Published in final edited form as:

Obesity (Silver Spring). 2020 February ; 28(2): 452–459. doi:10.1002/oby.22706.

A prospective study of the long-term inter-relationships among adiposity-associated biomarkers in women

Megu Y. Baden¹, Frank B. Hu^{1,2,3}, Tianyi Huang^{1,3}

¹Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA

²Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, MA

³Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA

Abstract

Objective: This study aimed to investigate the prospective inter-relationships among biomarkers that may provide mechanistic insights into obesity-related diseases.

Methods: We included 850 women in the Nurses' Health Study II with two fasting blood measurements (1996–1999 and 2010–2011) of adiponectin, leptin, soluble leptin receptor, insulin, retinol binding protein-4, high-sensitivity C-reactive protein (hsCRP), and interleukin-6. We examined biomarker inter-relationships in three ways: (1) cross-sectional associations at baseline and follow-up; (2) longitudinal associations of concurrent biomarker changes; and (3) prospective associations of each baseline biomarker with other biomarker changes.

Results: In cross-sectional analyses, most biomarkers were correlated after multivariable adjustment including BMI, with the strongest correlations observed between leptin and insulin and between hsCRP and interleukin-6. In longitudinal analyses, similar results were observed after multivariable adjustment including weight change. However, in prospective analyses, only three associations observed in cross-sectional and longitudinal analyses were consistently significant ($p < 0.05$). Every doubling in baseline adiponectin was associated with –9.0% insulin change. The corresponding estimate was 9.3% for baseline leptin and hsCRP change and 3.1% for baseline hsCRP and leptin change.

Conclusions: Baseline adiponectin concentrations were inversely associated with subsequent insulin change, whereas baseline leptin concentrations were positively associated with hsCRP change and vice versa.

Contact information of the corresponding author: Megu Y. Baden, Department of Nutrition, Harvard T. H. Chan School of Public Health, 665 Huntington Avenue, Boston, MA 02115, USA, Phone number: (617) 432-1333, mbaden@hsph.harvard.edu.

Author contributions:

MYB, FBH, and TH conceived and design this study. FBH participated in the data collection. MYB and TH analyzed the data. MYB drafted the manuscript. All authors critically revised the manuscript for important intellectual content and approved the final manuscript.

Disclosure: The authors declared no conflict of interest.

Data sharing statement: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Prior Presentation: The results were presented in the abstract form at the 79th Scientific Sessions of the American Diabetes Association, San Francisco, CA, on June 7–11, 2019.

Keywords

Adipokines; Adiponectin; Leptin; Insulin; C-Reactive Protein

Introduction

It is now recognized that adipose tissue acts as an important endocrine organ that synthesizes and secretes various adipokines, such as adiponectin, leptin, and retinol binding protein 4 (RBP-4) (1, 2, 3). These biologically active compounds play critical roles in the regulation of adipogenesis and energy metabolism (2, 4, 5). In turn, accumulation of adipose tissues, particularly excess visceral fat, induces dysregulation of these adipokines and leads to adverse metabolic responses including elevated inflammation and insulin resistance. Prior studies have consistently reported that obesity is associated with higher concentrations of leptin, insulin, RBP-4, C-reactive protein (CRP), and interleukin-6 (IL-6) and lower concentration of adiponectin and soluble leptin receptor (sOB-R) (1, 2, 3, 6, 7, 8). Further, these adiposity-associated biomarkers have been shown to predict future risk of cardiometabolic disease and cancer after adjustment for adiposity measures, suggesting their independent roles in disease development (9, 10, 11, 12, 13).

Given the diverse biologic functions of these adiposity-associated biomarkers, elucidating their inter-relationships is fundamental to understand the metabolic consequences of obesity. Although a number of cross-sectional studies reported strong correlations among these adiposity-associated biomarkers (8, 14, 15, 16, 17) and a few longitudinal studies reported several associations between changes in biomarkers (18, 19), the long-term directionality of their inter-relationships remains largely unclear. For example, it is unknown whether increased inflammation is associated with changes in insulin resistance or leptin resistance (or vice versa). Interestingly, experimental studies have revealed some mechanistic interactions among these adiposity-associated biomarkers. For instance, CRP can directly inhibit leptin effect to control satiety and promote leptin resistance (20).

To provide evidence for the long-term inter-relationships among adiposity-associated biomarkers in humans, using two repeated blood samples collected 11–15 years apart, we conducted the current analysis in 850 healthy women to examine cross-sectional, longitudinal, and prospective inter-relationships of seven adiposity-associated biomarkers, including adiponectin, leptin, sOB-R, insulin, RBP-4, high-sensitivity CRP (hsCRP), and IL-6.

Methods

Study Participants

The Nurses' Health Study II was established in 1989 among 116,686 US female registered nurses, aged 25–42 years. All women completed a baseline questionnaire, and their lifestyle factors and medical histories were updated by biennial follow-up questionnaires. In 1996–1999, 29,611 women provided a blood sample; of these, 15,982 women provided a second blood sample in 2008–2011. The same protocol was used for both collection, which has

been described in detail elsewhere (21). Briefly, participants had their blood drawn using a collection kit and shipped back with an ice pack by overnight courier to the laboratory, where it was processed and separated into plasma, red and white blood cells. All samples have been stored in liquid nitrogen freezers since collection.

For the current study, we randomly selected 850 women who provided two fasting (8 h since last meal at blood collection) blood samples 13 years apart (from 1996–1999 to 2010–2011) and were free from type 2 diabetes (T2D), cardiovascular diseases, and cancer (22). The study protocol was approved by the institutional review boards of the Harvard T. H. Chan School of Public Health and Brigham and Women’s Hospital. Completion of the self-administered questionnaire was considered to imply informed consent.

Plasma Biomarker Measurements

Detailed methods of plasma biomarker measurements have been described elsewhere (22, 23). Specifically, 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 (Minneapolis, MN, USA). Total adiponectin was assessed with a quantitative monoclonal sandwich ELISA (Alpco Diagnostics, Windham, NH, USA). Insulin was determined by an electrochemiluminescence immunoassay with the use of the Roche E modular system (Roche Diagnosis, Indianapolis, IN, USA). hsCRP was measured by an immunoturbidimetric assay (Denka Seiken, Tokyo, Japan). The baseline and follow-up samples from the same participant were assayed in the same batch to reduce variability. All assays included 10% quality controls, and the laboratory was blinded to participant characteristics and the status of the samples (i.e., baseline, follow-up or quality control samples). The mean inter assay coefficients of variation (CVs) were 10.1 % for adiponectin, 4.9 % for leptin, 11.1 % for sOB-R, 6.4 % for insulin, 10.3 % for RBP-4, 1.5 % for hsCRP, and 10.8 % for IL-6. To assess the biomarker stability over time, we calculated the crude intraclass correlation coefficient for each biomarker by using variance components (between-person and within-person variance) from a random-effects model (24). Most biomarker measurements were moderately stable over a mean follow-up of 13 years (intraclass correlation coefficients ranged from 0.41 for IL-6 to 0.76 for adiponectin, Table 1).

Covariate Assessments

The biennial questionnaires updated participants’ information on their lifestyle and other cardiometabolic risk factors including age, BMI, smoking status, physical activity, menopausal status, postmenopausal hormone use, hypertension, and hypercholesterolemia. BMI was calculated as kg/m^2 based on self-reported weight and height, which have previously been shown to have high reliability (25). Physical activity was assessed by metabolic equivalent task hours per week (MET-h/week) from a validated questionnaire (26). Dietary data were collected every four years using a semi-quantitative food frequency questionnaire (FFQ). Participants reported how often, on average, they had consumed defined portions of the 130 food items over the previous year using nine response categories ranging from “never or less than once/month” to “ six times/day”. The reliability and validity of the FFQ have been described previously (27, 28). Alcohol consumption (gram/

day) and total energy intake (kcal/day), as well as the Alternate Healthy Eating Index 2010 (AHEI-2010) scores that reflect overall dietary patterns (29), were derived from the FFQ. In the analysis, we considered the covariates that were collected concurrently with blood collection, i.e., assessed in either 1995 or 1999 (the closest to the first blood measurement) and in 2011 (close to the second blood measurement) following the previous study (22, 23).

Statistical Analyses

All biomarker concentrations were log₂-transformed to normalize distributions. Based on the generalized extreme studentized deviate many-outlier procedure, nine participants with extremely low or high biomarker concentrations were identified and excluded from the corresponding analyses (30). We analyzed the biomarker inter-relationships in three ways. First, Spearman partial correlations were used to examine the cross-sectional biomarker correlations at baseline and at follow-up separately, adjusting for age, BMI, total energy intake (in quintiles), AHEI-2010 scores (in quintiles), alcohol intake (in quintiles), smoking status, physical activity (in quintiles), menopausal status, postmenopausal hormone use, hypertension, and hypercholesterolemia. Second, we used multivariate general linear regression models to evaluate the longitudinal associations of biomarker changes, by modeling each biomarker change as the exposure and concurrent changes in other biomarkers as the outcomes. We adjusted for baseline age, baseline BMI (in quintiles), baseline corresponding exposure biomarker concentrations (in quintiles), baseline corresponding outcome biomarker concentrations (in quintiles), menopausal status, postmenopausal hormone use, smoking status, hypertension, hypercholesterolemia, baseline and changes in each of total energy intake (in quintiles), AHEI-2010 score, alcohol intake (in quintiles), physical activity (in quintiles), and weight change (in quintiles). Third, we used multivariate general linear regression models to evaluate the prospective associations of each baseline biomarker concentration as the exposure with changes in other biomarkers as the outcomes with adjustment for the same baseline covariates that were described above.

In the primary analysis, we considered the exposure as a continuous variable. For longitudinal analyses, we presented the association as the multivariable-adjusted percent differences of the outcome biomarker changes for every doubling of the percent changes of the exposure biomarker. For prospective analyses, we presented the associations as the multivariable-adjusted percent differences of the outcome biomarker changes for every doubling of the baseline exposure biomarker concentrations. These primary results were visualized in bar graphs. Secondly, we evaluated these associations by considering the exposures as categorical variables in quintile. Further, given the biological roles of these biomarkers in adiposity, we estimated the percent of the biomarker associations explained by adiposity measures by comparing the regression coefficients with versus without adjustment for weight change (in longitudinal analyses) or baseline BMI (in prospective analyses).

Analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC), and p value < 0.05 were considered statistically significant. Given the number of tests performed, we further conducted Bonferroni correction for multiple testing adjustment as a sensitivity analysis to identify the most robust associations.

Results

At baseline, participants were 45 ± 5 years of age and the mean BMI was 24.7 ± 3.6 kg/m². During a mean follow-up of 13 years (range: 11–15 years), all mean concentrations of seven adiposity-associated biomarkers increased (Table 2). In addition, participants on average had increased BMI (24.7 to 26.4 kg/m²), alcohol intake (3.9 to 6.2 g/day), physical activity (17.6 to 23.5 MET-h/week), prevalence of hypertension (11.3 to 29.2%) and hypercholesterolemia (15.2 to 34.9%), and decreased total energy intake (1888 to 1758 kcal/day) and cigarette smoking (4.0 to 1.4%) over the follow-up (Table 2). About half of the women underwent menopausal transition during this period (postmenopausal women: 21.2 to 71.9%), whereas the prevalence of postmenopausal hormone use decreased substantially (83.7 to 23.5%).

In cross-sectional analyses (Table 3A, 3B), most biomarkers were consistently correlated after multivariable adjustment including BMI ($p < 0.05$) both at baseline and at follow-up, with the strongest associations observed between leptin and insulin ($r = 0.34$ at baseline and 0.28 at follow-up), hsCRP and IL-6 ($r = 0.30$ and 0.33 , respectively), adiponectin and sOB-R ($r = 0.22$ and 0.29 , respectively), adiponectin and insulin ($r = -0.20$ and -0.26 , respectively), and leptin and hsCRP ($r = 0.25$ and 0.24 , respectively). These correlations remained significant after Bonferroni correction ($p < 0.05/21 = 0.002$).

In longitudinal analyses, most significant associations in cross-sectional analyses were similarly observed after multivariable adjustment including weight change, including associations between adiponectin and insulin (-19.3% [-27.4% to -10.2%] insulin change for every doubling in adiponectin percent change and -4.5% [-6.7% to -2.3%] adiponectin change for every doubling in insulin percent change) and leptin and hsCRP (18.1% [7.1% to 30.2%] hsCRP change associated with leptin change and 4.4% [1.8% to 7.0%] leptin change associated with hsCRP change) (Fig. 1, Table S1). Most associations remained significant after Bonferroni correction ($p < 0.05/6 = 0.008$). The percentage of these associations explained by weight change ranged from 8.1% for the association between hsCRP change and IL-6 change to 63.9% for the association between leptin change and IL-6 change (Table S1).

However, among those associations that were significant in cross-sectional and longitudinal analyses, only three associations remained statistically significant in prospective analyses after multivariable adjustment including baseline BMI, including the associations between baseline adiponectin and insulin change, baseline leptin and hsCRP change, and baseline hsCRP and leptin change (Fig. 2, Table S2). Every doubling in baseline adiponectin concentration was associated with -9.0% insulin change (95% CI, -15.5% to -2.0% , $p = 0.01$). The comparable estimate was 9.3% (95% CI, 0.1% to 19.5% , $p = 0.049$) for the association between baseline leptin and hsCRP change, and 3.1% (95% CI, 0.6% to 5.6% , $p = 0.02$) for the association between baseline hsCRP and leptin change. Baseline BMI explained 23.7% (95% CI, 11.1% to 43.6%) of the association between baseline adiponectin and insulin change, 25.9% (95% CI, 3.6% to 76.7%) of the association between baseline leptin and hsCRP change, and 20.1% (95% CI, 7.1% to 45.3%) of the association between baseline hsCRP and leptin change (Table S2). However, these associations were not significant after Bonferroni correction. Despite strong positive associations observed in the

cross-sectional and longitudinal analyses, there were no consistent positive associations in prospective analyses between adiponectin and sOB-R, adiponectin and hsCRP, leptin and insulin, and hsCRP and IL-6. Unexpectedly, baseline RBP-4 concentrations appeared to be predictive of changes in several adiposity-associated biomarkers including adiponectin, sOB-R and hsCRP ($p < 0.05$), although RBP-4 did not display consistent associations in the cross-sectional or longitudinal analysis.

Discussion

In the current analyses, while most adiposity-associated biomarkers were inter-related both cross-sectionally and longitudinally after multivariable adjustment, we found that baseline plasma concentrations of adiponectin, leptin, and hsCRP were predictive of 13-year changes in insulin, hsCRP, and leptin, respectively.

Our results from cross-sectional and longitudinal analyses were in line with previous studies, yielding remarkably similar estimates regarding the strength of the correlations. One prior study including 1,254 women who were free from diabetes in the Nurses' Health Study reported significant correlations between adiponectin and sOB-R ($r = 0.26$, $p < 0.01$), leptin and sOB-R ($r = -0.14$, $p < 0.01$), and leptin and CRP ($r = 0.18$, $p < 0.01$) after multivariable-adjustment including BMI (12). There was no significant association between leptin and insulin, possibly because this study included both fasting and non-fasting samples. Another cross-sectional analysis among 240 Polynesians showed that fasting serum leptin concentration was significantly associated with fasting insulin concentration (beta = 0.19, $p < 0.001$) after adjustment for sex, BMI, and their multiplicative interaction (31). In a cross-sectional study of 488 Japanese participants, serum fasting high molecular weight adiponectin concentration was inversely associated with insulin concentration (beta = -0.29, $p < 0.001$) after adjustment for age, sex, and BMI (17). In addition, Black et al. examined fasting biomarker concentrations in 1,203 Mexican Americans at baseline and a subset of 366 with repeated measurement at 4.6 years later, and reported significant correlations between CRP and leptin ($r = 0.39$ in cross-sectional analysis and $r = 0.19$ for longitudinal analysis) and between CRP and IL-6 ($r = 0.33$ for cross-sectional and $r = 0.29$ for longitudinal analysis) adjusting for age and sex in cross-sectional analysis and age, sex, and baseline body fat percentage in longitudinal analysis (18).

Only a few previous studies examined prospective relationships of adiposity-associated biomarkers. In the Whitehall II study, Herder et al. used the data from 7,683 British non-diabetic participants and revealed an inverse association between baseline adiponectin and 5-year change in insulin that was consistent with our results (19). However, they also reported positive associations of baseline hsCRP and IL-6 with insulin change and of baseline insulin with adiponectin change, which were not observed in the current study with longer follow-up. By contrast, Choi et al. found no association of baseline adiponectin with 3-year changes in insulin among 159 Korean school-aged boys (32). Li et al. followed 352 school-aged Chinese children for 10 years and reported that baseline RBP-4 was positively correlated with leptin and insulin at follow-up, but not with adiponectin at follow-up (33). These discrepancies might be explained by differences in study sample characteristics (e.g., age, sex, race, etc.), sample size, length of follow-up, and covariate adjustment. Compared with

previous studies, our results captured the longest follow-up over 13 years, evaluated the biomarker inter-relationships in multiple complementary ways, and considered a number of important behavioral and lifestyle factors related to obesity, which may represent the most comprehensive and robust associations to date.

Interestingly, we observed more significant associations between adiposity-related biomarkers in longitudinal analyses than in prospective analyses. This may be explained by the fact that longitudinal analyses were by nature cross-sectional and may capture concurrent changes in other factors that contributed to insulin resistance and inflammation, such as changes in visceral fat amount or menopausal status during the follow-up period (1, 2, 3, 7, 32, 34). By contrast, a single biomarker measurement at baseline may not adequately reflect the long-term pattern over 13 years of follow-up, as indicated by the moderate intraclass correlation coefficients for most biomarkers (e.g., 0.45 for insulin and 0.41 for IL-6). The potential misclassification by using a single baseline biomarker measure may attenuate the observed longitudinal associations with changes of other biomarker.

However, the strength of the prospective analysis is its ability to elucidate the directionality of the temporal relationships between adiposity-related biomarkers, which may imply the underlying biologic mechanisms and deepen our understandings of the roles of these biomarkers in the development of obesity-related diseases. For example, multiple lines of evidence support the inverse association between baseline adiponectin and insulin change. Adiponectin activates the 5-adenosine monophosphate-activated protein kinase in skeletal muscle and liver, stimulates glucose uptake in myocytes, and reduces molecules involved in gluconeogenesis in liver (35). Previous animal and human studies showed that adiponectin improves insulin sensitivity and is associated with lower risk of T2D (36, 37, 38). In longitudinal analyses with rhesus monkeys, plasma adiponectin decreased in parallel to the progression of insulin resistance (36). The adiponectin knockout mice exhibited severe diet-induced insulin resistance with reduced insulin-receptor substrate 1-associated phosphatidylinositol 3 kinase activity in muscle, and the adiponectin expression reversed insulin resistance (37). In humans, a meta-analysis of 13 prospective studies of 14,598 patients with 1–18 years follow-up showed the association of higher adiponectin with a lower risk of T2D (38). Similarly, a bidirectional, recursive relationship between leptin and hsCRP has also been implicated in prior experimental studies. Chen et al. reported that CRP bound directly to leptin and blocked its ability to activate signal transducer and phosphatidylinositol-3-kinase in cultured cells (20). They also showed that the administration of human CRP attenuated the effects of human leptin in ob/ob mice, and physiological concentrations of leptin stimulated CRP expression in human hepatocytes (20). To our knowledge, this is the first study to report the long-term bidirectional association between leptin and hsCRP in humans. Stenvinkel et al. reported the positive relation between initial CRP and 1-year leptin change in 36 chronic renal failure patients during peritoneal dialysis, although the interpretation should be cautious because the participants were all patients with chronic renal failure and received peritoneal dialysis (39). Two interventional studies showed that leptin administration increased CRP concentrations in people without obesity and with moderate obesity during 3–7 weeks (40, 41). Our results expand these short-term experimental findings and suggest that these mechanisms may persist for a long-term period in healthy women.

In addition, our results provide support for the link between obesity and obesity-related diseases such as T2D, cardiovascular disease, and cancer. Numerous evidence has shown that excess adiposity is associated with low concentrations of adiponectin and high concentrations of leptin and hsCRP (1, 2, 6). The observed inverse association of baseline adiponectin with insulin increase implies that lower adiponectin concentrations may be associated with future development of insulin resistance, an important precipitating factor for T2D, cardiovascular diseases and some cancers (9, 42). Similarly, the observed bidirectional positive association between leptin and hsCRP suggests that higher leptin concentrations may lead to elevated inflammation whereas higher hsCRP at baseline may contribute to leptin resistance in the future; both inflammation and leptin resistance have been implicated in the pathogenesis of T2D, cardiovascular disease and some cancers (2, 9, 10, 42). These findings were also corroborated by a recent study, which highlighted the dynamic biomarker correlation structures and the central role of leptin and insulin in diabetes development (43). Notably, these associations were attenuated but remained significant after adjustment for BMI and/or weight change, suggesting that these mechanisms were related to but not entirely explained by the effect of adiposity.

The strength of this study is the repeated measurements of adiposity-associated biomarkers and numerous validated covariates that enabled us to evaluate the long-term prospective inter-relationships of biomarkers independent of other adiposity-related factors. Leveraging the unique study design, we were able to conduct three types of analyses in parallel to identify the most robust biomarker associations. However, several limitations should be noted. First, generalizability may be limited because participants in our study were predominantly Caucasian registered nurses without T2D, cardiovascular diseases, and cancer. However, this characteristic can also be an advantage because health motivations and knowledge in nurses ensures the collection of high quality information on their lifestyle and other cardiometabolic risk factors. Second, although we controlled for various lifestyle and cardiometabolic risk factors, the possibility of residual confounding and measurement errors cannot be excluded due to the observational nature of the study using self-reported questionnaires. Third, the potential effect of long-term blood storage on the biomarker stability might lead to non-differential measurement errors and likely attenuate the observed associations in this study. However, the questionnaires used in the current study were highly validated against multiple information including diet records and biomarkers (26, 27, 44). In addition, the consistency of our results with other previous experimental and population-based studies suggests that the observed associations were unlikely to be entirely explained by residual confounding or measurement errors. Fourth, while the long follow-up period of 13 years minimized the potential for reverse causation in prospective analyses, it may not adequately capture some short-term associations. For example, one study following 84 diabetic patients reported that adiponectin increased significantly within the first three months of insulin therapy but remained steady after 6 months (45), suggesting that insulin status may be associated with short-term changes in adiponectin. This may explain why we did not observe an association between baseline insulin and 13-year changes in adiponectin in our analysis.

Conclusion

In conclusion, following 850 healthy women for 13 years showed that baseline adiponectin levels were inversely associated with subsequent changes in insulin levels, whereas baseline leptin levels were positively associated with subsequent changes in hsCRP and vice versa. Our results provide novel evidence for prospective inter-relationships in adiposity-associated biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Funding: This study has been supported by grants (UM1 CA176726, HL60712, and CA67262) from the National Institutes of Health. Dr. Baden is supported by a fellowship from the Manpei Suzuki Diabetes Foundation. Dr. Huang is supported by K01 HL143034.

References

1. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochemical and biophysical research communications* 1999;257:79–83. [PubMed: 10092513]
2. Carbone F, La Rocca C, Matarese G. Immunological functions of leptin and adiponectin. *Biochimie* 2012;94:2082–2088. [PubMed: 22750129]
3. Graham TE, Yang Q, Bluher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *The New England journal of medicine* 2006;354:2552–2563. [PubMed: 16775236]
4. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998;395:763–770. [PubMed: 9796811]
5. Tamori Y, Sakaue H, Kasuga M. RBP4, an unexpected adipokine. *Nature medicine* 2006;12:30–31; discussion 31.
6. Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obesity reviews : an official journal of the International Association for the Study of Obesity* 2013;14:232–244. [PubMed: 23171381]
7. Cartier A, Lemieux I, Almeras N, Tremblay A, Bergeron J, Despres JP. Visceral obesity and plasma glucose-insulin homeostasis: contributions of interleukin-6 and tumor necrosis factor-alpha in men. *The Journal of clinical endocrinology and metabolism* 2008;93:1931–1938. [PubMed: 18319319]
8. Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR. Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. *Obesity research* 2003;11:760–768. [PubMed: 12805397]
9. Khandekar MJ, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. *Nature reviews Cancer* 2011;11:886–895. [PubMed: 22113164]
10. 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. [PubMed: 14988254]
11. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *Jama* 2004;291:1730–1737. [PubMed: 15082700]
12. 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;59:611–618. [PubMed: 19959759]
13. Sun Q, Kiernan UA, Shi L, et al. Plasma retinol-binding protein 4 (RBP4) levels and risk of coronary heart disease: a prospective analysis among women in the nurses' health study. *Circulation* 2013;127:1938–1947. [PubMed: 23584360]

14. Shamsuzzaman AS, Winnicki M, Wolk R, et al. Independent association between plasma leptin and C-reactive protein in healthy humans. *Circulation* 2004;109:2181–2185. [PubMed: 15117839]
15. Bednarska-Makaruk M, Graban A, Wisniewska A, et al. Association of adiponectin, leptin and resistin with inflammatory markers and obesity in dementia. *Biogerontology* 2017;18:561–580. [PubMed: 28421328]
16. Tabak O, Simsek G, Erdenen F, et al. The relationship between circulating irisin, retinol binding protein-4, adiponectin and inflammatory mediators in patients with metabolic syndrome. *Archives of endocrinology and metabolism* 2017;61:515–523. [PubMed: 28977161]
17. Nakamura A, Miyoshi H, Ukawa S, et al. Serum adiponectin and insulin secretion: A direct or inverse association? *Journal of diabetes investigation* 2018;9:1106–1109. [PubMed: 29451743]
18. Black MH, Shu YH, Wu J, et al. Longitudinal Increases in Adiposity Contribute to Worsening Adipokine Profile over Time in Mexican Americans. *Obesity* 2018;26:703–712. [PubMed: 29427376]
19. Herder C, Faerch K, Carstensen-Kirberg M, et al. Biomarkers of subclinical inflammation and increases in glycaemia, insulin resistance and beta-cell function in non-diabetic individuals: the Whitehall II study. *European journal of endocrinology* 2016;175:367–377. [PubMed: 27491375]
20. Chen K, Li F, Li J, et al. Induction of leptin resistance through direct interaction of C-reactive protein with leptin. *Nature medicine* 2006;12:425–432.
21. Tworoger SS, Sluss P, Hankinson SE. Association between plasma prolactin concentrations and risk of breast cancer among predominately premenopausal women. *Cancer research* 2006;66:2476–2482. [PubMed: 16489055]
22. Huang T, Tobias DK, Hruby A, Rifai N, Tworoger SS, Hu FB. An Increase in Dietary Quality Is Associated with Favorable Plasma Biomarkers of the Brain-Adipose Axis in Apparently Healthy US Women. *The Journal of nutrition* 2016;146:1101–1108. [PubMed: 27075907]
23. Baden MY, Satija A, Hu FB, Huang T. Change in Plant-Based Diet Quality Is Associated with Changes in Plasma Adiposity-Associated Biomarker Concentrations in Women. 2019.
24. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. *Cancer Epidemiol Biomarkers Prev* 1995;4:649–654. [PubMed: 8547832]
25. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology (Cambridge, Mass)* 1990;1:466–473.
26. Chasan-Taber S, Rimm EB, Stampfer MJ, et al. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology (Cambridge, Mass)* 1996;7:81–86.
27. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *American journal of epidemiology* 1985;122:51–65. [PubMed: 4014201]
28. Salvini S, Hunter DJ, Sampson L, et al. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *International journal of epidemiology* 1989;18:858–867. [PubMed: 2621022]
29. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. *The Journal of nutrition* 2012;142:1009–1018. [PubMed: 22513989]
30. Rosner B Percentage Points for a Generalized ESD Many-Outlier Procedure. *Technometrics* 1983;25:165–172.
31. Zimmet P, Hodge A, Nicolson M, et al. Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study. *BMJ (Clinical research ed)* 1996;313:965–969.
32. Choi KM, Yannakoulia M, Park MS, et al. Serum adipocyte fatty acid-binding protein, retinol-binding protein 4, and adiponectin concentrations in relation to the development of the metabolic syndrome in Korean boys: a 3-y prospective cohort study. *The American journal of clinical nutrition* 2011;93:19–26. [PubMed: 21106915]
33. Li G, Esangbedo IC, Xu L, et al. Childhood retinol-binding protein 4 (RBP4) levels predicting the 10-year risk of insulin resistance and metabolic syndrome: the BCAMS study. *Cardiovascular diabetology* 2018;17:69. [PubMed: 29759068]

34. Hodson L, Banerjee R, Rial B, et al. Menopausal Status and Abdominal Obesity Are Significant Determinants of Hepatic Lipid Metabolism in Women. *Journal of the American Heart Association* 2015;4:e002258. [PubMed: 26432801]
35. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature medicine* 2002;8:1288–1295.
36. Hotta K, Funahashi T, Bodkin NL, et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* 2001;50:1126–1133. [PubMed: 11334417]
37. Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002;8:731. [PubMed: 12068289]
38. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. *Jama* 2009;302:179–188. [PubMed: 19584347]
39. Stenvinkel P, Lindholm B, Lonnqvist F, Katzarski K, Heimbürger O. Increases in serum leptin levels during peritoneal dialysis are associated with inflammation and a decrease in lean body mass. *Journal of the American Society of Nephrology : JASN* 2000;11:1303–1309. [PubMed: 10864587]
40. Mackintosh RM, Hirsch J. The effects of leptin administration in non-obese human subjects. *Obesity research* 2001;9:462–469. [PubMed: 11500526]
41. Hukshorn CJ, Lindeman JH, Toet KH, et al. Leptin and the proinflammatory state associated with human obesity. *The Journal of clinical endocrinology and metabolism* 2004;89:1773–1778. [PubMed: 15070944]
42. Paneni F, Costantino S, Cosentino F. Insulin resistance, diabetes, and cardiovascular risk. *Current atherosclerosis reports* 2014;16:419. [PubMed: 24781596]
43. Huang T, Glass K, Zeleznik OA, et al. A Network Analysis of Biomarkers for Type 2 Diabetes. *Diabetes* 2019;68:281–290. [PubMed: 30409783]
44. Yuan C, Spiegelman D, Rimm EB, et al. Relative Validity of Nutrient Intakes Assessed by Questionnaire, 24-Hour Recalls, and Diet Records as Compared With Urinary Recovery and Plasma Concentration Biomarkers: Findings for Women. *American journal of epidemiology* 2018;187:1051–1063. [PubMed: 29036411]
45. Zhang Q, Zhao X, Chen M, Fang Z, Chen Y, Wang Y. Weight gain and changes in plasma adiponectin and leptin concentrations after 12-month insulin intensive therapy for Chinese male patients with newly diagnosed type 2 diabetes. *Obesity research & clinical practice* 2016;10:553–563. [PubMed: 26422807]

Study Importance

What is already known?

- Obesity is associated with the dysregulation of adiposity-associated biomarkers.
- Adiposity-associated biomarkers have been shown to predict future risk of obesity-associated diseases including cardiometabolic disease and cancer.
- Although previous cross-sectional and longitudinal studies reported several associations between adiposity-associated biomarkers, their long-term prospective inter-relationships remain largely unclear.

What are the new findings?

- We examined cross-sectional, longitudinal, and prospective inter-relationships among adiposity-associated biomarkers using two repeated blood collections 11–15 years apart in 850 US women.
- Most adiposity-associated biomarkers were inter-related in both cross-sectional and longitudinal analyses.
- In prospective analysis, higher adiponectin concentration at baseline was associated with smaller insulin increase and there was a bidirectional positive association between leptin and C-reactive protein.

How might the results change the direction of research or the focus of clinical practice?

- Our results expanded the prior findings and elucidated the long-term temporal biomarker inter-relationships in healthy women.
- Our results provide implications for the biomarker-mediated pathways linking obesity and obesity-related diseases and suggest potential value of these biomarkers in predicting obesity-related conditions.

Figure 1. Associations between each biomarker changes with other biomarker changes. Values are multivariable-adjusted percent differences of biomarker changes per doubling of percent changes in each biomarker concentration. Error bars represent 95% confidence intervals. *indicates $p < 0.05$, **indicates significant association after Bonferroni correction ($p < 0.05/6 = 0.008$).

Figure 2. Associations between each baseline biomarker concentration with other biomarker changes.

Values are multivariable-adjusted percent differences of biomarker changes per doubling in each baseline biomarker concentration. Error bars represent 95% confidence intervals.

*indicates $p < 0.05$, **indicates significant association after Bonferroni correction ($p < 0.05/6 = 0.008$), and † indicates the consistent associations with cross-sectional and longitudinal analyses.

Table 1

Intraclass correlation coefficients of adiposity-associated biomarkers

	ICC	(95% CI)
Adiponectin	0.76	(0.73–0.79)
Leptin	0.75	(0.72–0.78)
sOB-R	0.73	(0.70–0.76)
Insulin	0.45	(0.39–0.50)
RBP-4	0.51	(0.46–0.56)
hsCRP	0.62	(0.57–0.66)
IL-6	0.41	(0.35–0.47)

sOB-R, soluble leptin receptor; RBP-4, retinol-binding protein 4; hsCRP, high-sensitivity C-reactive protein; IL-6, Interleukin 6.

Table 2

Age-standardized characteristics at baseline (1996–1999) and follow-up (2010–2011) in the Nurses' Health study II *

<i>n</i>	Baseline	Follow-up
	850	850
Age at blood collection [†] , y	45 ± 5	58 ± 4
BMI, kg/m ²	24.7 ± 3.6	26.4 ± 4.8
Weight, kg	66.6 ± 10.3	71.1 ± 13.1
Total energy intake, kcal/day	1888 ± 366	1758 ± 411
Alcohol, g/day	3.9 ± 5.7	6.2 ± 8.2
AHEI-2010	50.9 ± 8.6	65.1 ± 10.4
Current smokers, %	4.0	1.4
Physical activity, MET-h/week	17.6 ± 15.1	23.5 ± 22.1
Postmenopausal, %	21.2	71.9
Current PMH use in postmenopausal, %	83.7	23.5
Hypertension, %	11.3	29.2
Hypercholesterolemia, %	15.2	34.9
Plasma biomarkers		
Total adiponectin, µg/mL	7.0 [1.4]	7.4 [1.5]
Leptin, ng/mL	17.2 [1.8]	19.8 [2.0]
Soluble leptin receptor, ng/mL	24.7 [1.2]	24.8 [1.3]
Insulin, uU/mL	5.1 [1.6]	5.5 [1.8]
RBP-4, µg/mL	32.8 [1.2]	35.7 [1.2]
High-sensitivity CRP, mg/L	1.01 [2.57]	1.04 [2.59]
IL-6, pg/mL	0.87 [1.55]	0.91 [1.58]

* Values are means ± standard deviations (SDs) for continuous variables except plasma biomarkers, geometric means [geometric SDs] for all plasma biomarkers, and percentages for categorical variables; and are standardized to the age distribution of participants.

[†] Value is not age adjusted.

n, the number of observations; BMI, body mass index; AHEI-2010, Alternate Healthy Eating Index 2010; MET-h, metabolic equivalent task hours; PMH, postmenopausal hormone; RBP-4, retinol-binding protein-4; CRP, C-reactive protein; IL-6, interleukin-6.

Table 3A

Partial correlation coefficients among biomarker concentrations at baseline *

	Adiponectin	Leptin	sOB-R	Insulin	RBP-4	hsCRP	IL-6
Adiponectin	1.00						
Leptin	-0.11 **	1.00					
sOB-R	0.22 **	-0.20 **	1.00				
Insulin	-0.20 **	0.34 **	-0.18 **	1.00			
RBP-4	-0.11 **	0.06	-0.04	0.10 *	1.00		
hsCRP	-0.16 **	0.25 **	-0.02	0.14 **	0.10 *	1.00	
IL-6	-0.04	0.14 **	-0.09 *	0.07	-0.12 **	0.30 **	1.00

* Values are Spearman's partial correlation coefficients.

* indicates $p < 0.05$ and

** indicates significant association after Bonferroni correction ($p < 0.05/21 = 0.002$).

Correlations were adjusted for age, BMI, total energy intake, dietary habit, physical activity, alcohol intake, smoking status, menopausal status, postmenopausal hormone use, and history of hypertension and hypercholesterolemia. sOB-R, soluble leptin receptor; RBP-4, retinol-binding protein 4; hsCRP, high-sensitivity C-reactive protein; IL-6, Interleukin 6.

Table 3B

Partial correlation coefficients among biomarker concentrations at follow-up *

	Adiponectin	Leptin	sOB-R	Insulin	RBP-4	hsCRP	IL-6
Adiponectin	1.00						
Leptin	-0.15 **	1.00					
sOB-R	0.29 **	-0.23 **	1.00				
Insulin	-0.26 **	0.28 **	-0.19 **	1.00			
RBP-4	-0.14 **	0.06	-0.07 *	0.07 *	1.00		
hsCRP	-0.19 **	0.24 **	-0.003	0.11 **	-0.05	1.00	
IL-6	-0.06	0.12 **	-0.07 *	0.07	-0.05	0.33 **	1.00

* Values are Spearman's partial correlation coefficients.

* indicates $p < 0.05$ and

** indicates significant association after Bonferroni correction ($p < 0.05/21 = 0.002$).

Correlations were adjusted for age, BMI, total energy intake, dietary habit, physical activity, alcohol intake, smoking status, menopausal status, postmenopausal hormone use, and history of hypertension and hypercholesterolemia. sOB-R, soluble leptin receptor; RBP-4, retinol-binding protein 4; hsCRP, high-sensitivity C-reactive protein; IL-6, Interleukin 6.