Agreement between circulating IGF-I, IGFBP-1 and IGFBP-3 levels measured by current assays versus unavailable assays previously used in epidemiological studies

Chino S. Aneke-Nash [a,*], Clara Dominguez-Islas [b], Petra Bůžková [b], Qibin Qi [a], Xiaonan Xue [a], Michael Pollak [c], Howard D. Strickler [a], Robert C. Kaplan [a]

[a] Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY, United States
[b] Department of Biostatistics, University of Washington, Seattle, WA, United States
[c] Department of Medicine and Oncology, McGill University, Montreal, Quebec, Canada

A B S T R A C T

Objective: Levels of insulin-like growth factor (IGF) proteins are associated with the risk of cancer and mortality. IGF assays produced by Diagnostic Systems Laboratories (DSL) were widely used in epidemiological studies, were not calibrated against recommended standards and are no longer commercially available.
Design: In a split sample study among 1471 adults participating in the Cardiovascular Health Study, we compared values obtained using DSL assays with alternative assays for serum IGF-I (Immunodiagnostic Systems, IDS), IGFBP-1 (American Laboratory Products Company, ALPCO) and IGFBP-3 (IDS).
Results: Results were compared using kernel density estimation plots, quartile analysis with weighted kappa statistics and linear regression models to assess the concordance of data from the different assays. Participants had a mean age of 77 years. Results between alternative assays were strongly correlated (IGF-I, $r = 0.93$ for DSL versus IDS; log-IGFBP-1, $r = 0.90$ for DSL versus ALPCO; IGFBP-3, $r = 0.92$ for DSL versus IDS). Cross tabulations showed that participants were usually in the same quartile categories regardless of the assay used (overall agreement, 74% for IGF-I, 64% for IGFBP-1, 71% for IGFBP-3). Weighted kappa also showed substantial agreement between assays (k_w, 0.78 for IGF-I, 0.69 for IGFBP-1, 0.76 for IGFBP-3). Regressions of levels obtained with DSL assays (denoted X) to alternative assays were, IGF-I: 0.52X + 15.2 ng/ml, log-IGFBP-1: 1.01X − 1.73 ng/ml IGFBP-3: 0.87X + 791.1 ng/ml. Serum values of IGF-I, IGFBP-1 and IGFBP-3 measured using alternative assays are moderately correlated.
Conclusions: Care is needed in the interpretation of data sets involving IGF analytes if assay methodologies are not uniform.

1. Introduction

The insulin-like growth factor (IGF) axis is an evolutionarily conserved system with important biologic roles during embryonic development, growth and adulthood [1]. IGF-I has mitogenic and antiapoptotic activities and acts as the primary mediator of the effects of growth hormone. IGF-I also has insulin-like activity with direct effects on glucose and free fatty acid metabolism. IGF-I circulates in blood bound to six binding proteins (IGFBP-1 to IGFBP-6), and the acid-labile subunit, which together prolong IGF-I half-life and regulate its bioavailability [2,3]. Only approximately 1% of IGF-I circulates unbound to IGFBPs. IGFBPs also may have IGF-I independent functions that may include cell growth and apoptosis, as well as metabolism.

IGF-I and IGFBPs have been extensively studied in epidemiologic investigations. Several studies have associated circulating levels of these proteins with incident diabetes, heart failure, stroke, coronary heart disease, several types of cancer and overall mortality [4–12]. In addition, measures of IGF-I are used clinically to diagnose and assess response to treatment in those with growth hormone deficiency and acromegaly [13,14]. Several commercial assays for IGF-I, IGFBP-1, and IGFBP-3 are available.

Differences in assay performance may have contributed to some of the conflicting results from prior population studies investigating associations between IGF-related blood analytes and disease risk. Many, but not all, large-scale studies of IGF-related serum analytes used assays produced by one manufacturer, Diagnostics Systems Laboratory (DSL, Webster, TX) [15]. However, these assays have become unavailable. Moreover, they do not adhere to the consensus statement regarding standards in IGF assays [16,17]. In order to facilitate our own ongoing research, we compared values measured by currently available assays that follow the recommended calibration standards [17] to levels

* Corresponding author at: Albert Einstein College of Medicine, 1300 Morris Park Avenue, Belfer Building, Room 1306C, Bronx, NY 10461, United States.
E-mail address: chino.aneke@med.einstein.yu.edu (C.S. Aneke-Nash).

previously obtained with the DSL assays. It cannot be assumed a priori that different assay methods would yield comparable results. Comparisons were completed using linear and deming regression models as well as quantile analysis, since epidemiological studies often use quantiles for grouping individuals into categories in risk assessment studies.

In the Cardiovascular Health Study [18], we previously obtained measurements of circulating IGF-I, IGFBP-1 and IGFBP-3 levels using assays produced by DSL. Subsequently, other commercial assays have been used to measure IGF proteins in a subset of the CHS cohort. In a validation study conducted among over 1000 participants, we sought to evaluate the comparability of measurements of serum IGF-I, IGFBP-1 and IGFBP-3 levels.

2. Materials and methods

2.1. Study population

The CHS is a population-based, prospective cohort study of community-dwelling older adults aged ≥65 years [19]. The cohort consists of 5888 participants who were recruited from four U.S. communities (Washington County, MD; Allegheny County, PA; Forsyth County, NC; and Sacramento County, CA), using a randomly generated sampling frame derived from Medicare eligibility lists of the Health Care Financing Administration (HCFA). Standardized clinic examinations and questionnaires were administered annually. Informed consent was provided by participants in accordance with the institutional review board guidelines at their clinic site.

2.2. Specimen collection

After an 8–10 h fast, blood samples were collected from participants by trained phlebotomists. The samples were allowed to stand in room temperature for 30 min. They were then centrifuged at 3000 *g* at 4 °C temperature for 10 min. After centrifugation, the samples were put into −70 °C storage. Frozen samples packaged with frozen CO_2 in insulated styrofoam boxes were shipped weekly to the specimen repository at the Central Blood Analysis Laboratory (CBAL) at the University of Vermont (Burlington, VT). All refrigerators, −70 °C freezers and refrigerated centrifuges were monitored daily. Systemic errors in phlebotomy procedures, processing, shipping and storage were monitored by the CHS Coordinating Center [20]. Measurements of IGF-I, IGFBP-1 and IGFBP-3 were performed using stored fasting venous blood samples obtained during the 1996–1997 (year 9) examination cycle. These measurements were completed at the Cancer Prevention Research Unit, Lady Davis Research Institute of Jewish General Hospital [8] using the DSL, ALPCO and IDS assays described below.

2.3. Assays

Using stored blood specimens collected in during the year 9 CHS clinic visit (1996/1997), we performed IGF assays at the Cancer Prevention Research Unit, McGill University, Montreal [7]. [8].

2.3.1. Diagnostic Systems Laboratories assays

2.3.1.1. IGF-I. This assay involved acid–ethanol extraction to separate IGF-I from its binding proteins, followed by a sandwich ELISA assay. Intra-assay coefficient of variation was 1.49%.

2.3.1.2. IGFBP-1. This ELISA method had an intra-assay coefficient of variation of 1.88%.

2.3.1.3. IGFBP-3. This ELISA method had an intra-assay coefficient of variation of 1.77%. In 249 samples of specimens, repeat measures of IGF-I,

Table 1
IGF-I, IGFBP-1 and IGFBP-3 measurements obtained with old assay (all-stars study) and new assay (Kaplan study) stratified by gender and diabetes status.

	DSL assay IGF measures						
	Entire cohort	Men	Women	p-value	Non-diabetics	Diabetics	p-value
Log-IGF-I, ng/ml							
N	1158	371	787		1041	113	
Range	3.37 − 6.19	3.95 − 5.97	3.37 − 6.19		3.37 − 6.19	3.78 − 5.97	
Mean ± SD	5.05 ± 0.39	5.15 ± 0.34	5 ± 0.41	<0.0001	5.05 ± 0.39	5.07 ± 0.43	0.537
Log-IGFBP-1, ng/ml							
N	1384[a]	473	911		1241	138	
Range	0.03 − 4.3	1.59 − 5.58	1.16 − 5.51		1.16 − 5.51	1.44 − 5.58	
Mean ± SD	1.80 ± 0.79	3.38 ± 0.66	3.55 ± 0.69	<0.0001	3.48 ± 0.68	3.61 ± 0.76	0.059
IGFBP-3, ng/ml							
N	1334	448	886		1188	141	
Range	868 − 7230	893 − 5742	1025 − 6446		893 − 6446	1562 − 6374	
Mean ± SD	3719 ± 837	3049 ± 751	3551 ± 880	<0.0001	3376 ± 852	3446 ± 1025	0.438
	IDS and ALPCO IGF measures						
	Entire cohort	Men	Women	p-value	Non-diabetics	Diabetics	p-value
Log-IGF-I, ng/ml							
N	1158	371	787		1041	113	
Range	3.03 − 5.71	3.54 − 5.45	3.03 − 5.71		3.03 − 5.71	3.7 − 5.45	
Mean ± SD	4.56 ± 0.35	4.67 ± 0.3	4.5 ± 0.36	<0.0001	4.55 ± 0.35	4.59 ± 0.35	0.258
Log-IGFBP-1, ng/ml							
N	1384[a]	473	911		1241	138	
Range	1.16 − 5.58	0.06 − 4.3	0.03 − 4.18		0.03 − 4.18	0.06 − 4.3	
Mean ± SD	3.49 ± 0.69	1.64 ± 0.75	1.88 ± 0.8	<0.0001	1.8 ± 0.78	1.82 ± 0.89	0.719
IGFBP-3, ng/ml							
N	1334	448	886		1188	141	
Range	893 − 7178	1475 − 5891	868 − 6689		868 − 6575	2012 − 6689	
Mean ± SD	3385 ± 891	3474 ± 716	3849 ± 845	<0.0001	3715 ± 804	3803 ± 966	0.3

[a] Includes IGF levels 52 individuals with IGFBP-1 levels that were below the assay detectability threshold for the ALPCO assay. Values of half the lower limit of detection were assigned for these individuals.

IGFBP-1 and IGFBP-3 were obtained over the 2–3 year period of testing to assess the within-individual variability over time (Pearson Correlation coefficient (r) = 0.74–0.86) [9]. These assays were produced prior to the publication of recommended standards and thus, these assays were not calibrated against the WHO International Standard 02/254 [17].

2.3.2. Comparison assays

2.3.2.1. IGF-I. Measures were obtained using the IDS-iSYS Insulin like Growth Factor-I Assay (IDS-iSYS IGF-I), an automated chemiluminescence immunoassay provided by Immunodiagnostic Systems Ltd. (IDS, Boldon Business Park, Boldon, Tyne & Wear, England). Samples are incubated in an acidic solution to dissociate IGF-I from its binding proteins and the addition of excess of IGF-II prevents re-aggregation. After neutralization, the solution is further incubated with a biotinylated anti-IGF-I monoclonal antibody and an acridinium labeled anti-IGF-I monoclonal antibody. Magnetic particles labeled with streptavidin are added to the solution, incubated, and then captured using a magnet. IGF-I concentration is then determined by measuring the amount of light emitted by the acridinium label. This IGF assay is calibrated against the WHO International Standard 02/254 [17] and has no interference

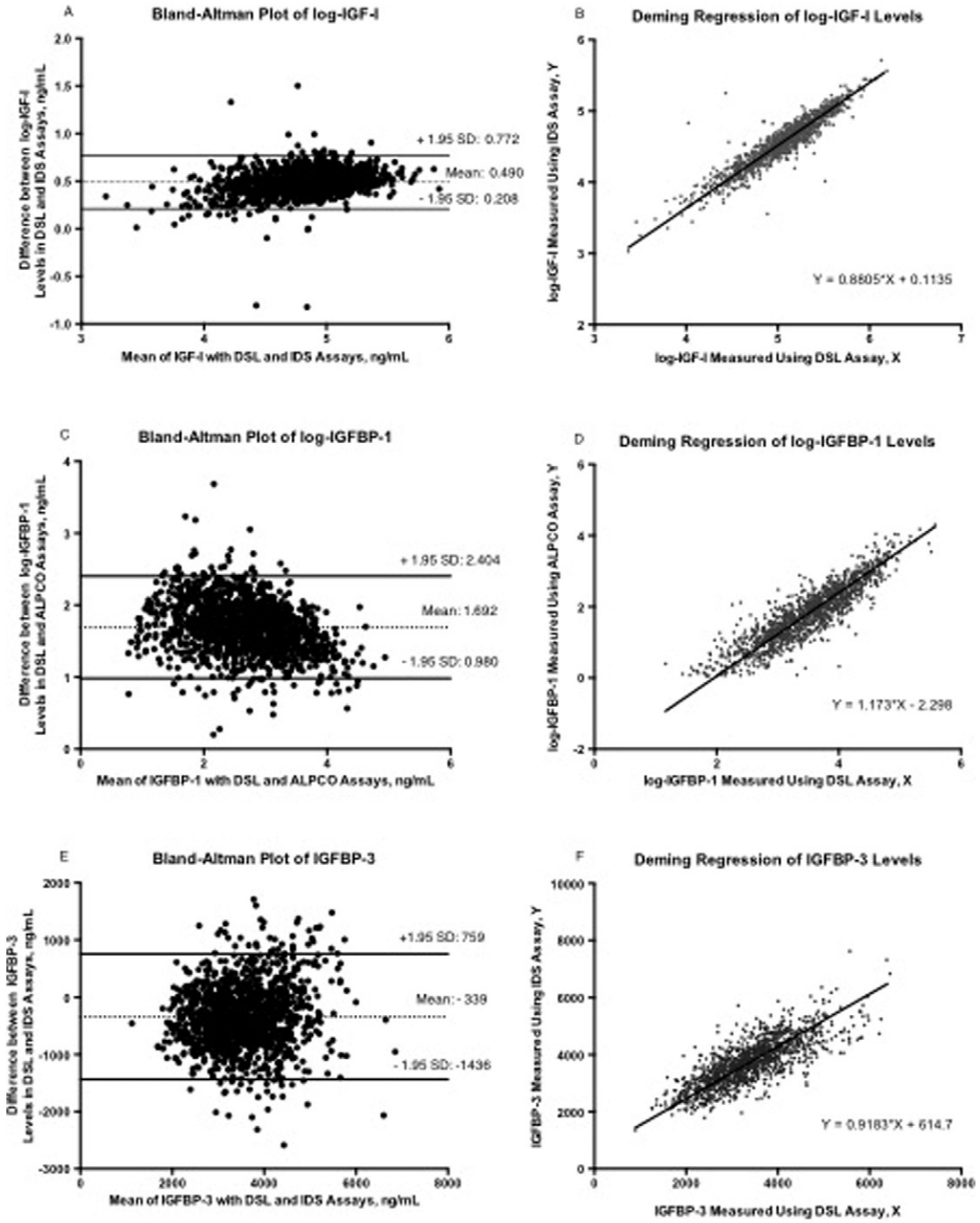

Fig. 1. Bland–Altman plots and scatter plot with fitted deming regression lines for log-IGF-I (A and B), log-IGFBP-1 (C and D) and IGFBP-3 (E and F). SD stands for standard deviation. Bland Altman plots (A, C, E) show plots of the mean difference between the two assay measures for each IGF protein on the X-axis and the difference between the two assays for each IGF protein on the Y axis. The dotted lines are the mean of the difference between the two assays, while the solid lines represent the 1.96 SD range of these differences. Deming regression graphs (B, D, F) show plots of a scatter of measures from the two assays superimposed with the regression lines. Measures from the older assays are plotted on the X-axis and the new assays on the Y-axis. The regression equation is also shown.

from IGF-II, insulin, proinsulin, and any of the IGFBPs (IGFBP-1 to IGFBP-6). The intra-assay coefficient of variation is 2.2% and the assay detects IGF-I levels in the range of 10–1200 ng/ml.

2.3.2.2. IGFBP-3. Measures were obtained using IDS-iSYS Insulin-Like Growth Factor Binding Protein—3 (IGFBP-3), an automated chemiluminescence immunoassay provided by Immunodiagnostic Systems Ltd. (IDS, Boldon Business Park, Boldon, Tyne & Wear, England) [21]. A diluent is used to dilute the original specimen sample. The solution is then incubated with a biotinylated anti-IGFBP-3 monoclonal antibody and an acridinium labeled anti-IGFBP-3 antibody. Magnetic particles labeled with streptavidin are added to the solution, incubated, and then captured using a magnet. IGFBP-3 concentration is then determined by measuring the amount of light emitted by the acridinium label. This IGFBP-3 assay if calibrated to the Reference Material: Insulin-Like Growth Factor Binding Protein—3 NIBSC code: 93/560 and has no cross-reactivity with IGF-I, IGF-II, proinsulin, insulin and any of the other IGFBPs. The intra-assay coefficient of variation is 1.94%. The assay detects IGFBP-3 levels in the range of 80–10,000 ng/ml and has no cross-reactivity with other IGFBPs.

2.3.2.3. IGFBP-1. Measures were obtained using a two-step sandwich ELISA assay method from American Laboratory Products Company (ALPCO, Keewaydin Drive, Salem, NH). The two antibodies in the two-step sandwich assay are a monoclonal IGFBP-1 antibody and a horseradish peroxidase tagged monoclonal IGFBP-1 antibody with specificity for a different region of IGFBP-1. The intra-assay coefficient of variation is 2.25% and this assay does not cross-react with any of the other IGFBPs. The assay detects IGFBP-1 levels in the range of 1–250 ng/ml and has no cross-reactivity with other IGFBPs.

2.4. Statistical analysis

Measures of IGF-I and IGFBP-1 were log-transformed to normalize the distribution and log-transformed values were used for all analyses. Descriptive statistics of IGF-I, IGFBP-1 and IGFBP-3 concentrations is presented for the entire analytic population and then separately for men and women and for diabetics and non-diabetics. For each biomarker, student's t-tests were used to compare differences in means of each IGF protein in men and women and in diabetics and non-diabetics. Bland Altman plots, which do not designate one assay as the gold standard, were used to visually assess the correlation between measures from the two assays. To evaluate the linear correlation between the values produced by the two assays, we calculated the Pearson correlation coefficient.

Epidemiological studies often follow the practice of using quantiles from the empirical distribution of biomarkers for grouping individuals into categories. Therefore, we also compared for each IGF-axis protein the quartile classifications obtained when using quartiles of measurements from the old and new assays. To evaluate the degree of agreement between these classifications we calculated a weighted Cohen's kappa coefficient, which ranges from 0 (maximum discordance) to 1 (perfect concordance) [22]. The following standard criteria were used to interpret the strength of agreement between measures: slight ($\kappa_w = 0.01–0.20$), fair ($\kappa_w = 0.21–0.40$), moderate ($\kappa_w = 0.41–0.60$), substantial ($\kappa_w = 0.61–0.80$), and almost perfect ($\kappa_w = 0.81–1.0$) [23]. Further, to evaluate the linear relationship between measures from the two assays, we fitted an Ordinary Least Squares (OLS) regression with the measurements obtained with the old assay as predictor of the measurements obtained from the new assay. To account for variation in both assays, a deming regression model was fit to further evaluate the relationship between measures from the two assays.

3. Results

The individuals studied had a mean age of 76.6 years, mean BMI of 27.2 and 12.8% were African American, 68% were female, and 9.8% had self-reported diabetes. Log-IGF-I, log-IGFBP-1 and IGFBP-3 levels were not significantly different among the diabetics and non-diabetics using measures from either assays (Table 1). Correlations between old and new assay levels were similar in the non-diabetic and diabetic populations for all three IGF proteins (non-diabetics: r = 89–93, diabetics r = 87–94). Men and women had statistically significant differences in both assay measures for all IGF proteins ($p < 0.0001$).

The range of log-IGF-I levels obtained with the DSL and IDS assays is similar (Table 1), although the measurements are highly correlated (Table 3: r = 0.93, 95% CI: 0.925–0.94). The Bland Altman plot shows a bias of 0.49 ng/ml, with a relatively wide range as indicated by the ±1.95 SD (Fig. 1A). The difference between measures from the two assays is greater at higher measures of log-IGF-I using both assays. Cross tabulations of IGF-I quartiles determined using the two assays showed that 74% of the participants were in exact concordant categories (Table 2), while all others varied by only one quartile, as reflected in the strong weighted kappa statistic ($k_w = 0.78$, "substantial" agreement). Deming regression analysis shows that the regression line does not deviate significantly from the regression lines (Table 3: $Y = 0.88^*X + 0.11$). Results from the linear regression model show that the estimated intercept and slope are 0.38 ng/ml (95% CI: 0.29, 0.48) and 0.83 ng/ml (95% CI: 0.81, 0.85), respectively, with this model explaining 87% of the variability observed in the measurements from the IDS assay.

The range of log-IGFBP-1 measures obtained from the DSL and ALPCO assays was very different; 3.4 ng/ml–6.2 ng/ml for the DSL assay and 1.2 ng/ml–5.6 ng/ml for the ALPCO assay (Table 1). Measures reported by both assays were highly correlated (Table 3: r = 0.90, 95% CI: 0.893, 0.912). Bland Altman plots show a bias of 1.69 ng/ml and the difference between measures from the two assays is higher at lower analytic values of log-IGFBP-1 (Fig. 1C). 52 individuals had IGFBP-1 results below the threshold for detection in the ALPCO assay while having available IGFBP-1 measurements from the DSL assay. They were included in the lowest ALPCO quartile in cross-tabulation analyses. Cross tabulations of log-IGFBP-1 quartiles of measures from the two assays show that 64% of the participants were in concordant categories (Table 2). Overall, the weighted kappa showed substantial agreement ($k_w = 0.69$). Deming regression analysis shows that the regression line does not deviate significantly from the regression lines (Table 3: $Y = 1.17^*X - 2.30$). Results from linear regression of log-transformed values were: estimated intercept: −1.73 ng/ml (95% CI:

Table 2
Cross-tabulation of quartiles of IGF protein levels measured using the old DSL assays and alternative assays (IDS for IGF-I and IGFBP-3, ALPCO for IGFBP-1). Values are row percentages. K_w indicates weighted kappa statistics.

DSL assay levels Quartiles	Alternative assay levels Quartiles (ng/ml)					
Log-IGF-I, (ng/ml) (n = 1158)	%				Total	K_w
	1	2	3	4		
1	86	13	0	1	100	
2	10	68	21	1	100	
3	1	17	66	15	100	
4	0	1	23	75	100	0.78
Log-IGFBP-1, (ng/ml) (n = 1436)	%				Total	K_w
	1[a]	2	3	4		
1	72	26	2	0	100	
2	13	52	32	4	100	
3	1	13	53	33	100	
4	0	1	12	87	100	0.69
IGFBP-3, (ng/ml) (n = 1471)	%				Total	K_w
	1	2	3	4		
1	88	10	2	0	100	
2	25	62	12	1	100	
3	0	28	59	13	100	
4	1	1	20	78	100	0.76

[a] Includes IGF levels 52 individuals with IGFBP-1 levels that were below the assay detectability threshold for the ALPCO assay. Values of half the lower limit of detection were assigned for these individuals.

Table 3
Results of regressions for IGF-1, IGFBP-1 and IGFBP-3 new assay measurements on old assay measurements.

	Estimate ± SE	95% IC	R-squared	Pearson correlation coefficient
Log-IGF-I				
OLS regression				
Intercept	0.383 ± 0.048	(0.288, 0.477)	0.87	0.933
Slope	0.827 ± 0.010	(0.808, 0.846)		
Deming regression				
Intercept	0.114 ± 0.051	(0.013, 0.214)		
Slope	0.881 ± 0.010	(0.861, 0.900)		
Log-IGFBP-1				
OLS regression				
Intercept	−1.730 ± 0.045	(−1.818, −1.643)	0.815	0.903
Slope	1.011 ± 0.013	(0.986, 1.036)		
Deming regression				
Intercept	−2.298 ± 0.05798	(−2.412 to −2.184)		
Slope	1.173 ± 0.01629	(1.142 to 1.205)		
IGFBP3-1				
OLS regression				
Intercept	791.1 ± 33.6	(725.2, 857.1)	0.846	0.92
Slope	0.865 ± 0.010	(0.846,0.883)		
Deming regression				
Intercept	614.7 ± 72.4	(472.7 to 756.7)		
Slope	0.918 ± 0.021	(0.878 to 0.959)		

−1.82, −1.64), slope: 1.011 ng/ml (95% CI: 1.986, 1.036). The linear regression model explained approximately 82% of the variation observed in the ALPCO assay measures.

The range of values reported by the DSL and IDS IGFBP-3 assays were similar and levels were highly correlated (Table 3: r = 0.92, 95% CI: 0.912, 0.927). Bland–Altman analysis showed a large bias of −339 with a wide range, as indicated by the ±1.95 SD (Fig. 1E). The difference between measures from the DSL and IDS assays did not vary at different levels of IGFBP-3. Additionally, cross tabulations of IGFBP-3 quartiles of measures from the two assays show that 71.1% of the participants were in concordant quartiles, (Table 2) with discordant values differing by no more than one level, as reflected in the weighted kappa statistic (k_w = 0.76). Deming regression analysis shows that the regression line does not deviate significantly from the regression lines (Table 3: Y = 0.92*X 615). Results from the linear regression model show that the estimated intercept and slope are 791.1 (95% CI: 725.2, 857.1) and 0.86 (95% CI: 0.85, 0.88), respectively, with this model explaining 85% of the variability observed in the measurements from the IDS assay.

4. Discussion

In this study, we evaluated the comparability of levels of serum IGF-I, IGFBP-1 and IGFBP-3 measured using the recently discontinued DSL assays to those of selected commercially assays (IDS for IGF-I and IGFBP-3 and ALPCO for IGFBP-1) that adhere to the recent recommendations for IGF standards [17].

The results showed that although each of the three sets of paired assays were highly correlated with one another, the magnitude of the values varied. These differences were greatest for IGFBP-1 and least for IGFBP-3. For all analytes, the IDS and ALPCO assay procedures were performed on the serum obtained at the same venipuncture as the samples used for the DSL assays, albeit the DSL aliquots had been frozen for fewer years at the time the assays were performed. However, studies have shown that IGF analytes are stable over long periods in sera frozen at −70 °C or lower [24]. Moreover, repeat measures of IGF-I, IGFBP-1 and IGFBP-3 were obtained over the 2–3 year period of testing in our laboratory to assess the within-individual variability over time (Pearson Correlation coefficient (r) = 0.74–0.86) [9]. Differences between measures from the two assays may be due to random variations or differences in assay sensitivity and antibody specificities for IGF-I and IGFBPs.

In all three cases, analysis of the IGF-axis data by quartile was shown to produce considerable agreement between paired assays. It has been common in published analyses of associations of IGF-related analytes with health and disease endpoints for the analytes to be analyzed based on categorical data (e.g., quartiles or quintiles). Several other studies have reported high correlation and agreement between measures of IGF-I from different assays [13,14,24,25], though the majority of these studies were in populations with growth hormone disorders. Some IGF-I assays only correlate at either low or high levels of IGF-I; however our data shows that the DSL and IDS IGF-I assays have relatively similar bias at varying levels of IGF-I. Assays for IGFBP-3 had the least bias while IGFBP-1 had the most bias at varying levels of each analyte. Correlation between results from different assays may depend on health status. For example, adequate correlation of results across alternative IGF-I assays has been observed in healthy subjects but not in those with diabetes [26]. However, this finding was not supported by a previous study [24]. In our population-based cohort, assay measure correlations were similar in the non-diabetic and diabetic populations for all three IGF proteins (non-diabetics: r = 89–93, diabetics: r = 87–94). Finally, we report linear and deming regression models that build upon the prior literature addressing this methodological problem [14,25,26]. The regression models were well-fit as indicated by a high r-square value.

Our study was completed using a cohort of individuals 65 years and older who have lower IGF-I levels; thus, our results should not be generalized to cohorts of varying age groups because assays may correlate at lower ranges but not in higher ranges of IGF protein levels. In addition, we recommend that whenever possible, all samples of a study should be run using a single method. This is particularly important for studies of population cohorts that identify disease associations associated with modest between-person differences within the normal ranges of IGF-related analytes.

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