characteristics between the baseline (1996–1999) and follow-up (2010–2011) collection period[1]

	Baseline (n = 831)	Follow-up (n = 831)
AHEI-2010 and its components[2]		
AHEI-2010	50.9 ± 8.6	64.8 ± 10.3*
Fruit,[3] servings/d	1.4 ± 0.6	1.6 ± 0.8*
Vegetables,[4] servings/d	3.0 ± 1.1	3.5 ± 1.4*
Nuts/legumes,[5] servings/d	0.4 ± 0.3	0.9 ± 0.7*
Red/processed meat,[6] servings/d	0.8 ± 0.4	0.9 ± 0.5
Sugar-sweetened beverage,[7] servings/d	1.0 ± 0.8	0.6 ± 0.6*
Alcohol,[8] drinks/d	0.3 ± 0.5	0.4 ± 0.6*
PUFAs, % calories	5.7 ± 1.3	7.7 ± 1.9*
trans Fat, % calories	1.3 ± 0.4	0.8 ± 0.2*
Long-chain fats, mg/d	177 ± 119	379 ± 267*
Whole grains, g/d	27 ± 13	41 ± 18*
Sodium, mg/d	2140 ± 50	1900 ± 509*
Plasma biomarkers		
Leptin, ng/mL	17.0 [1.8]	19.4 [2.0]*
sOB-R, ng/mL	24.8 [1.2]	25.0 [1.3]*
Total adiponectin, μg/mL	7.0 [1.4]	7.4 [1.5]*
Free leptin index[9]	0.7 [2.0]	0.8 [2.3]*
Leptin-to-adiponectin ratio	2.4 [2.1]	2.6 [2.5]*
Insulin, uU/mL	5.7 [1.6]	5.5 [1.7]
RBP-4, μg/mL	32.8 [1.2]	35.7 [1.2]*
CRP, mg/L	0.99 [2.55]	1.03 [2.58]
IL-6, pg/mL	0.87 [1.54]	0.91 [1.57]**
Other factors		
Age at blood collection, y	44.9 ± 4.5	57.9 ± 4.3
BMI, kg/m^2	24.6 ± 3.5	26.3 ± 4.7
Total energy intake, kcal/d	1870 ± 368	1760 ± 409
Physical activity, MET-h/wk	17.8 ± 15.2	23.5 ± 22.2
Current smokers, %	4	1
Postmenopausal, %	21	68
Current PMH use in postmenopausal, %	85	23
Hypertension, %	9	29
Hypercholesterolemia, %	13	35

[1] Values are means ± SDs for continuous variables except plasma biomarkers, geometric means [geometrics SDs] for all plasma biomarkers, and percentages for categorical variables. *,**Difference of dietary scores or biomarkers between baseline and follow-up, based on paired t test: *$P < 0.0001$, **$P < 0.05$. AHEI, Alternative Healthy Eating Index; CRP, C-reactive protein; MET-h, metabolic equivalent task hours; PMH, postmenopausal hormone; RBP-4, retinol-binding protein-4; sOB-R, soluble leptin receptor.
[2] Derived from FFQs assessed in 1995 (or 1999) and 2011, respectively.
[3] One serving is defined as 1 medium piece of fruit or 0.5 cup of berries (1 cup = 237 g).
[4] One serving is defined as 0.5 cup of vegetables or 1 cup of green leafy vegetables (1 cup = 237 g).
[5] One serving is defined as 1 oz (28 g) of nuts or 1 tablespoon (15 mL) of peanut butter.
[6] One serving is defined as 4 oz of unprocessed meat or 1.5 oz of processed meat (1 oz = 28 g).
[7] One serving is defined as 8 oz (1 oz = 28 g).
[8] One drink is defined as 4 oz of wine, 12 oz of beer, or 1.5 oz of liquor (1 oz = 28 g).
[9] Free leptin index is defined as the ratio of leptin to sOB-R.

TABLE 2 Cross-sectional association of biomarkers of inflammation and brain-adipose axis with AHEI-2010 in 1995 and 2011[1]

	AHEI-2010					% Change/10-point	
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	difference (95% CIs)	*P*-trend
At baseline, *n*	287	241	174	94	35		
At follow-up, *n*	49	86	161	235	300		
AHEI, median (range)	39.5 (18.5–45.5)	50.0 (46.0–53.5)	57.5 (54.0–61.5)	66.5 (62.0–71.5)	78.0 (72.0–98.5)		
Leptin, ng/mL							
Age-adjusted[2]	20.7 ± 1.0	19.6 ± 1.0	17.3 ± 1.0	17.7 ± 1.0	15.0 ± 1.0	−8 (−10, −5)	<0.0001
Multivariable[3]	19.0 ± 1.1	18.1 ± 1.1	15.9 ± 1.1	16.6 ± 1.1	14.3 ± 1.1	−7 (−9, −4)	<0.0001
Multivariable + BMI	18.8 ± 1.0	18.1 ± 1.0	16.8 ± 1.0	17.2 ± 1.0	15.6 ± 1.0	−4 (−7, −2)	0.0002
Soluble leptin receptor, ng/mL							
Age-adjusted	23.5 ± 1.0	24.7 ± 1.0	25.3 ± 1.0	25.4 ± 1.0	26.3 ± 1.0	3 (2, 4)	<0.0001
Multivariable	23.6 ± 1.0	24.6 ± 1.0	25.0 ± 1.0	25.0 ± 1.0	25.7 ± 1.0	2 (1, 3)	<0.0001
Multivariable + BMI	23.5 ± 1.0	24.2 ± 1.0	24.5 ± 1.0	24.5 ± 1.0	25.0 ± 1.0	2 (1, 2)	0.002
Adiponectin, μg/mL							
Age-adjusted	6.9 ± 1.0	7.2 ± 1.0	7.3 ± 1.0	7.6 ± 1.0	8.0 ± 1.0	4 (2, 5)	<0.0001
Multivariable	7.1 ± 1.0	7.4 ± 1.0	7.4 ± 1.0	7.6 ± 1.0	8.0 ± 1.0	3 (1, 4)	0.0003
Multivariable + BMI	7.0 ± 1.0	7.2 ± 1.0	7.2 ± 1.0	7.4 ± 1.0	7.7 ± 1.0	2 (1, 4)	0.002
Free leptin index[4]							
Age-adjusted	0.9 ± 1.1	0.8 ± 1.0	0.7 ± 1.0	0.7 ± 1.0	0.6 ± 1.1	−10 (−13, −7)	<0.0001
Multivariable	0.8 ± 1.1	0.7 ± 1.1	0.6 ± 1.1	0.7 ± 1.1	0.6 ± 1.1	−8 (−11, −5)	<0.0001
Multivariable + BMI	0.8 ± 1.1	0.7 ± 1.0	0.7 ± 1.0	0.7 ± 1.0	0.6 ± 1.1	−5 (−8, −2)	0.0002
Leptin-to-adiponectin ratio							
Age-adjusted	3.0 ± 1.1	2.7 ± 1.0	2.4 ± 1.0	2.3 ± 1.0	1.9 ± 1.1	−10 (−14, −7)	<0.0001
Multivariable	2.7 ± 1.1	2.4 ± 1.1	2.2 ± 1.1	2.2 ± 1.1	1.8 ± 1.1	−9 (−12, −6)	<0.0001
Multivariable + BMI	2.7 ± 1.1	2.5 ± 1.1	2.3 ± 1.1	2.4 ± 1.1	2.0 ± 1.1	−7 (−10, −4)	<0.0001
Insulin, uU/mL							
Age-adjusted	6.0 ± 1.0	5.4 ± 1.0	5.3 ± 1.0	5.0 ± 1.0	4.4 ± 1.0	−7 (−10, −5)	<0.0001
Multivariable	5.6 ± 1.1	5.1 ± 1.0	5.1 ± 1.0	4.9 ± 1.1	4.4 ± 1.1	−6 (−9, −3)	< 0.0001
Multivariable + BMI	5.7 ± 1.0	5.4 ± 1.0	5.5 ± 1.0	5.2 ± 1.0	4.8 ± 1.0	−4 (−6, −1)	0.003
RBP-4, μg/mL							
Age-adjusted	33.9 ± 1.0	34.2 ± 1.0	34.5 ± 1.0	34.4 ± 1.0	34.8 ± 1.0	1 (0, 2)	0.17
Multivariable	35.3 ± 1.0	35.4 ± 1.0	35.6 ± 1.0	35.3 ± 1.0	35.4 ± 1.0	0 (−1, 1)	0.95
Multivariable + BMI	35.3 ± 1.0	35.5 ± 1.0	35.7 ± 1.0	35.4 ± 1.0	35.6 ± 1.0	0 (−1, 1)	0.73
CRP, mg/L							
Age-adjusted	1.06 ± 1.07	0.99 ± 1.06	1.04 ± 1.06	0.95 ± 1.06	0.85 ± 1.07	−6 (−10, −1)	0.02
Multivariable	1.04 ± 1.10	0.95 ± 1.09	0.98 ± 1.09	0.90 ± 1.09	0.81 ± 1.09	−6 (−11, −1)	0.02
Multivariable + BMI	1.06 ± 1.09	1.01 ± 1.08	1.08 ± 1.08	1.00 ± 1.08	0.93 ± 1.09	−3 (−7, 2)	0.18
IL-6, pg/mL							
Age-adjusted	0.93 ± 1.03	0.89 ± 1.03	0.87 ± 1.03	0.87 ± 1.03	0.82 ± 1.04	−3 (−5, −1)	0.02
Multivariable	0.89 ± 1.05	0.86 ± 1.04	0.86 ± 1.04	0.87 ± 1.04	0.83 ± 1.05	−1 (−4, 1)	0.30
Multivariable + BMI	0.89 ± 1.04	0.88 ± 1.04	0.89 ± 1.04	0.91 ± 1.04	0.89 ± 1.05	0 (−2, 3)	0.85

[1] Values are least squares geometric means ± SEs from linear mixed model to account for within-person correlation between the 2 time points. AHEI, Alternative Healthy Eating Index; CRP, C-reactive protein; RBP-4, retinol-binding protein-4.
[2] Adjusted for age and time period.
[3] Adjusted for age, time period, menopausal status, postmenopausal hormone use, total energy intake, physical activity, and smoking.
[4] Free leptin index is defined as the ratio of leptin to soluble leptin receptor.

majority of women (90.1%) increased their dietary scores over follow-up, and only 1.6% decreased by ≥10 points. Women with the greatest improvement in dietary quality (highest quintile), compared with women with the least improvement (lowest quintile), had a significantly smaller increase in leptin (multivariable-adjusted percentage change: 13% compared with 42%, respectively; *P*-trend < 0.0001) and a greater increase in adiponectin (multivariable-adjusted percentage change: 14% compared with 6%, respectively; *P*-trend = 0.02) over follow-up. Similar to leptin, inverse trends were observed for free leptin index (*P*-trend = 0.0002) and leptin:adiponectin (*P*-trend < 0.0001). Although the highest quintile of AHEI score change was associated with a 4% increase in sOB-R concentration, the lowest quintile showed a slight decrease of −1% (*P*-trend = 0.04). The opposite was seen for CRP, with the percentage changes associated with the most and least improvement being −11% and 16%, respectively (*P*-trend = 0.02). These associations were attenuated after adjusting for concurrent weight change; only leptin, free leptin index, and leptin:adiponectin remained statistically significant. Improvement of dietary quality was not associated with changes in insulin, RBP-4, or IL-6 over time.

We did not observe significant differences by baseline AHEI score in the association between changes in dietary score and changes in biomarkers (all *P*-interaction ≥ 0.15; **Supplemental Table 1**). However, the impact of dietary improvement on changes in leptin, sOB-R, adiponectin, free leptin index, leptin:adiponectin,

TABLE 3 Percentage change in biomarker concentrations across quintiles of AHEI-2010 score change between 1995 and 2011[1]

	AHEI-2010 score change					
	Quintile 1 (n = 162)	Quintile 2 (n = 167)	Quintile 3 (n = 167)	Quintile 4 (n = 175)	Quintile 5 (n = 160)	P-trend
Median (range)	0.0 (−21.0 to 5.5)	9.0 (6.0–12.5)	16.0 (13.0–18.5)	22.5 (19.0–26.0)	31.5 (26.5–57.0)	
Leptin						
Multivariable[2]	42 (22, 66)	37 (18, 60)	24 (6, 44)	12 (−4, 32)	13 (−4, 32)	<0.0001
Multivariable + weight change[3]	33 (16, 52)	30 (14, 49)	22 (6, 39)	11 (−4, 27)	16 (1, 34)	0.002
Soluble leptin receptor						
Multivariable	−1 (−6, 4)	−1 (−6, 5)	0 (−5, 6)	0 (−5, 5)	4 (−1, 10)	0.04
Multivariable + weight change	0 (−5, 6)	0 (−5, 6)	1 (−4, 6)	0 (−5, 6)	4 (−2, 9)	0.23
Adiponectin						
Multivariable	6 (−2, 15)	6 (−2, 14)	5 (−3, 14)	10 (1, 19)	14 (5, 24)	0.02
Multivariable + weight change	8 (0, 17)	7 (0, 16)	6 (−1, 15)	11 (3, 20)	13 (5, 22)	0.14
Free leptin index[4]						
Multivariable	45 (20, 75)	35 (12, 62)	25 (4, 51)	15 (−5, 39)	11 (−9, 34)	0.0002
Multivariable + weight change	33 (13, 58)	27 (8, 50)	23 (4, 45)	13 (−5, 34)	15 (−3, 37)	0.01
Leptin-to-adiponectin ratio						
Multivariable	35 (11, 65)	27 (5, 54)	19 (−2, 44)	4 (−15, 28)	1 (−17, 23)	<0.0001
Multivariable + weight change	24 (4, 47)	19 (0, 40)	16 (−2, 38)	2 (−14, 22)	5 (−12, 26)	0.007
Insulin						
Multivariable	27 (4, 55)	29 (6, 56)	27 (5, 55)	19 (−3, 46)	17 (−5, 43)	0.23
Multivariable + weight change	22 (1, 49)	24 (3, 50)	27 (5, 53)	20 (−1, 46)	21 (−1, 47)	0.80
RBP-4						
Multivariable	8 (2, 13)	7 (1, 13)	5 (−1, 10)	6 (1, 12)	7 (1, 13)	0.75
Multivariable + weight change	7 (1, 13)	6 (1, 12)	4 (−1, 10)	6 (1, 12)	7 (1, 13)	1.00
CRP						
Multivariable	16 (−13, 54)	11 (−16, 47)	6 (−20, 40)	0 (−26, 34)	−11 (−34, 19)	0.02
Multivariable + weight change	8 (−18, 43)	5 (−20, 37)	4 (−21, 37)	−1 (−26, 31)	−9 (−31, 21)	0.14
IL-6						
Multivariable	19 (−1, 41)	12 (−6, 33)	17 (−2, 39)	18 (−2, 41)	12 (−7, 35)	0.73
Multivariable + weight change	17 (−2, 39)	11 (−7, 32)	16 (−2, 38)	18 (−2, 41)	13 (−6, 36)	0.98

[1] Values are adjusted least squares percent changes (95% CIs) in biomarker concentrations over time from general linear model. AHEI, Alternative Healthy Eating Index; CRP, C-reactive protein; RBP-4, retinol-binding protein-4.
[2] Adjusted for age, baseline dietary scores, baseline and change in total caloric intake and physical activity, change in smoking status, and change in menopausal status and postmenopausal hormone use.
[3] Concurrent weight change between 1995 and 2011.
[4] Free leptin index is defined as the ratio of leptin to soluble leptin receptor.

and CRP appeared more evident among women with lower baseline dietary quality. For example, among women with baseline AHEI ≤ 50, the percentage of increase in leptin over time was considerably greater (60% increase) for women with the least dietary improvement than it was for women with the most dietary improvement (16% increase; P-trend = 0.0002). However, among women with baseline AHEI > 50, there was less contrast in leptin increases: 27% and 11% for women with the least and most dietary improvement, respectively (P-trend = 0.10). By contrast, change in insulin (P-interaction = 0.15), which was not associated with change in AHEI score overall, showed a modest inverse association among women with baseline AHEI > 50 (P-trend = 0.05) but not among women with baseline AHEI ≤ 50 (P-trend = 0.99). No trends were observed for RBP-4 or IL-6 regardless of baseline AHEI score.

Discussion

In this sample of middle-aged women free of cardiovascular disease and diabetes over an average period of 13 y, higher dietary quality as assessed by AHEI-2010 was associated with significantly lower plasma concentrations of leptin, free leptin index, leptin:adiponectin, insulin, and CRP and with higher concentrations of sOB-R and adiponectin. Women who improved the quality of their diets over this time period also had more favorable changes in these biomarkers of the brain-adipose axis (except insulin), independent of baseline dietary quality. However, at least some of the association was explained by concurrent weight change. RBP-4 and IL-6 were not associated with overall dietary quality or change in dietary quality.

Our findings are consistent with inverse associations between healthy dietary patterns and leptin, insulin, and CRP, and their positive associations with adiponectin, as reported in prior cross-sectional studies (22–27). Compared with the dietary patterns evaluated in these studies (e.g., the prudent pattern or the Mediterranean dietary pattern), which recommend higher intakes of fruit, vegetables, and whole grains and lower intakes of red meat and saturated fats, the high-quality dietary pattern characterized by AHEI-2010 additionally takes into account the potential health effects by PUFAs, protein sources, and sugar-sweetened beverages from emerging evidence of their associations with chronic diseases (29). These differences may contribute to the somewhat stronger and more consistent associations in the

current study. We also observed higher sOB-R and lower free leptin index and leptin:adiponectin with higher dietary quality, which have not been evaluated previously. However, the significant inverse relation between healthy dietary patterns and IL-6 reported in some previous studies (22, 25–27, 35) was only observed in our age-adjusted model. Of note, the association with IL-6 was substantially attenuated in several studies after adjusting for BMI or waist circumference (22, 25, 27).

To our knowledge, no study has examined long-term changes in dietary quality in relation to changes in these plasma biomarkers. Our within-person change-to-change analysis confirmed and strengthened the cross-sectional results and provided further support for the health benefits of a high-quality diet. We also noted that the benefits of improving dietary quality on plasma biomarkers were independent of the baseline dietary quality, although the benefits appeared suggestively stronger for women starting with a poor-quality diet. Because women initially high in dietary quality had less room for improvement, lack of exposure variation may have prevented us from detecting more significant trends in these women. However, because most women in the study sample improved their diet during follow-up, our analysis could not adequately evaluate diets worsening in quality. As expected, several biomarkers were not significantly associated with dietary quality change after further adjusting for weight change.

Interestingly, we observed that leptin (and the free leptin index) increased over time across all categories of dietary quality change although diet improved (to a lesser or greater extent) among nearly all the women. Although this may reflect changes in body fat, hormonal milieu, and other nondietary factors over the 13 y of observation (36, 37), it is worth noting that the increase in leptin concentrations was smallest for women with the greatest dietary improvement. Similarly, a small increase in adiponectin occurred even among women with limited dietary improvement, consistent with the well-documented positive association between age and adiponectin (14). However, the increase was more substantial for women who had the most dietary improvement. These data suggest that improving dietary quality may potentially delay development of age-related leptin resistance and may amplify age-related increase of adiponectin. Further, insulin was only cross-sectionally, but not longitudinally, associated with dietary quality. It is possible that long-term changes in insulin may be predominantly determined by the onset of certain preclinical conditions, such as insulin resistance or prediabetes. Reverse causation may also obscure the inverse association, if women with certain metabolic conditions such as prediabetes or metabolic syndrome characterized by elevated fasting insulin were more likely to improve their dietary quality. Therefore, the impact of dietary improvement on insulin may be more likely to be observable among women at lower risk of developing metabolic abnormalities, such as those with high baseline dietary quality among whom we observed a modest inverse association.

Our observations have important biological implications. First, although many of these molecules are secreted by adipose tissues, they have regulatory feedbacks on energy intake and adipocyte differentiation through their receptors located in the CNS (7). Such brain-adipose crosstalk is further influenced by adiposity. For example, in obese individuals, the leptin transport system across the blood-brain barrier is compromised, a possible mechanism that impairs the appetite-suppressing effects of leptin and contributes to leptin resistance (38). The interplay between these biomarkers and adiposity corresponds to the consistent attenuation of the associations after adjusting for BMI or weight change in our analysis. Second, there are substantial overlaps in the physiologic functions between brain-adipose biomarkers and inflammatory cytokines, such as the insulin-sensitizing and anti-inflammatory properties for adiponectin and the proinflammatory role for leptin (39, 40). Conversely, IL-6 has been implicated as a brain-adipose cytokine to inhibit appetite (7, 41), although IL-6 did not show a strong association with dietary quality in our study. In addition, CRP can directly bind to leptin to block its effect on satiety (21). Third, despite the strong correlation with obesity, these biomarkers have previously been associated with insulin resistance, type 2 diabetes, and cardiovascular disease, independent of adiposity (14, 16–19, 31, 42–45). This suggests that these biomarkers are involved in various biological pathways that may serve as potential mechanisms underlying the associations of dietary quality with weight gain and cardiometabolic disorders (1–4, 29).

The current study has several strengths. We had repeated biomarker measurements and dietary assessments to evaluate the long-term longitudinal changes and associations between these changes. A number of plasma biomarkers of brain-adipose axis (e.g., leptin, sOB-R, adiponectin), which had been evaluated in separate studies, were available simultaneously in the current study. This allowed us to evaluate the associations with physiologically relevant measures, including free leptin index and leptin:adiponectin. We also collected detailed and regularly updated covariate information to control for potential time-varying confounders. However, the generalizability of the results may be limited, because our study population was homogeneous, consisting of predominantly white, registered nurses. Particularly, few women in this sample lowered their dietary quality over time, the impact of which should be explored in future studies. The focus on apparently healthy women with potentially strong health-related motivations (e.g., volunteer to provide blood samples twice) also limited the ability to generalize our results to other populations. In addition, there may be potential misclassification of changes in dietary quality or biomarkers, given that our assessment was based on differences between only 2 discrete time points. However, we administered similar FFQs for dietary assessment, and the samples from the same individual were assayed in the same batch to reduce variability. Despite a number of important lifestyle factors considered in the analysis, the possibility of residual confounding cannot be excluded. Nevertheless, the strong, biologically plausible associations between dietary quality and biomarker concentrations observed consistently in both cross-sectional and longitudinal analyses were unlikely to be explained by misclassification or residual confounding.

In conclusion, higher or improved dietary quality is associated with more favorable profiles of plasma biomarkers of the brain-adipose axis. Our results provide further evidence and mechanistic insight at the biomarker level to support that a high-quality diet may be beneficial to prevent weight gain, type 2 diabetes, and cardiovascular disease. Additional investigation is needed to elucidate whether these plasma biomarkers mediate the association between dietary quality and weight gain or other cardiometabolic diseases.

Acknowledgments

TH and FBH designed and conducted the study; DKT developed the dietary quality score; TH performed the statistical analyses and drafted the manuscript; TH, DKT, AH, NR, SST, and FBH interpreted the data and critically revised the manuscript, as well as assume full responsibility for the analyses and interpretation of these data; and TH, DKT, SST, and FBH

had full access to all study data. All authors read and approved the final manuscript.

References

1. Fung TT, Pan A, Hou T, Chiuve SE, Tobias DK, Mozaffarian D, Willett WC, Hu FB. Long-term change in diet quality is associated with body weight change in men and women. J Nutr 2015;145:1850–6.
2. 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.
3. Beunza JJ, Toledo E, Hu FB, Bes-Rastrollo M, Serrano-Martinez M, Sanchez-Villegas A, Martinez JA, Martinez-Gonzalez MA. Adherence to the Mediterranean diet, long-term weight change, and incident overweight or obesity: the Seguimiento Universidad de Navarra (SUN) cohort. Am J Clin Nutr 2010;92:1484–93.
4. Gao SK, Beresford SA, Frank LL, Schreiner PJ, Burke GL, Fitzpatrick AL. Modifications to the Healthy Eating Index and its ability to predict obesity: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr 2008;88:64–9.
5. Shai I, Schwarzfuchs D, Henkin Y, Shahar DR, Witkow S, Greenberg I, Golan R, Fraser D, Bolotin A, Vardi H, et al. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. N Engl J Med 2008;359:229–41.
6. Nordmann AJ, Nordmann A, Briel M, Keller U, Yancy WS, Jr., Brehm BJ, Bucher HC. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. Arch Intern Med 2006;166:285–93.
7. Shimizu H, Mori M. The brain-adipose axis: a review of involvement of molecules. Nutr Neurosci 2005;8:7–20.
8. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature 2006;444:847–53.
9. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292–5.
10. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, Considine RV. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. Lancet 1996;348:159–61.
11. Myers MG, Jr., Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: distinguishing cause from effect. Trends Endocrinol Metab 2010;21:643–51.
12. Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metab 2003;88:1730–6.
13. Gerozissis K. Brain insulin and feeding: a bi-directional communication. Eur J Pharmacol 2004;490:59–70.
14. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003;46:459–69.
15. Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS. Adiponectin acts in the brain to decrease body weight. Nat Med 2004;10:524–9.
16. Finucane FM, Luan J, Wareham NJ, Sharp SJ, O'Rahilly S, Balkau B, Flyvbjerg A, Walker M, Hojlund K, Nolan JJ, et al. Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. Diabetologia 2009;52:2345–9.
17. Thorand B, Zierer A, Baumert J, Meisinger C, Herder C, Koenig W. Associations between leptin and the leptin / adiponectin ratio and incident Type 2 diabetes in middle-aged men and women: results from the MONICA / KORA Augsburg study 1984–2002. Diabet Med 2010;27:1004–11.
18. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, Wason CJ, Oberbach A, Jansson PA, Smith U, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006;354:2552–63.
19. Yang Q, Graham TE, Mody N, Preitner F, Peroni OD, Zabolotny JM, Kotani K, Quadro L, Kahn BB. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005;436:356–62.
20. Stenlöf K, Wernstedt I, Fjällman T, Wallenius V, Wallenius K, Jansson JO. Interleukin-6 levels in the central nervous system are negatively correlated with fat mass in overweight/obese subjects. J Clin Endocrinol Metab 2003;88:4379–83.
21. Chen K, Li F, Li J, Cai H, Strom S, Bisello A, Kelley DE, Friedman-Einat M, Skibinski GA, McCrory MA, et al. Induction of leptin resistance through direct interaction of C-reactive protein with leptin. Nat Med 2006;12:425–32.
22. Fargnoli JL, Fung TT, Olenczuk DM, Chamberland JP, Hu FB, Mantzoros CS. Adherence to healthy eating patterns is associated with higher circulating total and high-molecular-weight adiponectin and lower resistin concentrations in women from the Nurses' Health Study. Am J Clin Nutr 2008;88:1213–24.
23. Yannakoulia M, Yiannakouris N, Melistas L, Kontogianni MD, Malagaris I, Mantzoros CS. A dietary pattern characterized by high consumption of whole-grain cereals and low-fat dairy products and low consumption of refined cereals is positively associated with plasma adiponectin levels in healthy women. Metabolism 2008;57:824–30.
24. Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC, Hu FB. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. Am J Clin Nutr 2001;73:61–7.
25. Lopez-Garcia E, Schulze MB, Fung TT, Meigs JB, Rifai N, Manson JE, Hu FB. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2004;80:1029–35.
26. Fung TT, McCullough ML, Newby PK, Manson JE, Meigs JB, Rifai N, Willett WC, Hu FB. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2005;82:163–73.
27. Nettleton JA, Steffen LM, Mayer-Davis EJ, Jenny NS, Jiang R, Herrington DM, Jacobs DR, Jr. Dietary patterns are associated with biochemical markers of inflammation and endothelial activation in the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Clin Nutr 2006;83:1369–79.
28. Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, Giugliano D. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. JAMA 2003;289:1799–804.
29. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142:1009–18.
30. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. Cancer Res 2006;66:2476–82.
31. Sun Q, van Dam RM, Meigs JB, Franco OH, Mantzoros CS, Hu FB. Leptin and soluble leptin receptor levels in plasma and risk of type 2 diabetes in U.S. women: a prospective study. Diabetes 2010;59:611–8.
32. Silha JV, Nyomba BL, Leslie WD, Murphy LJ. Ethnicity, insulin resistance, and inflammatory adipokines in women at high and low risk for vascular disease. Diabetes Care 2007;30:286–91.
33. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semi-quantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
34. 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.
35. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Dietary patterns and markers of systemic inflammation among Iranian women. J Nutr 2007;137:992–8.
36. Staiger H, Tschritter O, Machann J, Thamer C, Fritsche A, Maerker E, Schick F, Haring HU, Stumvoll M. Relationship of serum adiponectin and leptin concentrations with body fat distribution in humans. Obes Res 2003;11:368–72.
37. Wildman RP, Wang D, Fernandez I, Mancuso P, Santoro N, Scherer PE, Sowers MR. Associations of testosterone and sex hormone binding globulin with adipose tissue hormones in midlife women. Obesity (Silver Spring) 2013;21:629–36.

38. Banks WA. Is obesity a disease of the blood-brain barrier? Physiological, pathological, and evolutionary considerations. Curr Pharm Des 2003;9:801–9.
39. Tschritter O, Fritsche A, Thamer C, Haap M, Shirkavand F, Rahe S, Staiger H, Maerker E, Haring H, Stumvoll M. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 2003;52:239–43.
40. Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. J Am Soc Nephrol 2004;15:2792–800.
41. Wallenius K, Wallenius V, Sunter D, Dickson SL, Jansson JO. Intracerebroventricular interleukin-6 treatment decreases body fat in rats. Biochem Biophys Res Commun 2002;293:560–5.
42. Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, Sattar N. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 2001;104:3052–6.
43. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes 2004;53:693–700.
44. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836–43.
45. Rubins HB, Robins SJ, Collins D, Nelson DB, Elam MB, Schaefer EJ, Faas FH, Anderson JW. Diabetes, plasma insulin, and cardiovascular disease: subgroup analysis from the Department of Veterans Affairs high-density lipoprotein intervention trial (VA-HIT). Arch Intern Med 2002;162:2597–604.