Intra-individual Variation of Plasma Adipokine Levels and Utility of Single Measurement of These Biomarkers in Population-Based Studies

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Abstract

Adipokines, soluble mediators produced by adipocytes, may link adipose tissue to the inflammatory, metabolic, and immune dysregulation that characterize many obesity-related diseases. The stability of plasma adipokine levels within individuals, their seasonal variability, intercorrelations, and relationships to well-established measures of adiposity are incompletely defined. We measured levels of 12 adipokines [interleukin 1β (IL-1β), IL-6, IL-8, tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor-1 (PAI-1), high-sensitivity C-reactive protein (hsCRP), monocyte chemoattractant protein-1 (MCP-1), nerve growth factor (NGF), leptin, adiponectin, hepatocyte growth factor (HGF), and resistin] in four seasonal random plasma samples of 48 male participants of a population-based cohort study. The representativeness of single measurements was assessed by correlating the adipokine levels of a single, random sample with the mean levels from the remaining three samples using a bootstrap approach and using intra-class correlation coefficients (ICC). Spearman correlations between adipokine levels, age, body mass index (BMI), and waist-to-hip ratio (WHR) were estimated. Correlations between plasma adipokine levels from one random sample and the mean of the remaining three seasonal samples ranged from 0.57 to 0.89. Over the 1-year study period, the ICCs for adipokine levels ranged from 0.44 (PAI-1) to 0.83 (HGF). IL-8, MCP-1, and resistin levels were positively associated with age; HGF and PAI-1 levels were correlated with BMI and WHR. This study suggests that adipokine levels in a single blood sample may be useful biomarkers of inflammation in population-based studies of obesity-related disease. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2464–70)

Introduction

Evidence has accumulated from recent studies that obesity is associated with increased circulating levels of cytokines, chemokines, and acute-phase proteins, and with the activation of pro-inflammatory pathways (1). Adipokines constitute a growing list of soluble polypeptides that are involved in complex signaling between white adipose tissue and systemic inflammatory, metabolic, and immunoregulatory pathways (2). Several subgroups of adipokines are currently recognized: (*a*) classic cytokines and chemokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, IL-10, and tumor growth factor-β (TGF-β); (*b*) acute-phase proteins such as haptoglobin, serum amyloid-A, C-reactive protein, and plasminogen activator inhibitor-1 (PAI-1); and (*c*) other adipocyte- or macrophage-derived proteins, including leptin, nerve growth factor (NGF), monocyte chemoattractant protein-1 (MCP-1), resistin, and adiponectin (1, 3-7). An increasing number of other candidate adipokines are also being reported, including hepatocyte growth factor (HGF), the angiogenic protein vascular endothelial growth factor (VEGF), migration-inhibitory factor (MIF), and the iron-regulatory peptide hepcidin (3, 8).

Obesity has emerged as an important risk factor for many chronic diseases and cancers. Numerous studies have suggested that the low-grade inflammation associated with obesity (9, 10) contributes to the pathogenesis of many chronic diseases or conditions, including atherosclerotic vascular disease, cancer, and components of the metabolic syndrome such as insulin resistance and type 2 diabetes mellitus (3-6, 11, 12).

Although the levels of many pro-inflammatory adipokines have been shown to correlate with obesity, the variability of adipokine levels within individuals, their intercorrelations, and the relationships of some adipokines to BMI and waist-to-hip ratio (WHR) have not been fully characterized. If adipokine levels are reliable measures of inflammation, the measurement of specific adipokine levels may be useful in epidemiologic studies and may significantly improve methods of characterizing disease risk among overweight and obese individuals. The purpose of this analysis was therefore to facilitate future studies by evaluating the stability of a wide range of plasma adipokine levels in a sample of middle-aged

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and elderly men over time and to better define their correlations with other adipokines and to accepted measures of adiposity.

Materials and Methods

Subjects. The subjects in this study were a subset of participants in the Shanghai Men's Health Study (SMHS), a population-based cohort study consisting of 61,582 men that was conducted in Shanghai, China, from April 2002 to June 2006. The overall participation rate in the SMHS was 74.1%, non-participation was mainly due to refusals (21.1%), absence during the study period (3.1%), and miscellaneous reasons, including poor health or hearing difficulties (1.7%). All men residing in the eight communities of urban Shanghai chosen for the study and who were between the ages of 40 and 74 years were eligible for the SMHS.

The current study was based on the data of the SMHS dietary validation study, which included administration of two food frequency questionnaires (FFQ), 12 monthly 24-hour dietary recalls and collection of four seasonal blood and urine samples (13). Participants of the validation study were randomly selected from the SMHS rosters of study subjects who lived in two of the eight study communities; the response rate was 69.3%.

Of 214 subjects recruited by the dietary validation study, 196 subjects (91.6%) completed all associated surveys. Of these, 48 were randomly selected from those who had provided one blood sample in each season (four samples in total) throughout the year for the current study. These 48 individuals will henceforth be referred to as the adipokine study sample. Information on major obesity-related chronic diseases was obtained by asking study subjects at the baseline survey whether a physician had ever diagnosed them as having type 2 diabetes mellitus, cardiovascular disease, and/or stroke. Anthropometric measurements, including weight, height, and circumferences of the waist and hips, were taken at baseline recruitment according to a standard protocol by trained interviewers who were retired medical professionals. The study was approved by the Institutional Review Boards of all participating institutions, and all subjects provided written informed consent.

Analysis of Adipokine Levels. Blood samples were collected from study subjects using BD Vacutainer serum tubes at the time of their in-person interview. Samples were transported in portable insulated bags containing ice packs (at 0-4°C) and processed by centrifugation within 6 h of collection. Plasma was stored at −70°C.

All adipokine levels with the exception of high-sensitivity C-reactive protein (hsCRP) levels were determined by immunoassay using the LINCO*plex* kit (Luminex xMAP Technology) at the Vanderbilt Hormone Assay & Analytical Services Core. Human Serum Adipokine Panel B (HADK2-61K-B) was used for IL-1β, IL-6, IL-8, TNF-α, MCP-1, HGF, leptin, and NGF, and Human Serum Adipokine Panel A (HADK1-61K-A) was used for adiponectin, resistin, and total PAI-1. The sensitivities of these assays were 0.1 pg/mL for IL-1β, 1.6 pg/mL for IL-6, 0.2 pg/mL for IL-8, 0.14 pg/mL for TNF-α and MCP-1, 19.2 pg/mL for HGF, 50.9 pg/mL for leptin, 2.5 pg/mL for NGF, 145.5 pg/mL for adiponectin, 6.7 pg/mL for resistin, and 1.3 pg/mL for PAI-1. The levels of hsCRP were measured using the ACE High Sensitivity C-Reactive Protein Reagent (ACI-22) on ACE Clinical Chemistry System (Alfa Wassermann, Inc.) following the manufacturer's protocol. The minimum detectable concentration of hsCRP by this method is 0.1 mg/L. Coefficients of variation (CV) for intra-assay variation ranged from 1.4% to 7.9%, and CVs for inter-assay variation were <21%. All four seasonal samples from each study participant were measured in the same batch.

Statistical Analysis. Subjects with an undetectable level of adipokines were assigned an averaged value

Table 1. Comparison of adipokine study participants with the parent cohort and dietary validation study participants on selected baseline characteristics

	Current study (n = 48)	Diet validation study (n = 196)	SMHS cohort (n = 61,582)
Age (y), mean ± SD	54.8 ± 9.19	54.8 ± 9.59	54.9 ± 9.74
Body mass index (kg/m^2), mean ± SD	24.0 ± 2.89	23.8 ± 2.78	23.7 ± 3.07
WHR, mean ± SD	0.894 ± 0.049	0.899 ± 0.052	0.900 ± 0.057
Education (%)			
≥College	2 (4.2)	15 (7.0)	4,102 (6.8)
High school	20 (41.7)	73 (34.3)	20,369 (33.6)
Middle school	15 (31.3)	75 (35.2)	21,885 (36.0)
≤Elementary school	11 (22.9)	50 (23.5)	14,362 (23.7)
Income per capita (yuan/mo)*			
<500	6 (12.5)	23 (10.8)	7,734 (12.6)
500-<1,000	18 (37.5)	93 (43.7)	26,174 (42.6)
1,000-<2,000	18 (37.5)	74 (34.7)	21,576 (35.1)
≥2,000	6 (12.5)	23 (10.8)	5,971 (9.7)
Regular alcohol consumption, n (%)	14 (29.2)	62 (29.1)	18,032 (29.3)
Regular cigarette smoking, n (%)	27 (56.3)	131 (61.5)	36,103 (58.6)
Total energy intake (kcal/d), mean ± SD	1,987 ± 495.8	1,982 ± 495.8	1,909 ± 458.1
Interval between last meal and plasma collection (h)	4.7 ± 3.96		
Medication ingestion within past 24 h (yes, %)	44.3		
Number of cigarettes smoked within past 24 h, n †	14.2 ± 6.26		

*At the time of study recruitment, $1 was equivalent to 8.2 yuan.
†Among smokers (58.8%).

Table 2. Median adipokine levels by season and stability of single spot measurements using ICCs and the bootstrap method

	Medians*						ICC †	*r* (95%CI) †,‡
	All seasons	Winter	Spring	Summer	Fall	*P*§		
Classic cytokines								
IL-1β	0.13	0.12	0.14	0.13	0.14	0.815	0.77	0.84 (0.78-0.90)
IL-6	3.21	3.01	3.21	3.77	3.12	0.848	0.73	0.81 (0.74-0.87)
IL-8	2.70	2.95	2.57	2.91	2.64	0.806	0.51	0.63 (0.49-0.75)
TNF-α	1.96	1.70	1.82	2.11	2.01	0.476	0.48	0.62 (0.52-0.77)
Acute-phase proteins								
PAI-1	13.51	13.64	14.20	13.08	14.10	0.626	0.44	0.57 (0.39-0.71)
hsCRP	3.0	5.0	1.0	3.5	3.0	0.685	0.59	0.70 (0.58-0.81)
Inflammation-related proteins								
MCP-1	114.99	123.25	116.10	108.90	110.95	0.244	0.70	0.83 (0.76-0.88)
NGF	11.86	11.80	11.80	11.80	13.51	0.782	0.69	0.78 (0.70-0.85)
Leptin	2.82	2.48	3.27	2.89	2.74	0.726	0.74	0.84 (0.69-0.90)
Adiponectin	4.20	4.06	4.55	3.93	4.91	0.944	0.81	0.89 (0.76-0.94)
Miscellaneous proteins								
HGF	411.67	322.44	380.40	527.84	472.10	0.520	0.83	0.89 (0.83-0.94)
Resistin	5.58	5.34	5.63	5.35	5.92	0.185	0.49	0.62 (0.41-0.81)

NOTE: *r*, correlation between a randomly chosen sample for each of the 48 study participants and the mean from the remaining three seasonal samples based on bootstrapping analysis.
Abbreviation: 95% CI, 95% confidence interval.
*Values are medians, $n = 48$ men who participated in the adipokine study and had one sample drawn each season (192 measurements for all seasons). Units for adipokine levels are picograms per milliliter, with the exception of PAI-1, leptin, and resistin (ng/mL), adiponectin (μg/mL), and hsCRP (mg/L).
†From log-transformed data.
‡Correlations between a randomly chosen individual measurement and averaged measurements, estimated using the bootstrap method.
§ANOVA test.

between zero and the sensitivity of each adipokine assay. The ANOVA test was applied to compare the mean level of each adipokine for the four seasonal samples. We estimated the correlation coefficients and their 95% confidence intervals for a randomly chosen individual measurement with the mean of the remaining three measurements to evaluate the representativeness of the single measurement using the bootstrap method with 2,000 repeats in each case (13). Using log-transformed data, intra-class correlation coefficients (ICC) were also estimated to further evaluate the stability and seasonal variability of individual plasma adipokine levels.

To estimate correlations between adipokine values, we calculated Spearman correlation coefficients. Finally, for each adipokine, we also calculated Spearman correlations between mean adipokine levels and age, BMI, and WHR. The Wilcoxon rank sum test was used to compare mean adipokine levels between subjects with and without a self-reported history of specific chronic diseases (including type 2 diabetes mellitus, cardiovascular disease, and stroke) and between current smokers and nonsmokers. All statistical analyses were carried out using SAS statistical software (version 9.1, SAS Institute).

Results

The general characteristics of the 48 subjects selected for the adipokine study were, in general, similar to the 196 subjects recruited into both the dietary validation study and the entire SMHS cohort, including age, anthropometric measurements, and general demographic and major lifestyle characteristics (Table 1).

Adipokine levels drawn during different seasons were generally similar for all of the adipokines analyzed (Table 2). Median adipokine levels for all seasons were as follows: IL-1β, 0.13 pg/mL; IL-6, 3.21 pg/mL; IL-8, 2.70 pg/mL; TNF-α, 1.96 pg/mL; PAI-1, 13.51 ng/mL;

Table 3. Spearman's correlations between levels of 12 adipokines

	Classic cytokines				Acute-phase proteins		Inflammation-related proteins				Other proteins	
	IL-1β	IL-6	IL-8	TNFα	PAI-1	hsCRP	MCP-1	NGF	Leptin	Adiponectin	HGF	Resistin
IL-1β	1.00	0.40	0.28	0.34	0.20	−0.02	0.16	0.36	0.01	0.07	0.13	0.18
IL-6		1.00	0.26	0.19	0.29	0.33	−0.05	0.45	−0.17	0.02	0.18	0.26
IL-8			1.00	0.46	0.27	−0.14	0.31	0.38	−0.03	0.10	0.15	0.23
TNF-α				1.00	0.18	−0.02	0.47	0.17	0.17	−0.07	0.57	0.26
PAI-1					1.00	0.11	0.20	0.33	0.01	0.12	−0.16	0.21
hsCRP						1.00	−0.16	0.02	0.07	−0.14	0.04	0.12
MCP-1							1.00	0.07	0.16	0.07	0.27	0.14
NGF								1.00	−0.11	0.17	0.09	0.15
Leptin									1.00	−0.15	0.30	0.07
Adiponectin										1.00	−0.07	0.04
HGF											1.00	0.21
Resistin												1.00

Table 4. Associations between levels of 12 adipokines and selected demographic factors

	n	IL-1β	IL-6	IL-8	TNF-α	PAI-1
Correlation* age	48	0.01*	0.05	0.16	0.06	−0.01
BMI	48	0.02	0.21	0.07	0.06	0.42
WHR	48	0.21	0.41	0.21	0.20	0.56
Mean difference †						
History of chronic disease ‡						
Yes	4	0.51 (0.20) ‡	3.61 (2.30)	3.89 (3.86)	2.23 (0.80)	15.11 (71.57)
No	44	1.95 (4.77)	5.97 (7.66)	4.41 (6.51)	2.38 (2.37)	15.95 (87.09)
Current smoking						
No	21	**0.52 (1.29)**	**3.47 (3.16)**	4.08 (5.63)	2.05 (0.63)	**14.40 (7.48)**
Yes	27	**2.66 (5.62)**	**7.57 (9.08)**	4.60 (6.84)	2.62 (2.97)	**17.02 (92.1)**

*Spearman correlation coefficient [values in bold are statistically significant ($P < 0.05$)].
†Values presented are mean (SD; values in bold are statistically significant ($P < 0.05$) for the Wilcoxon rank sum test).
‡Self-reported history of diabetes mellitus, cardiovascular disease, and stroke.

hsCRP, 3.0 mg/L; MCP-1, 114.99 pg/mL; NGF, 11.86 pg/mL; leptin, 2.82 ng/mL; adiponectin, 4.20 ng/mL; HGF, 411.67 pg/mL; resistin, 5.58 pg/mL. No significant seasonal variations in plasma adipokine levels were observed. ICCs calculated using log-transformed data for four random plasma samples across four seasons ranged from 0.44 (PAI-1) to 0.83 (HGF). Correlations between randomly selected single-spot adipokine levels and the mean of the remaining three seasonal samples ranged from 0.57 for PAI-1 to 0.89 for adiponectin and HGF.

Correlation coefficients for all of the pro-inflammatory and other adipokine levels analyzed are presented in Table 3. Among adipokines in the classic cytokine group, the correlation ranged from 0.19 to 0.46. The correlation was relatively low among adipokines in acute-phase proteins (PAI-1 and hsCRP), inflammatory-related proteins (MCP-1, NGF, leptin, and adiponectin), and other proteins (HGF and resistin). On the other hand, correlations between adipokines in the classic cytokine group with other groups of adipokines were relatively high. PAI-1, NGF, and resistin were correlated with classic cytokines with a range of 0.17 to 0.45. MCP-1 was correlated with IL-8 ($r = 0.31$) and TNF-α ($r = 0.47$). HGF was correlated with TNF-α ($r = 0.57$).

Age was positively correlated with mean levels of IL-8 ($r = 0.16$), MCP-1 ($r = 0.18$), and resistin ($r = 0.19$; Table 4). BMI and WHR were correlated with mean HGF levels ($r = 0.34$ and 0.51, respectively) and PAI-1 levels ($r = 0.42$ and 0.56, respectively). In addition, WHR was associated with levels of IL-6 ($r = 0.41$), MCP-1 ($r = 0.54$), NGF ($r = 0.48$), and leptin ($r = 0.53$). Men who reported a history of diabetes mellitus, cardiovascular disease, and/or stroke had significantly higher levels of MCP-1, leptin, and resistin in plasma as compared with men who did not report a history of these diseases ($P < 0.05$). Current smokers also had higher mean plasma levels of IL-1β, IL-6, and PAI-1, but lower leptin levels than did nonsmokers ($P < 0.05$).

Discussion

In this study involving middle-aged and elderly Chinese men, we observed high stability and minimal seasonal variability of plasma adipokine levels based on the results of ICC calculations and bootstrap analyses. Despite the small proportion of participants in our study who reported a history of atherosclerotic vascular disease or type 2 diabetes mellitus, significantly higher levels of several pro-inflammatory adipokines were observed in individuals with these chronic, obesity-related diseases, consistent with previous reports. Overall, our results suggest that adipokine levels in a random blood sample could be a representative measurement of adipokine levels over a relatively long period and could be used as reliable biomarkers of obesity-related inflammation in population-based cohort studies.

If within-person variability in biomarker levels is random, then knowledge of the correlation in a population of a single, individual measurement with the average of multiple measurements can be used to correct for attenuation of relative risk estimates, due to the fact that a single measurement non-differentially misclassifies subjects with respect to their true average exposure (14). We found that individual, random adipokine levels were highly correlated with mean measurements in our study sample within and across seasons. Many adipokines, including IL-1β, IL-6, NGF, HGF, MCP-1, leptin, and adiponectin, were also shown to have reasonably high ICC values (ranging from 0.69 to 0.83), suggesting small within-person variability and greater variability between individuals, whereas IL-8, TNF-α, resistin, PAI-1, and hsCRP showed relatively low ICC values (ranging from 0.44 to 0.59). To evaluate the influence of observed measurement errors in blood adipokines on relative risk estimates for future etiologic studies, we estimated the observed relative risk (RR_{ob}) that would be observed given true relative risks by multiplying the natural logarithm of the specified true relative risks (RR_{true}) with the ICC and exponentiated the result ($RR_{ob} = EXP[ICC \times \ln RR_{true}]$; ref. 15). For example, if the observed ICC was 0.5 and the true relative risks of the association between disease and an adipokine were 1.5, 2.0, and 2.5, the observed relative risk would be attenuated 81.3%, 70%, and 62.8%, respectively.

With few exceptions, the intercorrelations we observed are consistent with what is known about these adipokines from *in vitro* and some human studies. The release of TNF-α, IL-6, IL-1β, and IL-8 occurs as part of a complex and coordinated inflammatory response associated with obesity and may trigger an ongoing vicious cycle of inflammation as a result of the paracrine and autocrine effects of several of these pro-inflammatory cytokines (16, 17). TNF-α has also been reported to have a strong stimulatory effect on the expression of genes belonging to the neuroptrophin family, such as NGF

Table 4. Associations between levels of 12 adipokines and selected demographic factors (Cont'd)

hsCRP	MCP-1	NGF	Leptin	Adiponectin	HGF	Resistin
−0.17	0.18	−0.09	0.05	0.10	0.01	0.19
−0.29	0.27	0.25	0.23	0.06	0.34	−0.07
−0.21	0.54	0.48	0.53	−0.29	0.51	0.24
0.04 (0.07)	**135.1 (31.8)**	17.8 (14.5)	**5.17 (2.89)**	4.60 (3.62)	513.4 (187.0)	**9.88 (3.94)**
0.13 (0.22)	**111.1 (40.0)**	27.6 (43.8)	**3.39 (2.33)**	5.38 (4.09)	482.9 (391.0)	**7.52 (7.71)**
0.05 (0.06)	113.2 (40.4)	18.5 (18.9)	**4.33 (2.25)**	5.11 (3.89)	437.1 (212.1)	7.05 (5.69)
0.17 (0.26)	113.1 (39.6)	33.3 (53.0)	**2.93 (2.38)**	5.47 (4.17)	524.2 (467.7)	8.24 (8.62)

(18, 19). We did not find a direct correlation between NGF with TNF-α levels in our study, but we did note correlations between TNF-α and IL-1β, IL-6, and IL-8. One of the pleiotropic effects of adipocyte-mediated TNF-α production is postulated to be the up-regulation of MCP-1 mRNA expression because recruitment of monocytes and macrophages to adipose tissue is an important component of the associated inflammatory response (18), and we observed a high correlation between TNF-α and MCP-1 levels in this study. Although TNF-α and IL-6 are potent inhibitors of adiponectin expression and secretion in human adipose biopsy tissue and cultured adipocytes *in vitro* (20), *in vivo* effects may differ, as we did not find a high correlation between adiponectin levels and levels of TNF-α or IL-6, possibly due to lower levels of pro-inflammatory mediators in our study. An inverse association between the levels of leptin (a pro-inflammatory adipokine) and adiponectin (a predominantly anti-inflammatory cytokine) were observed, as in previous studies (21-23). Our finding of a positive correlation between TNF-α and HGF is also consistent with TNF-α–stimulated HGF release from adipose tissue and increased levels of HGF in obesity (24). Positive correlations between PAI-1 levels, an acute-phase protein important in vascular hemostasis (25, 26), and the levels of many inflammatory mediators (TNF-α, IL-6, IL-1β, IL-8, MCP-1, and NGF) in our study are similarly consistent with its proposed function. As in previous studies, we found IL-6 and hsCRP levels to be correlated as well (16, 27). The correlation of resistin levels with the levels of many pro-inflammatory adipokines in this study also supports the hypothesis that resistin plays a role in obesity-related insulin resistance and type 2 diabetes mellitus (28, 29).

Abdominal adiposity in particular has been associated with type 2 diabetes and insulin resistance and with significantly increased levels of IL-6 (30), MCP-1 (31), and leptin (24, 32). The levels of these adipokines were correlated with WHR, and higher levels of several adipokines (MCP-1, leptin, and resistin) were associated with a history of specific chronic diseases, including type 2 diabetes mellitus, in our study. Plasma levels of MCP-1, resistin, and IL-8 were also positively correlated with age, and levels of HGF and PAI-1 were correlated with both BMI and WHR as reported previously (24, 33-36). Although increased hsCRP levels have been linked to diabetes, cardiovascular disease, and hypertension in Caucasian (37, 38) and Asian (32, 39) populations, we did not see a statistically significant difference in hsCRP levels between men with and without self-reported chronic disease, possibly due to the low prevalence of chronic disease in our study sample. Smoking was correlated with higher levels of IL-1β, IL-6, and PAI-1, but with lower levels of leptin (40, 41). Cigarette smoking has been associated with lower leptin levels in other studies as well, but it has been suggested that reduced leptin levels in smokers may reflect their reduced fat mass rather than an effect of smoking itself (42). This issue requires further study.

Plasma adipokine levels in the present study were lower overall than those reported in previous studies. Levels of IL-1β, TNF-α, and PAI-1 were somewhat lower in our study than levels reported elsewhere (7, 42), and the median leptin level (2.8 ng/mL) was also lower than the levels reported in the Health ABC cohort (5.5 ng/mL), the San Antonio Center for Biomarkers of Risk of Prostate cohort (7.6 ng/mL; refs. 7, 43), and in some clinical studies (24, 37, 43, 44). Similarly, the median adiponectin level (4.2 μg/mL) was 2- to 7-fold lower than levels observed previously (7, 21, 24, 37, 44, 45). In contrast, plasma IL-6 levels (3.21 pg/mL) were in the range reported elsewhere (1.04-14.5 pg/mL; refs. 7, 24, 37, 44, 45). These observed differences could be due to a variety of differences between participants in our study and other populations in whom adipokine levels have been measured. Participants in our study were younger (mean age, 54.8 years) and had lower mean BMI (24.0 kg/m^2) than in many previous studies and were exclusively male. Significant differences in the prevalence of obesity and in body fat distribution between men and women and between different racial/ethnic groups may underlie some of this variation (45). Our study also included relatively healthy individuals (only 8% of men reported a history of diabetes mellitus or atherosclerotic vascular disease). Finally, adipokine assay methods (RIA, ELISA, or RIA kits, etc.) may also have differed between our study and many of these previous studies. DuPont et al. (46) examined the correlation of ELISA and multiplex bead array assays of adipokine levels and showed excellent correlations between ELISA and Luminex for seven cytokines (IL-1β, IL-4, IL-5, IL-6, IL-10, IFN-γ, and TNF-α). Nevertheless, the authors also reported significant variation between some other cytokine concentrations determined by ELISA and multiplex kits.

Limitations of our study include its small sample size and potentially significant differences from other populations to which results may be generalized. Although we found that adjustment for a broad category of medication use during the week preceding the biological sample collection did not change the results, specific information on drugs that influence adipokine levels was unavailable. Despite the noticeable strength of this

study of including four seasonal samples from each subject, it is still possible that samples obtained during a 1-year time frame are not truly representative of lifetime levels of exposure. Nevertheless, knowledge of the stability of adipokine levels within individuals and their intercorrelations will enable epidemiologists to design future studies to better delineate population differences that may be relevant to obesity- and inflammation-related diseases. We believe that this analysis of a wide range of adipokines/cytokines in a sample of men from a large, population-based cohort study provides important information for epidemiologists planning to undertake such studies.

In conclusion, our study suggests that plasma adipokine levels are stable over time within individuals, and that random levels are acceptable substitutes for the mean level. These findings suggest that random plasma adipokine levels are reliable and potentially useful biomarkers of obesity-related inflammation in large-scale epidemiologic studies.

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