Adiponectin (also named apM1, Arcp-30, AdipoQ, or GBP-28), an abundant protein produced by adipose tissue, is regarded as one of the adipokines, which include leptin, resistin, interleukin-6, tumor necrosis factor-α, and plasminogen activator inhibitor-1, and other constituents necessary for endocrinologically active adipose tissue (1–4). Decreased adiponectin concentrations have been associated with the fundamental components of the metabolic syndrome (3, 4), i.e., insulin resistance and type 2 diabetes (2, 5–10), hypertension, endothelial dysfunction (11, 12), and increased susceptibility to atherosclerosis (13, 14). Despite its intriguing associations with the metabolic syndrome, the clinical use of adiponectin as an analyte remains to be defined. In addition to early inhouse methods, most recent studies have been performed with ELISA-based methods or a RIA-based commercial method by Linco Research (8). However, reports comparing the separate methods have not been published, and the introduction of a commercially available ELISA-based method could enable even wider implementation of adiponectin measurements in the implicated fields of research.

This study was undertaken to evaluate the analytical properties of an adiponectin assay based on a standard ELISA platform and to compare the results with the commercially available RIA-based assay.

A total of 59 individuals from the personnel of the Turku University Central Hospital Laboratories (TUCH Laboratories) and their family members volunteered for this study. A brief questionnaire providing information about age (mean, 42.9 years; range, 18–65 years), sex (18 males and 41 females), height and weight (mean body mass index, 25.2 kg/m²; range, 18.8–36.3 kg/m²), chronic illnesses, regular medications, family history of diabetes and/or known cardiovascular disease, smoking, and menstrual status of the female participants was obtained. Only three of the participants had type 2 diabetes, and none were smokers. The samples and the patient information were collected according to the revised Declaration of Helsinki.

After an overnight fast, a venous blood sample was drawn from the saphenous vein of each participant, and the separated serum was stored frozen in −20°C before sampling.

The adiponectin assay (prod. no. GBP-28; Fujirebio Inc.) evaluated in this study uses purified native adiponectin from human serum in both calibrators and controls (low and high). Serum samples and controls need to be diluted 1:441 (first dilution was 10 μL of sample in 200 μL of dilution buffer; subsequent dilutions were 10 μL of previous dilution plus 200 μL of dilution buffer) before sampling, whereas the calibrator solutions (2, 5, 10, 25, and 50 μg/L) are ready to use. Dilution buffer is used as the zero calibrator. The test is an enzyme immunometric assay based on a standard 96-well microtiter plate, and the antibody used to coat the wells (solid phase) is the same Fab anti-adiponectin antibody that is used in peroxidase-labeled form in the reaction solution. The generated absorbances are read at 450 nm (PlateReader; Wallac/Perkin-Elmer), and the results obtained by the reader need to be multiplied by 441 to compensate for the

### Table 1. Linearity characteristics of the adiponectin ELISA.

<table>
<thead>
<tr>
<th>Adiponectin in sample, mg/L</th>
<th>Expected/observed, %</th>
<th>Equation for the regression line</th>
<th>R²</th>
<th>S&lt;sub&gt;ir&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.8</td>
<td>116.1–125</td>
<td>y = 39.7x + 0.72 mg/L</td>
<td>0.9888</td>
<td>2.167 (P &lt; 0.0001)</td>
</tr>
<tr>
<td>15.1</td>
<td>107.9–111.4</td>
<td>y = 16.8x + 0.02 mg/L</td>
<td>0.9988</td>
<td>0.486 (P &lt; 0.0001)</td>
</tr>
<tr>
<td>3.18</td>
<td>95.6–107.9</td>
<td>y = 3.1x + 0.02 mg/L</td>
<td>0.9982</td>
<td>0.0627 (P &lt; 0.0001)</td>
</tr>
</tbody>
</table>

*Linearity was tested by serially diluting and measuring three patient samples, by multiplying the observed results by the dilution factor to calculate expected/observed percentages, and by estimating the trend by linear regression analysis.
Other instrumentation needed to perform the assay include a plate washer (PlateWash; Wallac/Perkin-Elmer) and precision pipettes for volumes of 10–200 µL (BioHit). The RIA to measure serum adiponectin that was used for comparison was obtained from Linco Research. Certified Reference Material (CRM)-470 protein calibrator was used to evaluate the general concentration of adiponectin (NCCLS, Community Bureau of Reference, Commission of the European Communities, Brussels, Belgium).

All statistical calculations were performed with the SPSS 11.5 statistical software package (SPSS Inc.), except for the Deming regression [EP_Suite 9-A for Windows™ (15)]. A Bland–Altman plot was used to evaluate agreement.

The time required to perform the assay was ~4 h. The intraassay variations (CV) were 2.7–4.1%, as evaluated by assaying 3 different samples in 10 replicates in a single assay. The interassay variations (CV) were 3.7–5.8%, as evaluated by measuring the CRM-470 (11.4 mg/L), the low (2.73 mg/L) and high (14.3 mg/L) controls of the ELISA, an internal control serum pool from TUCH Laboratories (18.5 mg/L), and one patient sample (13.8 mg/L) in duplicate in five consecutive assays. The imprecision thus obtained was acceptable.

Results of the linearity study are presented in Table 1. The linearity of the assay was considered generally acceptable, although a slight curving of the slope was detected in the serial dilution of the high-normal sample (34.8 mg/L). This was considered not to diminish the reliability or potential usefulness of the assay because the potential interest regarding decision-making or risk assessment lies at the low-normal end of the concentration distribution (10).

Recovery was tested by diluting patient samples 1:1 (50 µL plus 50 µL) with the 5, 25, and 50 µg/L calibrators. The observed recoveries were 91.9–106.8%, which were in agreement with the observed linearity and were also acceptable. The functional detection limit, defined as the concentration at which the duplicate-sample CV exceeded 20%, was 0.36 mg/L. The analytical detection limit was 0.28 mg/L. This was estimated as the concentration equal to the mean absorbance of 10 replicates of the zero calibrator plus 3 SD. These values were well below the observed (mean, 14.13 mg/L; range, 2.92–34.9 mg/L in this study) and reported (2–20 mg/L by the manufacturer) lower end of the range of serum adiponectin concentrations. The concentration of the CRM-470 protein calibrator was 11.4 mg/L, which was close to the observed mean in our study population.

The stability of adiponectin in serum samples was evaluated by subjecting a sample to eight freeze-thaw cycles and assaying the sample after each cycle. The initial result from the sample was 15.7 mg/L, and the subsequent results [mean (SD), 16.3 (0.8) mg/L; CV = 4.9%] were similar. There was no significant drift, as evaluated by comparing results from the controls assayed at both ends of the plates. These characteristics are valuable when considering the potential of the assay as a tool for scientific and possible routine clinical work.

The physiologic and clinical data were not used to exclude individuals because it was necessary to obtain samples covering as large a range of adiponectin concentrations as possible. Therefore, the study population was inappropriately heterogeneous to be used for determination of reference values or for investigation of clinical associations between adiponectin concentrations and the clinical information obtained on the questionnaire. For this purpose, substantially larger study populations and
well-controlled multivariant or longitudinal study settings would be imperative.

The agreement between the two methods was evaluated by correlating results from patient samples and control solutions of the two assays, and a Bland-Altman plot was constructed to visualize the differences (Fig. 1). Despite the fairly good correlation, the trend observed in the Bland-Altman plot together with the large $S_{\text{bias}}$ (2.19 mg/L for the samples) prevents direct comparison of the results. The Bland-Altman plot (Fig. 1) further indicates that the difference is composed of both constant and proportional components.

To further evaluate the nature of the differences, we performed a Deming regression analysis because there are neither commutable primary calibrators nor reference methods available to evaluate the trueness of results of adiponectin measurements. In the Deming regression analysis, both patient and control samples were included, and it yielded a slope of 0.7670 (SE = 0.0352), an intercept of $-2.740$ (0.8557 mg/L), and a correlation coefficient ($r$) of 0.9420 ($P<0.0001$). The overall imprecision in duplicate measures as demonstrated by the constant SD was 0.627 mg/L for the ELISA and 1.761 mg/L for the RIA methods (Deming analysis). These results indicate similar dynamic performances, but clear differences in calibration between the methods. The surprisingly large variation between duplicate measurements on the RIA method possibly caused the Deming regression slope to be slightly better (0.7670 vs 0.7161) and the correlation coefficient slightly weaker ($r = 0.9420$ vs 0.9673) than those of the traditional correlation analysis (patients and controls together). Together with the practical disadvantages associated with the use of radiolabeled reagents, this may be interpreted as an advantage for the ELISA method over the RIA-based method in the research laboratory.

In conclusion, this study confirmed that the enzyme immunometric assay evaluated here is a robust and an easily implementable tool for measuring adiponectin concentrations on a standard platform in clinical laboratory research.

The Fujirebio Inc. (Tokyo, Japan) made this study possible by kindly donating the reagents needed to perform the adiponectin ELISA assay.

References


Proteinuria and Hypertension Are Independent Factors Affecting Fetal DNA Values: A Retrospective Analysis of Affected and Unaffected Patients, Akihiko Sekizawa,∗ Antonio Farina,†,‡ Yumi Sugito,§ Ryu Matsuoka,∥ Mariko Iwatsuki,¶ Hiroshi Saito,¶ and Takashi Okai∥ († Department of Obstetrics and Gynecology, Showa University School of Medicine, 1-5-6 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan; ‡ Institute of Embryology, Obstetrics and Gynecology, University of Bologna, Bologna, Italy; ∥ author for correspondence: fax 81-33784-8355, e-mail sekizawa@med.showa-u.ac.jp)

Cell-free fetal DNA circulates in the plasma of pregnant women (1–3). Increased concentrations of fetal DNA have been reported in the plasma of pregnant women with various complications of pregnancy, including pre-eclampsia (PE) (4–6), preterm labor (7), invasive placenta (8), hyperemesis gravidarum (9), and aneuploidy (10–12). We recently reported that fetal DNA in maternal plasma is derived primarily from villous trophoblasts bordering the intervillous spaces, which are filled with maternal blood (13). Because trophoblast damage may be involved in the pathogenesis of PE, it may be possible to use fetal DNA as a marker to monitor the severity of PE. In this study, we assessed the relationship between fetal DNA concentrations in maternal plasma and clinical evidence of PE, such as proteinuria and/or hypertension.

We conducted a retrospective study in which the control group included 116 women with uncomplicated male pregnancies between 28 and 40 weeks of gestation. Controls were matched with 45 consecutive pregnancies in