Adiponectin: Stability in Plasma over 36 Hours and Within-Person Variation over 1 Year, *Tobias Pischon,[1,2*] Gökhan S. Hotamisligil,[1] and Eric B. Rimm[1,3,4]* ([1] Department of Nutrition and Division of Biological Sciences and [3] Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115; [2] Franz-Volhard-Clinic, Charité, Humboldt-University, 13125 Berlin, Germany; [4] Channing Laboratory, Department of Medicine, Harvard Medical School and Brigham & Women's Hospital, Boston, MA 02115; * address correspondence to this author at: Department of Nutrition, Harvard School of Public Health, 665 Huntington Ave., Boston, MA 02115; e-mail tpischon@hsph.harvard.edu)

Adiponectin (Arcp30, AdipoQ, apM1, or GBP28), a novel 247-amino acid peptide, is secreted predominantly by adipocytes and accounts for ~0.05% of total serum proteins *(1–4)*. It is induced early in adipocyte differentiation *(1)*, consists of an N-terminal collagenous and a C-terminal globular domain, and shares homology to subunits of complement factor C1q *(1, 3)*. Adiponectin expression is reduced in obesity and type 2 diabetes, and plasma concentrations of adiponectin are inversely related to body weight and insulin concentrations *(5–8)*. Treatment with adiponectin improves insulin sensitivity in mouse models of insulin resistance *(9, 10)*, and in adiponectin knockout mice, adiponectin substitution can reverse diet-induced insulin resistance *(11)*. Adiponectin is also inversely associated with other traditional cardiovascular risk factors, such as blood pressure, heart rate, total cholesterol, LDL-cholesterol, and triglycerides *(12, 13)*. In addition, recent studies suggest that it may have antiatherogenic and antiinflammatory properties *(14–19)*. Adiponectin may therefore be an important blood biomarker to assess in large-scale epidemiologic studies of several chronic diseases.

To gain a reliable risk estimate with a single blood measurement, the within-person variability over time should be small compared with the between-person variability *(20)*. In addition, the stability of a valid biological marker should not be substantially affected by length of storage or temperature *(21–25)*. Ideally, serum or plasma from whole blood should be separated immediately and stored in deep freeze. In large epidemiologic studies, however, blood specimens are often collected at different times and locations and transported on ice over several hours or days to central laboratories for processing and storage.

The aims of the present study were to evaluate the stability of human adiponectin concentrations in blood specimens collected and stored on ice packs for up to 36 h before processing and to assess the reproducibility of human adiponectin concentrations over a period of 1 year.

The stability of adiponectin was assessed from samples collected in EDTA (6 male and 6 female volunteers) and sodium-heparin (12 female volunteers) Vacutainers, both after a 12-h overnight fast. The samples were collected in three 10-mL Vacutainers and stored with ice packs in Styrofoam containers. This process emulated the conditions we used to collect more than 60 000 blood samples mailed to our laboratory from cohort members of the Health Professionals Follow-up Study (HPFS), the Nurses' Health Study, and the Nurses' Health Study II *(25)*. Time to process was defined as 0, 24, and 36 h after venipuncture. Samples were centrifuged and aliquoted for storage in liquid nitrogen (−150 °C) at each of the three time points. Each sample was assigned a different identification number and was randomly placed in the analysis batch with respect to the three different processing time periods.

In a separate pilot study we randomly selected 300 men from the HPFS and collected two EDTA samples 1 year apart, using methods described elsewhere *(25)*. From this pilot collection, we chose a subsample of 20 men to test the 1-year reproducibility of adiponectin. Similar to the stability study, each sample was assigned a different identification number and was randomly placed in the analysis batch with respect to the date of blood collection. In the main HPFS, information about health and disease status is assessed biennially by a self-administered questionnaire, starting in 1986 *(26)*. All participants gave written informed consent, and the study was approved by the Harvard School of Public Health Human Subjects Committee Review Board.

Plasma adiponectin was measured by competitive RIA using a commercial reagent set (Linco Research Inc.), utilizing a highly purified antibody raised against recombinant human adiponectin. The samples were analyzed in duplicate, with one reagent set each for the EDTA stability samples, the sodium-heparin stability samples, and the EDTA reproducibility samples. We included additional samples to assess the intraassay variation and found CVs of 16% (n = 4) for the EDTA stability samples, 26% (n = 6) for the sodium-heparin stability samples, and 20% (n = 2) for the reproducibility samples.

Adiponectin values were ln-transformed to improve the normality of the distribution and are presented as geometric means and 95% confidence intervals (CIs). The Student paired *t*-test of geometric means was used to compare the adiponectin concentrations measured at different time-to-process periods and to compare the concentrations in 2000 and 2001. Intraclass correlation coefficients were calculated by ANOVA to assess the stability of adiponectin over time and to assess the reproducibility from the year 2000 to 2001 *(20)*. To account for changes in body weight over time, we adjusted our reproducibility analysis for body mass index (BMI), calculated as the ratio of body weight (reported in pounds in the HPFS questionnaire in the years 2000 and 2002) to body height (reported to the closest inch) squared and expressed as kg/m^2, using analysis of covariance. Changes in BMI were tested by the Student paired *t*-test. Pearson correlation coefficients and partial correlation coefficients were calculated to assess the relationship between BMI, age, and adiponectin concentrations. All analyses were conducted with SAS 6.12 (SAS Institute Inc.). All *P* values presented are two-sided, and *P* values <5% were considered statistically significant.

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Table 1. Adiponectin concentrations in samples processed 0, 24, and 36 h after blood collection in EDTA and sodium heparin.

	EDTA samples (n = 12)			Sodium-heparin samples (n = 12)		
	Geometric mean	95% CI	P^a	Geometric mean	95% CI	P^a
Adiponectin, mg/L						
0 h	13.07	9.17–18.61		13.70	10.67–17.59	
24 h	13.11	9.94–17.28	0.96	14.33	11.34–18.11	0.44
36 h	15.70	11.37–21.67	0.05	13.87	11.00–17.49	0.85

[a] Compared with adiponectin concentrations at 0 h (paired *t*-test of geometric means).

We found that in the sodium-heparin samples, adiponectin concentrations were not significantly different in those specimens analyzed after 24 or 36 h compared with those which were processed immediately (Table 1). In the EDTA samples, plasma concentrations were similar 0 and 24 h after blood collection but slightly increased after 36 h ($P = 0.05$). The overall intraclass correlation coefficients of the samples processed at 0, 24, and 36 h after blood collection were 0.85 (95% CI, 0.67–0.95) in the EDTA blood samples and 0.85 (0.67–0.95) in the sodium-heparin samples. The intraclass correlations for separate time intervals (0–24, 0–36, and 24–36 h) were in similar ranges (data not shown). Stratified by gender, adiponectin concentrations in the EDTA samples did not change significantly over 36 h (data not shown), and the overall intraclass correlation coefficient was 0.46 (−0.03 to 0.88) in men and 0.71 (0.27–0.95) in women.

Adiponectin concentrations decreased over a period of 1 year, from 17.90 (14.39–22.70) to 15.86 (13.02–19.33) mg/L ($P = 0.03$); however, this was somewhat accounted for by changes in BMI. After adjustment for BMI, there was no significant difference ($P = 0.09$) between adiponectin concentrations obtained in the years 2000 [17.64 (14.71–21.15) mg/L] and 2001 [16.10 (13.42–19.30) mg/L]. Furthermore, the two measurements were highly correlated (intraclass correlation coefficient, 0.85; 95% CI, 0.66–0.94; BMI-adjusted, 0.84; 95% CI, 0.65–0.94). Mean BMI increased from 25.5 ± 2.8 to 25.9 ± 3.2 kg/m^2 ($P = 0.13$). There were significant inverse associations between the adiponectin concentrations in 2000 and 2001 and BMI in 2000 and 2002 ($r = -0.47$; $P = 0.04$ and $r = -0.45$; $P = 0.04$, respectively). After adjustment for age, these correlations became slightly stronger ($r = -0.54$; $P = 0.02$, and $r = -0.48$; $P = 0.04$, respectively). Age itself was not significantly related to the adiponectin concentrations ($r = 0.23$; $P = 0.33$ for 2000 and $r = 0.14$; $P = 0.55$ for 2001). The mean age of our sample was 59 years (range, 53–65 years).

A multitude of factors potentially affects the assessment of biological markers, leading to imprecision of results. Storage time and temperature are important factors that may affect assay stability of blood and may bias results in either direction. Therefore, documentation of specimen stability in typical study conditions is essential in large-scale epidemiologic studies. Furthermore, a single assessment of a biochemical indicator may be susceptible to short-term variation and not reflect true long-term exposure. Random measurement error generally tends to decrease correlation and regression coefficients in epidemiologic studies toward 0 and bias relative risks toward 1. In our study, despite considerably high intraassay CVs, we found intraclass correlation coefficients for adiponectin of ~0.85, indicating excellent reproducibility *(20)*. Assuming true relative risks between adiponectin and chronic diseases of 1.5, 2.0, and 2.5, an intraclass correlation coefficient of this magnitude would lead to observed relative risks [$RR_{observed} = \exp(\ln RR_{true} \times r_{intraclass}$] of 1.4, 1.8, and 2.2 *(27)*, indicating only a modest risk reduction. Furthermore, our results may be an underestimate of the true intraclass correlation coefficient, as more precise laboratory methods to determine adiponectin concentrations become available. Our study indicates that packing and transporting adiponectin blood samples on ice for up to 36 h is unlikely to produce any systematic error, although adiponectin concentrations were slightly increased 36 h after blood collection in the EDTA samples. We speculate whether the dissociation of adiponectin from polymeric to monomeric forms might be a reason for this observation *(28)*. However, this finding may also be attributable to chance because similar changes were not observed in the sodium-heparin samples.

In conclusion, we found that human adiponectin concentrations are stable in whole blood stored in EDTA or sodium-heparin Vacutainers when placed on ice packs and stored in Styrofoam containers for up to 36 h. Furthermore, after accounting for changes in BMI, individual blood adiponectin concentrations did not significantly change over a period of 1 year but showed a high degree of reproducibility. These findings suggest that a single adiponectin measurement may be sufficient for risk assessment in epidemiologic studies. Other possible factors affecting the stability and reproducibility of adiponectin, such as long-term storage, temperature, and repeated freeze-thaw cycles, should also be considered *(29–32)*.

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High Stability of Markers of Cardiovascular Risk in Blood Samples, *Erik J. Giltay,[1*] Johanna M. Geleijnse,[1] Evert G. Schouten,[1] Martijn B. Katan,[2] and Daan Kromhout[3]* ([1] Division of Human Nutrition & Epidemiology, Wageningen University, 6700 EV Wageningen, The Netherlands; [2] Wageningen Center for Food Sciences (M.B.K.), 6700 AN Wageningen, The Netherlands; [3] National Institute of Public Health and the Environment, 3720 BA Bilthoven, The Netherlands; * address correspondence to this author at: Division of Human Nutrition and Epidemiology, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands; fax 31-317-483342, e-mail giltay@dds.nl)

Biomarkers representing long-term risk of coronary heart disease are widely used in epidemiologic research. Procedures for blood sampling and processing need to be efficient and cost-effective to enable large sample sizes for greater statistical power and precision. Transfer of blood samples by mail is less expensive, easier to perform, and less time-consuming than fast courier services or asking patients to attend a central laboratory for blood sampling. This approach may be incorporated into routine practice and may reduce the likelihood of missing data and random error *(1, 2)* and eliminate intercenter variability. Although the stabilities of many analytes at different temperatures and different time intervals have been reported *(3–7)* and some studies have mimicked transportation conditions *(2, 8)*, to our knowledge neither the effects of transfer by a next-working-day mail service nor the effects on the fatty acid (FA) composition are known. We therefore investigated the variability and reliability of risk factors for coronary heart disease in blood samples delivered by a next-working-day mail service, which is available within most Western countries.

We studied 20 healthy volunteers (7 men and 13

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