Short Communication

Within-Individual Stability of Obesity-Related Biomarkers among Women

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Abstract

Objective: **Novel biomarkers including proinflammatory cytokines and adipokines are being explored as potential mediators of cancer and other obesity-related conditions. Prospective studies linking biomarker levels with disease outcomes often measure biomarkers at a single time point and assume that the within-individual variation in levels is small compared with the interindividual variation. However, this assumption is seldom tested.**

Methods: **This study examined the within-individual stability over time of plasma adiponectin, resistin, leptin, plasma activator inhibitor type 1, hepatocyte growth factor, tumor necrosis factor α, interleukin 6, and insulin among healthy young women.**

Results: **The study included 17 women (9 Black non-Hispanic, 2 Black Hispanic, 2 White Hispanic, and 4 other race/ethnicity) with mean age of 32.3 years, mean body mass index of 31.2 kg/m^2, and 76% prevalence of smoking. Analysis of intraclass correlation (ICC) suggested high to moderate correlation over repeated samples taken over 3 years in levels of resistin (ICC = 0.95), hepatocyte growth factor (0.91), plasma activator inhibitor type 1 (0.84), adiponectin (0.73), insulin (0.62), and leptin (0.58). ICCs were weaker for levels of proinflammatory cytokines, tumor necrosis factor α (0.39), and interleukin 6 (0.47).**

Conclusion: **In this population of minority young females with a high prevalence of overweight and smoking, several obesity-related endocrine markers were stable over a period of 3 years. This supports the feasibility of longitudinal studies relating these biomarkers to the future occurrence of cancer and other health consequences of obesity.** (Cancer Epidemiol Biomarkers Prev 2007;16(6):1291–3)

Introduction

Obesity has long been recognized as a risk factor for early mortality, cancer, and other common chronic diseases including coronary heart disease, stroke, and diabetes (1, 2). Recent data suggest that adipose tissue is not merely a passive depot for energy storage but also an endocrine organ that may contribute to pathogenic processes by directly or indirectly affecting levels and activity of hormones, inflammatory mediators, or adipokines, which are newly identified proteins that are largely or solely derived from adipose tissue. Studies in human populations relating circulating levels of obesity-related biomarkers to risk of incident diseases are an important part of the effort to uncover mechanisms responsible for the adverse health consequences of obesity. Biomarkers being explored as potential mediators of obesity-related conditions include insulin, proinflammatory cytokines, and adipokines such as adiponectin, leptin, and resistin. In population studies, a single biomarker measurement is often obtained under the assumption that the value is representative for that individual and that any within-individual variation is small compared with the interindividual variation. An improved understanding of the within-individual stability of obesity-related biomarkers is important for designing and interpreting epidemiologic and clinical studies (3-6). In a population of predominantly minority young females, with a high overall level of adiposity and high prevalence of smoking, we examined the within-individual correlation over a period of 3 years in fasting levels of adiponectin, resistin, leptin, plasma activator inhibitor type 1 (PAI-1), hepatocyte growth factor (HGF), tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and insulin.

Subjects were 17 women participating as HIV-uninfected controls in the multicenter Women's Interagency HIV Study (WIHS; ref. 7). WIHS is a longitudinal cohort study of HIV-infected and HIV-uninfected women initiated in 1994 that was designed to investigate the natural history of HIV infection. The present analyses included HIV-uninfected women who were similar to HIV-infected women on race, socioeconomic status, and HIV risk behaviors, including injection drug use, and sexual history. Since 1994, WIHS participants have completed semiannual study visits including standardized assessments of medical history, clinical measurements, and venous blood sampling (fasting specimens were obtained after 1999). Human subjects research approval was obtained from all participating institutions, and all subjects provided informed consent.

For the present investigation, we selected 17 HIV-uninfected participants at the Bronx, New York WIHS site who provided fasting blood samples at annual intervals over 3 years, were confirmed to be HIV-negative at baseline and follow-up visits, and were without a history of hepatitis B or hepatitis C infection. Baseline characteristics of women included in the present analysis included mean age, 32.3 years (range, 19-47 years);

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Table 1. Geometric mean levels (95% CIs) of obesity-related biomarkers at baseline, 1 y, and 3 y

	Baseline visit	Year 1 visit	Year 3 visit
	Geometric mean (95% CI)	Geometric mean (95% CI)	Geometric mean (95% CI)
Adiponectin, μg/mL	6.20 (4.54-8.48)	6.85 (5.19-28.22)	6.98 (5.24-32.71)
Resistin, pg/mL	7,555.27 (4,836.52-11,802.29)	8,184.52 (5,129.01-59,825.00)	7,608.79 (4,927.12-46,355.65)
Leptin, ng/mL	9.78 (5.91-16.19)	11.53 (7.40-68.05)	11.22 (7.25-72.62)
TNF-α, pg/mL	2.89 (1.81-4.59)	3.01 (1.66-36.50)	2.61 (2.00-6.99)
IL-6, pg/mL	7.39 (2.66-20.58)	7.63 (2.29-224.12)	5.72 (2.39-54.80)
PAI-1, pmol/L	213.50 (146.16-310.00)	239.62 (149.81-2,121.37)	242.31 (212.89-790.39)
HGF, pg/mL	190.23 (114.24-316.77)	197.64 (119.13-1,483.12)	174.99 (112.74-1,013.32)
Insulin, pmol/L	34.03 (25.70-44.45)	38.20 (29.86-141.68)	31.95 (25.00-143.76)

9 Black non-Hispanic, 2 Black Hispanic, 2 White Hispanic, and 4 other race/ethnicity; current smoking, 76%; and mean body mass index (BMI), 31.2 kg/m^2.

For this study, we tested repeated blood samples collected during three study visits, spaced ~1 to 2 years apart (2002, 2003, and 2005). Fasting venous blood samples were collected in 8.0-mL sodium citrate Vacutainer Cell Preparation Tubes (Becton Dickinson). These tubes were centrifuged at room temperature at 1,500 to 1,800 × *g* for at least 20 min within 6 h of collection. Plasma aliquots (1.0 mL) were maintained in −70°C storage. In March 2006, the frozen aliquots were shipped to the Laboratory for Clinical Biochemistry Research, University of Vermont, for analysis. Analytes were measured using the Lincoplex human adipokine panels A and B, which are multiplex assay kits based on the Luminex xMAP technology (Linco Research, Inc.).[4] Panel A (insulin, adiponectin, PAI-1, and resistin) was done on specimens after 1:400 dilution; panel B analytes (IL-6, TNF-α, leptin, and HGF) are less abundant in plasma and were assayed in undiluted samples. All study samples were tested in duplicate in 1 day, with the samples of an individual assayed together on the same plate so that interassay variability was minimized. Levels of adipokines measured by the Lincoplex assays have been shown to correlate well with those obtained from the standard ELISA-based methods (r = 0.97 for adiponectin, 0.95 for leptin, 0.73 for PAI-1, 0.93 for resistin, 0.84 for TNF-α, and 0.83 for IL-6).[5] In a separate study in which plasma samples from 12 individuals were assayed five times in different days using the Lincoplex adipokine panels A and B, the interassay coefficients of variation were 12.6% for adiponectin, 10.3% for PAI-1, 13.5% for resistin, 5.9% for leptin, 13.6% for HGF, 12.8% for TNF-α, 22.6% for IL-6, and 6.8% for insulin. We also conducted analyses of IL-1β and found that the assay reproducibility was poor. Thus, we excluded IL-1β from the present analyses.

Laboratory values were log transformed and presented as geometric means and 95% confidence intervals (95% CI). Linear mixed models were used to assess systematic changes in values over time and to assess the associations between clinical and laboratory variables. Within-person stability of laboratory values over 3 years was calculated using the intraclass correlation coefficient (ICC), both for the overall data across three visits and for pairwise between-visit comparisons. A one-way random effects nested ANOVA model was used to separate the total variance (σ_T^2) into three components: (*a*) between-subject variance (σ_G^2); (*b*) intraindividual variance (σ_I^2); and (*c*) analytic variance (σ_A^2), such that $\sigma_T^2 = \sigma_G^2 + \sigma_I^2 + \sigma_A^2$. The proportion of total variance attributable to between-subject, intraindividual, and analytic variations was derived as R_G, R_I, and R_A, respectively (e.g., $R_A = \sigma_A^2/\sigma_T^2$). Analytic variation was based on within-run variance from samples assayed in duplicate. The ICC for each inflammatory marker over a 3-year period was estimated as σ_G^2/σ_T^2. The criterion for statistical significance was defined as two-sided $P < 0.05$.

Geometric mean levels of biomarkers (Table 1) did not increase or decrease across the baseline (year 0), year 1, and year 3 samples; the lack of temporal trends in levels was confirmed by linear mixed models. Analysis of ICC (Table 2) suggested high to moderate correlation over the three repeated samples in levels of resistin (overall ICC = 0.95), HGF (0.91), PAI-1 (0.84), adiponectin (0.73), insulin (0.62), and leptin (0.58). ICCs were somewhat weaker for levels of the proinflammatory cytokines TNF-α (overall ICC = 0.39) and IL-6 (0.47). For all assays, analytic variability was ≤2.5% of the total variability. Adjustment for BMI, age, or race/ethnicity did not change the ICC substantially.

We assessed the correlations between biomarkers and level of adiposity. Leptin and insulin levels were higher and adiponectin levels were lower with increasing BMI. Per 1 unit higher BMI, leptin was 10.4% higher ($P < 0.0001$), insulin was 4.6% higher ($P < 0.0001$), and adiponectin was 3.2% lower ($P = 0.03$). Pearson correlations were significant ($P < 0.01$) for leptin-BMI (r = 0.71) and insulin-BMI (r = 0.64). Correlations with BMI were of borderline significance for adiponectin ($r = -0.37$) and HGF (r = 0.29), whereas resistin, PAI-1, IL-6, and TNF-α had weak correlations with BMI ($|r| < 0.11$) and were not statistically significant.

This study confirms prior data from the Health Professionals Follow-up Study suggesting that adiponectin level is highly stable within individuals over 1 year (n = 20 men, ICC = 0.85; ref. 5). In the present analysis, we found an ICC for adiponectin that was only slightly lower than this (ICC = 0.73) over an extended period of 3 years follow-up. High molecular weight and low molecular weight adiponectin complexes may be of greater biological relevance than total adiponectin, although assessment of levels of high molecular weight and low molecular weight adiponectins was beyond the scope of this study (8). Resistin and HGF levels had remarkably high intraindividual stability in this study (resistin, ICC = 0.95; HGF, ICC = 0.91). For leptin, we found an ICC of 0.58 over 3 years, suggesting poorer within-individual correlation than previously shown among men in the Health Professionals

Table 2. ICCs (95% CIs) for repeated measures of obesity-related biomarkers over 3 y

	Overall	Baseline vs year 1	Baseline vs year 3
	ICC (95% CI)	ICC (95% CI)	ICC (95% CI)
Adiponectin	0.73 (0.53-0.89)	0.75 (0.53-0.97)	0.82 (0.66-0.98)
Resistin	0.95 (0.92-0.98)	0.96 (0.92-0.99)	0.96 (0.91-0.99)
PAI-1	0.84 (0.69-0.93)	0.85 (0.71-0.99)	0.85 (0.72-0.99)
Leptin	0.58 (0.31-0.80)	0.50 (0.13-0.87)	0.58 (0.25-0.91)
HGF	0.91 (0.86-0.97)	0.94 (0.88-0.99)	0.91 (0.83-0.99)
IL-6	0.47 (0.19-0.74)	0.33 (0.01-0.77)	0.62 (0.32-0.92)
TNF-α	0.39 (0.10-0.69)	0.41 (0.01-0.82)	0.58 (0.26-0.91)
Insulin	0.62 (0.38-0.83)	0.45 (0.06-0.85)	0.77 (0.57-0.97)

[4] http://www.luminexcorp.com/01_xMAPTechnology/
[5] http://www.med.uvm.edu/lcbr/

Follow-up Study (ICC = 0.74, over 4 years; ref. 3) and Janus (ICC = 0.83, over 1 year; ref. 6) cohorts. Within-individual correlations for the proinflammatory cytokines IL-6 and TNF-α were more modest than correlations for levels of adiponectin, HGF, leptin, and resistin. Prior studies, however, have suggested higher within-individual correlations for IL-6 and TNF-α than that found here (3, 4). This may have been due to differences in laboratory methods, as we used multiplex assays rather than the more standard ELISA techniques. Notably, our population was female, predominantly Black, and had a high prevalence of smoking and overweight. The high level of adiposity in the present cohort was reflected in substantially lower adiponectin levels and higher resistin and leptin levels compared with prior cohorts. These characteristics may have influenced the levels of inflammation and the longitudinal correlations associated with inflammation markers. It is possible that these obesity-related biomarkers are less stable among individuals with a lower level of adiposity or unstable weight as compared with the women in the present study. The inability to stratify results by race/ethnicity due to small sample size is a limitation of the present study. Several of the biomarkers that were examined may be influenced by genes that have different distributions across race/ethnicity groups (9-11). The lack of follow-up data beyond 3 years is another limitation.

In conclusion, this study found that several obesity-related biomarkers are stable within individuals over a period of 3 years. Our study examined a population of young, predominantly minority females, with a high overall level of adiposity and high prevalence of smoking. This population has not been well represented in prior studies of within-person biomarker variability. The results support the rationale for conducting long-term population studies relating baseline levels of selected obesity-related biomarkers to risk of cancer and have important implications for the study of factors that mediate the association of obesity with cancer and other conditions.

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