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Analytical and biological variability in biomarker measurement in the Hispanic Community Health Study/Study of Latinos

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Abstract

Background—Biomarker variability, which includes within-individual variability (CV_I), between-individual variability (CV_G) and methodological variability (CV_{P+A}) is an important determinant of our ability to detect biomarker-disease associations. Estimates of CV_I and CV_G may be population specific and little data exists on biomarker variability in diverse Hispanic populations. Hence, we evaluated all 3 components of biomarker variability in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL) using repeat blood collections (n=58) and duplicate blood measurements (n = 761 – 929 depending on the biomarker).

Methods—We estimated the index of individuality (II) $((CV_I+CV_{P+A})/CV_G)$ for 41 analytes and evaluated differences in the II across sexes and age groups.

Results—Biomarkers such as fasting glucose, triglycerides and ferritin had substantially higher inter-individual variability and lower II in HCHS/SOL as compared to the published literature. We

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also found significant sex-specific differences in the II for neutrophil count, platelet count, hemoglobin, % eosinophils and fasting glucose. The II for fasting insulin, post oral glucose tolerance test glucose and cystatin C was significantly higher among the 18–44 y age group as compared to the 45+ y age group.

Conclusions—The implications of these findings for determining biomarker- disease associations in Hispanic populations need to be evaluated in future studies.

Keywords

Analytical variation; biomarker variability; Hispanics

INTRODUCTION

Reliable measurement of biomarkers and ability to compare changes in biomarker values over time is of great importance to epidemiological studies that are designed to evaluate cross sectional and longitudinal changes in the incidence and prevalence of various diseases. Thus, it is important for epidemiological studies to estimate the background variation of biomarkers. The Hispanic Community Health Study/Study of Latinos (HCHS/SOL) is a population-based cohort study designed to examine risk factors for chronic diseases such as cardiovascular disease (CVD), stroke, asthma, chronic obstructive lung disease, sleep disorders, dental caries and periodontal disease, hearing impairment and tinnitus, diabetes, kidney and liver disease, and cognitive impairment. The HCHS/SOL recruited 16415 self-identified Hispanic/latino adults (Cuban, Dominican, Mexican, Puerto Rican, Central American, and South American backgrounds) aged 18 to 74 y from randomly selected households in four US communities (Bronx, New York; Chicago, Illinois; Miami, Florida; San Diego, California) between March 2008 and June 2011. Details about the sample design and cohort selection have been previously described [1]. Fasting blood samples, an oral glucose tolerance test and spot urine samples were collected for measurement of various biomarkers that included liver enzymes such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ- glutamyl transferase (GGT), kidney function biomarkers such as serum creatinine, cystatin C, urinary creatinine, microalbumin and urinary albumin/creatinine ratio, lipid biomarkers such as serum total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C), diabetes related biomarkers such as glycated hemoglobin, fasting/post oral glucose tolerance test glucose and insulin, iron related biomarkers such as serum total iron, serum ferritin, serum transferrin and total iron binding capacity, inflammatory biomarkers such as, high sensitive C reactive protein (hsCRP), and a complete blood count with differential white blood cell count.

Previous studies have shown that linear and logistic regression models that are commonly used in the analysis of epidemiological data, produce biased estimates of the association between biomarker and disease outcomes when the biomarker has low repeatability [2]. The major sources of variability in biomarker measurement include within-individual variability, between-individual variability and methodological variability. The methodological variability encompasses (a) process (pre-analytical) variability such as variability in blood drawing, field center processing (including centrifuging and freezing) and shipping (b)

laboratory assay (analytical) variability and (c) post-analytical variability (e.g. errors in data transmission etc.). Though there are several studies evaluating the sources of variability for a large number of biomarkers [3–6] in predominantly Caucasian populations, only limited data are available on the variability of biomarkers in a diverse Hispanic population [7–11]. While methodological variability can be improved by better analytical techniques and standardization of biospecimen collection and processing procedures, between and within individual variability may be determined by the characteristics of the population being studied and likely will differ across various epidemiological studies. Since the repeatability of a biomarker measurement determines its association with disease outcomes in epidemiological studies, the HCHS/SOL conducted a study to estimate the within-individual biologic variability, between-individual variability and methodological variability in the HCHS/SOL biomarker measurement.

MATERIAL AND METHODS

Study Design

IRB approval for the HCHS/SOL study was obtained at each field center, the coordinating center and the central laboratory. Fasting blood samples were obtained following a standardized venipuncture protocol by staff at the HCHS/SOL baseline clinic visit. Approximately 80 ml of blood and 10 ml of urine specimens were collected according to the standardized protocol [12,13]. All biospecimens were processed at the field centers into multiple 500 μl serum and plasma aliquots and frozen at −80°C. The serum tubes were kept at room temperature for 30–45 min prior to centrifugation to allow for clotting while the citrate and EDTA anticoagulated plasma tubes were processed within 15 min of blood collection. The anticoagulated tubes were centrifuged at 3000 × *g* for 30 min at 15°C while the serum tubes were centrifuged at 3000 × *g* for 10 min at 15°C. Urine samples were kept refrigerated after collection at 4°C and processed within 12 h of collection. After thoroughly mixing the urine samples aliquots of neutral urine, alkaline and acidic urine were prepared and frozen at −80°C. Frozen specimens were shipped on dry ice to the central laboratory at the University of Minnesota weekly. The frozen aliquots were used to analyze a variety of biochemical markers at baseline [12]. An unprocessed EDTA tube was shipped daily at 4°C to the central laboratory for measurement of complete blood counts. The central laboratory maintains a biorepository of plasma, serum, genomic DNA, RNA and urine for future analysis. The HCHS/SOL QC committee implemented the Within-Individual Variation study (all procedures and most questionnaires) in 58 volunteer participants to estimate the within-individual variability. In addition, the study implemented the Sample Handling study to obtain 5% duplicate biospecimens to monitor over time the variability in the measurement of various biochemical analytes. A detailed description of all the analytes measured in this study can be found in study manual 7a publically available at https://www2.cscc.unc.edu/hchs/manuals-forms.

Within-Individual Variation study—Following the HCHS/SOL protocol, all blood and urine samples were collected from 58 participant volunteers (Bronx (n=14), Chicago (n=12), Miami (n=15), and San Diego (n=15)) at 2 time points; first at baseline and then approximately a little over a month later. The repeatability study started 6 months after

HCHS/SOL baseline clinic start-up and recruitment was completed over 30 months. One individual in this study had end stage renal disease, six individuals had self-reported history of diabetes and all other participants were healthy volunteers. This study recruited equal numbers of men and women between the ages of 18–44 and 45–74.

Sample Handling study—Over the entire HCHS/SOL study collection period (36 months), a QC duplicate sample was obtained during the participant's clinic visit by either drawing 1 to 3 additional tube(s) of blood, or by dividing a urine sample into separate containers. The QC duplicate samples were collected after all the study samples (9 blood tubes and 1 urine specimen) were collected. The tourniquet was released within 2 min to minimize hemoconcentration. The duplicate samples were then processed at the field centers using the same method as for the original samples. These additional duplicate specimens were labeled with a *phantom* participant ID that was indistinguishable from other ID numbers, so that the laboratory was blinded to the replicate samples. In other words, the Sample Handling study did not collect duplicate collections for all 10 tubes for from a single participant. Instead, six participants were needed to provide a complete set of 10 QC duplicate specimens for a phantom ID. Therefore, 3,980 participants contributed to the pool of 5,545 duplicate specimens with 432 individuals contributing 2 specimens and 38 contributing three specimens. A duplicate urine sample required that the participant provide at least 15 ml of urine. A total of 12 ml were divided among six 2.0 ml vials for determination of creatinine and albumin levels by the Central Laboratory, and four aliquots were stored for future analyses. Thus, depending on the biomarker, 761 to 929 HCHS/SOL participants contributed duplicate samples for the Sample Handling Study. For data analysis, results on each duplicate specimen were matched to the corresponding participant results at the Coordinating Center using the Phantom ID Form which links both IDs completed by field center technicians.

STATISTICAL ANALYSIS

Before any analysis was done on the Sample Handling Study and the Within-Individual Variation Study, the data was initially screened for possible mismatches (e.g., sample mislabeling) and excluded from further analyses. Biomarkers with skewed distributions were log-transformed. We used scatterplots and Bland-Altman plots to visually check linearity and constant variance, and to identify outliers (defined as difference from mean > 3SD). Analyses reported exclude outliers. The biomarker's total variance (σ^2) was partitioned into 3 components: the within-individual variance (σ^2_I), the between-individual variance (σ^2_W) and the methodological variance (σ^2_{P+A}; combination of process and analytical variance). Specifically, we used data from the Within-Individual Variation Study to estimate the total within-individual variance (σ^2_I) (which includes both biological variation within individuals and methodological variation) and data from the Sample Handling study to estimate both the between-individual variance (σ^2_G) and methodological variance (σ^2_{P+A}). We used the Sample Handling Study to estimate between-individual variability in the HCHS/SOL since the Sample Handling Study was a random sample of the HCHS/SOL cohort and more closely mirrors the biomarker distribution in the HCHS/SOL cohort. These three variance components were estimated using linear mixed models with random intercepts using

maximum likelihood estimation[14], an extension of the ANOVA models used previously[15] that assumes our participants come from a random sample of a larger population about whom we want to make inference. While the model structure, as seen below, was identical it is important to note that 2 separate models were fit, one for the Within-Individual Variation Study and one for the Sample Handling Study. Thus, the definition of Y_{ij}, as defined as the jth biomarker measurement on the ith individual, was different in the Within-Individual Variation and Sample Handling studies. Albumin/creatinine ratio was highly skewed and hence was log-transformed. The between-individual variance was the variance of the random effect term, b_{0i}, and the within-individual variance and the methodological variance were estimated as the variance of the random error term e_{ij}.

$$Y_{ij}=\beta_0+b_{0i}+e_{ij}$$

The process and analytical variance was assumed to include both the process (pre and post-analytical) and laboratory assay (analytical) variability. However, we were able to calculate the laboratory assay (analytical) variability (σ^2_A) using the observed variability in biomarker measurement in control samples (independent from the study samples) that were analyzed in at least 20 consecutive analytical runs prior to start of the study. The assay performance was monitored during the course of the study using laboratory controls and participation in external proficiency tests through the College of American Pathology (CAP). These control samples showed that the assay performance remained unchanged during the duration of the study. For each biomarker, we estimated the within-individual coefficient of variation (CV_I) based on data from the Within-Individual Variation Study as the standard deviation (the square root of the within-individual variance component) multiplied by 100 and divided by the average value (the average value being the mean of the average of the original and the repeat measurement). Similarly, the between-individual and process and analytical coefficient of variation (CV_G and CV_{P+A} respectively) were estimated based on data from the Sample Handling Study. In the case of the between-individual variation, the mean value used to calculate the CV was the mean value of the blinded duplicate data. The process and analytical CV (CV_{P+A}) was estimated using the standard deviation expressed as a percent of the mean of the blinded duplicate pairs. We used these CV values to estimate desirable imprecision ($CV_I/2$), desirable bias ($0.25*[(CV_I)^2+(CV_G)^2]^{1/2}$) and total error (1.65*(desirable imprecision) + (desirable bias)). We calculated a statistic commonly used in clinical pathology literature called the index of individuality (II)[15] using the equation $II = (S_I+S_{P+A})/S_G$ where $S_G + S_{P+A} = (CV^2_I+ CV^2_{P+A})^{1/2}$ and $S_G = (CV^2_G)^{1/2}$. Finally we performed all the above described analyses stratified by sex and age group (18–44 and 45–74 y). To test for significant differences in the index of individuality between sex and age groups, an approximate permutation test was conducted using 500 rearrangements of the dataset [16]. Reassignments of both age and gender categories were done by random assignment while ensuring the age and gender distributions were identical to the true population. An α level of 0.05 was used to determine statistical significance.

RESULTS

Table 1 shows the within-individual variability (CV_I), the between-individual variability (CV_G), the combined process and analytical variability (CV_{P+A}) and the analytical variability (CV_A) for all the biomarkers measured at HCHS/SOL baseline. Overall, most of the biomarkers met one of the most widely used criteria for acceptable level of analytical precision (CV_A < desirable imprecision), with the exception of serum cystatin (2.9% vs. 2.6%), serum creatinine (4.1% vs. 3.6%) and total iron binding capacity (TIBC) (2.7% vs. 2.3%). The overall process and analytical error was lower than the total error (1.65* desirable imprecision + desirable bias) for all the analytes. The index of individuality ranged from 0.11 to 1.36 indicating a relatively large range across all these analytes. Subsequently, we compared the CV_I, CV_G and the index of individuality observed in HCHS/SOL with the corresponding values in NHANES or other published studies (Table 2). A majority of analytes showed substantially higher CV_G in HCHS/SOL as compared to previously published studies while the CV_I in HCHS/SOL was comparable to published literature (Table 2). This is reflected by the lower II in HCHS/SOL as compared to other studies (Table 2). The index of individuality did not differ substantially for the majority of the analytes across both sexes with some exceptions (Table 3). The index of individuality was significantly lower in women as compared to men for neutrophil count ((0.47 vs. 0.94; p=0.002), fasting insulin (0.32 vs. 0.58; p<0.0001), platelet count (0.28 vs. 0.40; p=0.02) and hemoglobin (0.36 vs. 0.48; p=0.04). The index of individuality was significantly higher among women as compared to men for fasting glucose (0.21 vs. 0.10; p=0.03) and % eosinophils (0.54 vs. 0.32; p=0.04). Though not statistically significant, the index of individuality for urinary creatinine was also substantially higher among women as compared to men (0.93 vs. 0.56; p=0.07). Analysis stratified by age group (18–44 vs. 45+ y) showed that the index of individuality was significantly higher among the 18–44 y age group as compared to the 45–74 age group for fasting insulin (0.57 vs. 0.30; p=0.002), post OGTT glucose (0.80 vs. 0.43; p=0.002), logarithmically transformed urinary albumin/creatinine ratio (0.66 vs. 0.27; p=0.002) and cystatin C (0.35 vs. 0.23; p=0.03) (Table 4).

DISCUSSION

This study found that the between-individual variability was substantially higher in the HCHS/SOL population as compared to published literature [4] including NHANES [17] while the within-individual variability was comparable to other studies. These findings are also reflected in the substantially lower index of individuality in HCHS/SOL as compared to other studies. Notable examples of analytes with the substantially higher CV_G and lower index of individuality in HCHS/SOL as compared to the published literature and the variability estimates from the NHANES study include fasting glucose, triglycerides and ferritin. The index of individuality for these biomarkers was 0.16, 0.29 and 0.19 respectively in the HCHS/SOL while the corresponding values in the published literature are 0.66–0.78 [4, 17], 0.51–0.61 [4,17] and 0.95 [4]. Small sample sizes in many studies evaluating biomarker variability, exclusive inclusion of patients on treatment for certain biomarkers (e.g. inclusion of diabetics for estimating variability in glucose), variability between different assays used for measurement of analytes and inclusion of hospital based samples as compared to a random subset of the general population) are possible explanations for the

observed differences. However, our study also included some individuals with diabetes, mild elevation of liver enzymes and/or end stage renal disease. So inclusion of patients with clinical disease alone is not sufficient to explain the observed differences. Differences in study design, where estimates for CV_I and CV_G were obtained from different participants in HCHS/SOL while several of the other studies obtained estimates for CV_I and CV_G from the same participants is another potential explanation for the observed differences. A study design similar to that used in HCHS/SOL is also commonly used in several epidemiological studies such as NHANES [17–19] and the Atherosclerosis Risk in Communities (ARIC) [11] as CV_G estimates can usually be obtained on a much larger group of people as compared to the CV_I The NHANES, [17–19] which is a population-based study and estimated CV_I and CV_G from different participants also reported different estimates for some biomarkers as compared to HCHS/SOL. This suggests that the time interval between the 2 measurements to estimate within-individual variability (average of 19 days in NHANES as compared to an average of 44 days in HCHS/SOL) and racial/ethnic differences in the 2 populations (majority non-Hispanic whites in NHANES) are other possible explanations for the observed differences in variability observed for various analytes. The CV_I estimate in HCHS/SOL and NHANES is also limited by the nonrandom, self-selected design and may also contribute to the observed differences between the 2 studies.

In the context of clinical medicine, as formally evaluated by Harris [20], when the index of individuality for a particular biomarker is low (<0.6), the participant's test results stay within the population-based reference range. In the context of epidemiological studies, the lower index of individuality (and corresponding higher reliability coefficients) suggest that single point measurements of these analytes may more accurately reflect long term homeostatic set points for these analytes [15] in the diverse Hispanic population as compared to other racial/ethnic subgroups. Participants in the HCHS/SOL study have substantially higher between-individual variability as compared to published data while the within-individual variability estimates were similar to published literature The higher between-individual coefficients of variability observed in HCHS/SOL may also indicate substantial heterogeneity in biomarker distributions across Hispanic backgrounds. However, the design of the HCHS/SOL study where people with specific Hispanic backgrounds were recruited from specific field centers does not allow us to completely distinguish between field center specific effects and Hispanic background group effects. The immediate implications of the lower index of individuality (conversely higher reliability coefficient) is that epidemiological studies in diverse Hispanic populations may require smaller sample sizes to detect significant associations of magnitudes similar to those detected in other populations and that adjustment for Hispanic background may be necessary to minimize confounding of any observed biomarker-disease associations.

As previously reported in published literature, we confirmed lower mean levels of several biomarkers such as hemoglobin [21], neutrophil count [22,23] and fasting glucose [24] among women as compared to men while women had higher platelet count [23, 25] and fasting insulin levels as compared to men. Though no previous studies have reported higher mean fasting insulin concentrations among women as compared to men, a previous study has shown higher insulin sensitivity among women as compared to men [26]. We also

observed sex specific differences in the index of individuality, with fasting glucose having significantly higher index of individuality among women as compared to men while hemoglobin, platelet count, neutrophil count and fasting insulin all having lower index of individuality among women as compared to men. The NHANES reported no sex-specific differences in the index of individuality for fasting glucose or the hematological parameters such as hemoglobin, platelet count and neutrophil count [19]. In contrast, the NHANES reported sex specific differences in the between-individual variance for several analytes such as ferritin, creatinine and ALT though no sex specific differences were noted in the HCHS/SOL[18],[19]. These results suggest that, at least for some commonly used biomarkers, sex specific differences in the index of individuality and reliability coefficient may affect ability to detect associations of similar magnitude between a biomarker and an outcome in the 2 sexes in a diverse Hispanic population. This study also demonstrates higher index of individuality among younger individuals as compared to older age groups for fasting insulin, cystatin C, logarithmically transformed urinary albumin/creatinine ratio and OGTT glucose. These findings are consistent with previously published data on serum creatinine that shows inter-individual variability for serum creatinine increasing with age[27]. However, given the large number of comparisons made in this study, these findings need to be confirmed in other studies. Both the NHANES and the HCHS/SOL studies found no differences in the index of individuality across age groups for the hematological parameters evaluated in both the studies [18].

We also evaluated the specific components of process and analytical variability by separating out the analytical variability (CV_A) and the variation due to the pre-analytical differences in processing of blood samples. For most analytes, the pre-analytical variation is minimal and a majority of the analytical variation (60%–100%) is due to analytical measurement error and reflect the rigorous implementation of standardized protocols for collection and processing of biospecimens in the HCHS/SOL. Few specific analytes, such as the urinary albumin/creatinine and high sensitivity CRP remain sensitive to small variations in collection and processing of biospecimens and procedures. Further refinement of the protocol to minimize the time delay between urine collection and processing of urinary specimens may lead to lower process and analytical variation for the urinary analytes. A majority of the hematological variables show that approximately half of the overall methodological variability is due to pre-analytical variation in this study. In the HCHS/SOL, the whole blood samples were shipped to the central laboratory within 24–72 hours after collection and complete blood counts were performed in a central laboratory. The sample shipping to the central laboratory likely increases the contribution of pre-analytical variation to the overall method variation. Hence this observation for the hematological variables may not be applicable to other clinical scenarios where the blood is processed soon after sample collection. Of note, cystatin C and creatinine, 2 widely used measures to estimate kidney function, showed that the analytical variability of these assays were higher than the optimal imprecision estimated by the within person variability. Both cystatin C and creatinine were measured in a CLIA certified laboratory and results of external proficiency testing (performed every 3 months) for both analytes showed that the results were within acceptable limits for both analytes with no evidence of long term laboratory assay shifts or drifts. Intra-individual CV and inter-individual CVs are properties of the population being studied and

can vary based on population characteristics (age, sex, ethnic distribution etc.). Thus, though the analytical CVs for the cystatin C and serum creatinine were within acceptable limits in terms of analytical precision as estimated by the external proficiency testing samples and similar to the analytical CVs reported in NHANES (serum creatinine: 4.1% in HCHS/SOL vs. 4.6% in NHANES), based on the distribution of the intra-individual and inter-individual CVs, the analytical goals for the HCHS/SOL study demand more stringent control of analytical variation for these 2 analytes. This highlights an important issue; while it is desirable for all analytes to meet these analytical goals, some of the currently available in-vitro diagnostics methods may not be able to meet the specifications for individual research studies. As described previously the higher analytical variability in serum creatinine may lead to clinical misinterpretation of creatinine based eGFR values that are used for staging chronic kidney disease [28]. The impact of higher analytical variability on the ability of the HCHS/SOL study to accurately classify participants into various categories of kidney function needs to be further evaluated.

In summary, our study shows significant differences in parameters such as the index of individuality and the reliability coefficient between the diverse Hispanic population in HCHS/SOL and the published literature. We further document sex and age specific differences for many biomarkers in this Hispanic population. The implications of these findings for determining associations between biomarkers and various disease outcomes, repeated measurement of several biomarkers and disease classification need to be evaluated in future studies.

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Highlights

- There is little data on background biomarker variability in Hispanic populations.
- US based Hispanics show higher between-person variability for several biomarkers.
- US based Hispanics also show lower index of individuality for several biomarkers.

Table 1

Estimates for within-individual, between-individual and process and analytical variabilities in HCHS/SOL

	HCHS/SOL Within-Individual Variation Study			HCHS/SOL Sample Handling Study					Desirable imprecison	Desirable Bias	Total Error
	N	Mean (SD_I)	CV_I	N	Mean (SD_G)	CV_G between	$CV_{(P+A)}$ process+ analytical	CV_A	II			
LIVER ENZYME MEASURES												
Alanine aminotransferase (U/l)	50	25.0 (6.03)	24.1	791	26.1 (1.61)	72	6.2	5	0.35	12.05	18.98	38.86
Aspartate aminotransferase (U/l)	51	23.1 (3.68)	15.9	787	23.9 (1.7)	49.04	7.1	6	0.36	7.95	12.89	26.01
GGT (U/l)	49	31.2 (7.14)	22.9	774	31.8 (1.95)	95.3	6.1	3.6	0.25	11.45	24.50	43.40
KIDNEY FUNCTION MEASURES												
Cystatin C (mg/l)	49	0.81 (0.04)	5.2	771	0.78 (0.03)	25.63	3.6	2.9	0.25	2.60	6.54	10.83
Creatinine (mg/dl)	49	0.83 (0.06)	7.3	797	0.84 (0.06)	39.23	6.8	4.1	0.25	3.65	9.98	16.00
Urine creatinine, random (mg/dl)	51	131 (57.16)	43.6	917	146.6 (4.59)	55.07	3.1	1.4	0.79	21.80	17.56	53.53
Urine microalbumin, random (mg/dl)	44	17.1 (8.26)	48.2	885	35.9 (5.84)	481.41	16.3	4.8	0.11	24.10	120.95	160.72
Log Albumin/creatinine ratio (mg/g)	45	2.2 (0.40)	17.8	879	2.1 (0.12)	48.06	5.4	–	0.39	8.90	12.81	27.50
LIPID MEASURES												
Total cholesterol (mg/dl)	51	199 (13.55)	6.8	786	197.7 (5.85)	20.04	3	2.2	0.37	3.40	5.29	10.90
Triglycerides (mg/dl)	50	126.3 (23.62)	18.7	788	133.8 (5.23)	66.81	3.9	2.8	0.29	9.35	17.34	32.77
HDL-cholesterol (mg/dl)	50	50.4 (3.22)	6.4	791	49.9 (1.59)	25.7	3.2	2.6	0.28	3.20	6.62	11.90
LDL-cholesterol (mg/dl)	51	122.8 (11.87)	9.7	768	121 (4.54)	27.42	3.7	3.6	0.38	4.85	7.27	15.27
DIABETES RELATED MEASURES												

	HCHS/SOL Within-Individual Variation Study			HCHS/SOL Sample Handling Study					Desirable imprecison	Desirable Bias	Total Error
	N	Mean (SD_I)	CV_I	N	Mean (SD_G)	CV_G between	$CV_{(P+A)}$ process+ analytical	CV_A	II			
Insulin, fasting (mU/l)	48	12.8 (3.19)	25	766	12.9 (1.61)	75.85	12.5	6	0.37	12.50	19.97	40.59
Insulin, post OGTT (mU/l)	37	77.5 (24.45)	31.6	919	86.9 (7.3)	96.11	8.4	5.9	0.34	15.80	25.29	51.36
Glucose, fasting (mg/dl)	50	95.1 (4.92)	5.2	779	105.1 (2.96)	36.26	2.8	2.6	0.16	2.60	9.16	13.45
Glucose, post OGTT (mg/dl)	37	110.4 (22.35)	20.2	928	122.2 (4.06)	36.07	3.3	1.6	0.57	10.10	10.34	27.00
% Glycated Hemoglobin	57	5.6 (0.13)	2.2	924	5.8 (0.05)	20.39	0.8	0.6	0.12	1.10	5.13	6.94
IRON RELATED MEASURES												
Ferritin (ug/l)	48	90.7 (16.41)	18.1	762	114.7 (5.87)	96.53	5.1	2.9	0.19	9.05	24.55	39.49
Iron (ug/dl)	50	80.6 (23.65)	29.3	786	88.5 (2.75)	37.54	3.1	3.4	0.79	14.65	11.91	36.08
Total Iron Binding Capacity (TIBC) (ug/dl)	50	314.5 (14.43)	4.6	789	322.5 (10.39)	14.79	3.2	2.7	0.38	2.30	3.87	7.67
% Transferrin saturation	49	25.9 (7.57)	29.3	786	28.2 (0.76)	41.57	2.7	2.4	0.71	14.65	12.71	36.89
INFLAMMATORY MEASURES									0.00	0.00	0.00	
High-sensitivity C-Reactive Protein (mg/l)	50	3.7 (2.04)	54.9	789	3.8 (0.19)	136.5	4.9	2.5	0.4	27.45	36.78	82.07
HEMATOLOGICAL MEASURES												
Red Blood Count (×10e12)	56	4.7 (0.12)	2.5	920	4.7 (0.08)	8.69	1.7	0.9	0.35	1.25	2.26	4.32
White Blood Count (×10e9)	53	6.6 (0.79)	11.8	839	6.5 (0.36)	28.63	5.5	1.8	0.46	5.90	7.74	17.48
Platelet Count (×10e9)	56	264.5 (18.61)	7	919	251.5 (9.52)	26.08	3.8	2.4	0.31	3.50	6.75	12.53
Hemoglobin (g/dl)	55	13.5 (0.36)	2.7	920	13.7 (0.25)	10.53	1.8	0.8	0.31	1.35	2.72	4.95
% Hematocrit	56	41.4 (1.39)	3.4	920	41.8 (0.79)	9.16	1.9	1.2	0.42	1.70	2.44	5.25

	HCHS/SOL Within-Individual Variation Study			HCHS/SOL Sample Handling Study						Desirable imprecison	Desirable Bias	Total Error
	N	Mean (SD_I)	CV_I	N	Mean (SD_G)	CV_G between	$CV_{(P+A)}$ process+ analytical	CV_A	II			
Mean Corpuscular Volume (fl)	53	88.6 (1.53)	1.7	920	88.9 (0.6)	6.46	0.7	0.8	0.29	0.85	1.67	3.07
Mean Corpuscular Hemoglobin (pg)	55	29.1 (0.34)	1.2	921	29.1 (0.28)	7.08	0.9	0.8	0.21	0.60	1.80	2.79
Mean Corpuscular Hemoglobin Concentration (g/dl)	55	32.7 (0.85)	2.6	924	32.8 (0.36)	4.21	1.1	1	0.67	1.30	1.24	3.38
% Red Cell Distribution Width	54	13.6 (0.32)	2.3	929	13.7 (0.11)	9.25	0.8	0.7	0.27	1.15	2.38	4.28
% Neutrophils	54	55.2 (6.97)	12.6	835	54.6 (3.45)	19.27	6.3	1.7	0.73	6.30	5.76	16.15
% Lymphocytes	54	33.1 (5.82)	17.6	832	33.5 (2.7)	25.45	8	2.4	0.76	8.80	7.74	22.26
% Monocytes	53	8.2 (1.5)	18.3	835	8.1 (1.02)	27.5	12.6	4.9	0.81	9.15	8.26	23.36
% Eosinophils	53	3.0 (1.04)	34.5	838	2.9 (0.52)	93.29	17.9	6.7	0.42	17.25	24.87	53.33
% Basophils	54	0.6 (0.44)	72	832	0.5 (0.37)	73.75	69.8	1.1	1.36	36.00	25.77	85.17
Neutrophil Count (×10e9)	52	3.7 (0.62)	16.8	832	3.6 (0.35)	39.59	9.7	2.7	0.49	8.40	10.75	24.61
Lymphocyte Count (×10e9)	52	2.1 (0.25)	12.3	832	2.1 (0.16)	28.39	7.6	2.8	0.51	6.15	7.73	17.88
Monocyte Count (×10e9)	52	0.5 (0.08)	16.6	844	0.5 (0.07)	32.09	14	6.2	0.67	8.30	9.03	22.73
Eosinophil Count (×10e9)	53	0.2 (0.05)	27.5	845	0.2 (0.04)	100.99	19.9	7	0.34	13.75	26.17	48.85
Basophil Count (×10e9)	54	0.0 (0.03)	126.4	840	0.0 (0.03)	146	129.1	2.1	1.24	63.20	48.28	152.56

CV_I = coefficient of variation for within-individual variability

CV_G = coefficient of variation for between-individual variability. Mean values of the blinded duplicate

$CV_{(P+A)}$ = coefficient of variation for process and analytical variability

CV_A = coefficient of variation for analytical variability

Maximum desirable analytical imprecision = $(CV_I/2)$

Desirable accuracy $(0.25*[(CV_I)^2+(CV_G)^2]^{1/2})$

total error (1.65*(desirable imprecision) + (desirable accuracy))

II is the Index of Individuality

The sample size reported excluded outliers defined as paired differences in absolute value greater than three standard deviations.

N = Number of participants in the Within-Individual Variation study and the Sample Handling study.

Table 2

Comparison of within-individual and between-individual variabilities in HCHS/SOL and other studies

	HCHS/SOL			NHANES[17–19]			Ricos database[4–6]		
	CV_I	CV_G	II	CV_I	CV_G	II	CV_I	CV_G	II
LIVER ENZYME MEASURES									
Alanine aminotransferase (U/l)	24.1	72	0.35	20.3,23.7	43.4,50.2	0.47,0.47	19.4	41.6	0.47
Aspartate aminotransferase (U/l)	15.9	49.04	0.36	15.5,15.1	27.1,29.1	0.52,0.57	12.3	23.1	0.53
GGT (U/l)	22.9	95.3	0.25	16.2,16.2	56.7,59.8	0.27,0.29	13.4	42.15	0.32
KIDNEY FUNCTION MEASURES									
Cystatin C (mg/l)	5.2	25.63	0.25				5	13	0.38
Creatinine (mg/dl)	7.3	39.23	0.25	6.7,6.8	27.6,18.7	0.36,0.24	11	23	0.4
Urine creatinine, random (mg/dl)	43.6	55.07	0.79	43	61.3	0.7	36.3	32.4	1.12
Urine microalbumin, random (mg/dl)	48.2	481.41	0.11						0.65
Log Albumin/creatinine ratio (mg/g)	17.8	48.06	0.39						
LIPID MEASURES									
Total cholesterol (mg/dl)	6.8	20.04	0.37	6.8,8.2	21.5,22.3	0.37,0.33	5.95	15.3	0.39
Triglycerides (mg/dl)	18.7	66.81	0.29	28.9,28.8	56.9,56.8	0.51,0.49	19.9	32.7	0.61
HDL-cholesterol (mg/dl)	6.4	25.7	0.28	12.4	28.3	0.44	7.3	21.2	0.34
LDL-cholesterol (mg/dl)	9.7	27.42	0.38						0.38
DIABETES RELATED MEASURES									
Insulin, fasting (mU/l)	25	75.85	0.37	25.2	55.9	0.45	21.1	58.3	0.36
Insulin, post OGTT (mU/l)	31.6	96.11	0.34						
Glucose, fasting (mg/dl)	5.2	36.26	0.16	7.1,8.3	11.3,12.5	0.66,0.64	4.5	5.8	0.78
Glucose, post OGTT (mg/dl)	20.2	36.07	0.57						
% Glycated Hemoglobin	2.2	20.39	0.12	1.5	9.6	0.16	1.85	5.7	0.33
IRON RELATED MEASURES									
Ferritin (ug/l)	18.1	96.53	0.19	17.9	73.7	0.24	14.2	15	0.95
Iron (ug/dl)	29.3	37.54	0.79	31.5	39.4	0.76	26.5	23.2	1.14
Total Iron Binding Capacity (TIBC) (ug/dl)	4.6	14.79	0.38	9.1	15	0.56			
% Transferrin saturation	29.3	41.57	0.71				3	4.3	0.7
INFLAMMATORY MEASURES									

	HCHS/SOL			NHANES[17–19]			Ricos database[4–6]		
	CV_I	CV_G	II	CV_I	CV_G	II	CV_I	CV_G	II
High-sensitivity C-Reactive Protein (mg/l)	54.9	136.5	0.4				49.7	89.23	0.56
HEMATOLOGICAL MEASURES									
Red Blood Count (×10e12)	2.5	8.69	0.35						
White Blood Count (×10e9)	11.8	28.63	0.46				11.4	21.3	0.54
Platelet Count (×10e9)	7	26.08	0.31				9.1	21.9	0.42
Hemoglobin (g/dl)	2.7	10.53	0.31				2.85	6.8	0.42
% Hematocrit	3.4	9.16	0.42				2.7	6.41	0.42
Mean Corpuscular Volume (fl)	1.7	6.46	0.29				1.4	4.85	0.29
Mean Corpuscular Hemoglobin (pg)	1.2	7.08	0.21				1.4	5.2	0.27
Mean Corpuscular Hemoglobin Concentration (g/dl)	2.6	4.21	0.67				1.06	1.2	0.88
% Red Cell Distribution Width	2.3	9.25	0.27				5.7	1.8	0.61
% Neutrophils	12.6	19.27	0.73						
% Lymphocytes	17.6	25.45	0.76						
% Monocytes	18.3	27.5	0.81						
% Eosinophils	34.5	93.29	0.42						
% Basophils	72	73.75	1.36						
Neutrophil Count (×10e9)	16.8	39.59	0.49				17.1	32.8	0.52
Lymphocyte Count (×10e9)	12.3	28.39	0.51				10.2	35.3	0.29
Monocyte Count (×10e9)	16.6	32.09	0.67				17.8	49.8	0.36
Eosinophil Count (×10e9)	27.5	100.99	0.34				21	76.4	0.27
Basophil Count (×10e9)	126.4	146	1.24				28	54.8	0.51

CV_I = coefficient of variation for within-individual variability

CV_G = coefficient of variation for between-individual variability. Mean values of the blinded duplicate

Table 3

Sex specific estimates for within-individual and between-individual variability in HCHS/SOL

	FEMALES								MALES							
	HCHS/SOL Within-Individual Variation Study		HC HS/SOL Sample Handling Study						HC HS/SOL Within-Individual Variation Study		HCHS/SOL Sample Handling Study					
	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]
LIVER ENZYME MEASURES																
Alanine aminotransferase (U/l)	32	28.3	485	22.7 (1.58)	77.58	7	0.38	0.55	18	19.3	307	31.4 (1.72)	61.46	5.5	0.33	0.46
Aspartate aminotransferase (U/l)	31	11.7	484	22.0 (1.71)	47.59	7.8	0.3	0.56	19	17.2	304	26.8 (1.73)	48.12	6.5	0.38	0.63
GGT (U/l)	30	24.2	475	25.9 (1.77)	100.72	6.8	0.25	0.30	18	12.6	299	41.2 (2.21)	82.61	5.4	0.17	0.29
KIDNEY FUNCTION MEASURES																
Cystatin C (mg/l)	30	5.6	467	0.75 (0.03)	22.57	3.7	0.3		19	4.7	302	0.83 (0.03)	27.95	3.2	0.2	
Creatinine (mg/dl)	31	8.3	486	0.75 (0.06)	43.36	7.4	0.26	0.39	18	5.9	307	0.99 (0.05)	28.61	5.5	0.28	0.25
Urine creatinine, random (mg/dl)	32	54.6	548	128.7 (4.79)	58.87	3.7	0.93		19	26.0	367	173.6 (3.64)	46.49	2.1	0.56	
Urine microalbumin, random (mg/dl)	28	59.5	514	22.8 (1.74)	391.03	7.6	0.15		16	22.7	363	44.9 (4.36)	506.06	9.7	0.05	
Log Albumin/creatinine	2	18.0	51	2.2	42.3	4.2	0.3		17	17.0	36	2.0	56.2	6.5	0.39	
ratio (mg/g)	8		2	(0.09)	4		2				3	(0.13)	7			
LIPID MEASURES																
Total cholesterol (mg/dl)	32	6	482	198.1 (5.96)	19.77	3	0.34	0.33	19	8.1	304	197.2 (5.69)	20.49	2.9	0.42	0.32
Triglycerides (mg/dl)	31	18.6	484	126.4 (5.26)	63.99	4.2	0.3	0.47	19	18.7	305	145.7 (6.6)	68.86	4.5	0.28	0.51
HDL-cholesterol (mg/dl)	32	5.9	483	51.8 (1.62)	25.36	3.1	0.26		19	8.9	308	46.8 (1.54)	24.93	3.3	0.38	

	FEMALES								MALES							
	HCHS/SOL Within-Individual Variation Study		HC HS/SOL Sample Handling Study						HCHS/SOL Within-Individual Variation Study		HCHS/SOL Sample Handling Study					
	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]
LDL-cholesterol (mg/dl)	32	8.8	471	121.2 (4.55)	27.69	3.8	0.35		19	11.2	297	120.7 (4.51)	27.04	3.7	0.44	
DIABETES RELATED MEASURES																
Insulin, fasting (mU/l)	31	22.3	447	13.4 (1.72)	80.7	12.9	0.32		18	36.9	319	12.3 (1.44)	66.78	11.7	0.58	
Insulin, post OGTT (mU/l)	21	30.3	548	94.4 (7.8)	92.63	8.3	0.34		16	31.8	368	75.7 (5.9)	100.76	7.8	0.32	
Glucose, fasting (mg/dl)	32	6.9	452	102.5 (2.88)	34.64	2.8	0.21	0.66	19	2.6	327	108.6 (3.06)	37.96	2.8	0.1	0.63
Glucose, post OGTT (mg/dl)	21	16.6	552	127.1 (4.34)	34.91	3.4	0.48		16	26	373	115.2 (3.39)	37.21	2.9	0.7	
% Glycosylated Hemoglobin	35	2	553	5.8 (0.06)	20.62	1	0.11		21	2	373	5.8 (0.05)	19.98	0.8	0.11	
IRON RELATED MEASURES																
Ferritin (ug/l)	29	21.9	464	74.4 (5.54)	101.92	7.4	0.23		19	14.4	299	180.4 (7.31)	74.98	4.1	0.2	
Iron (ug/dl)	31	26.9	477	80.7 (2.38)	37.57	2.9	0.72	0.74	19	33.6	307	101 (3.28)	34.02	3.2	0.99	0.80
Total Iron Binding Capacity (TIBC) (ug/dl)	32	5.4	482	329.3 (10.42)	14.93	3.2	0.42	0.53	19	4.5	308	311.7 (10.49)	13.87	3.4	0.41	0.61
% Transferrin saturation	31	305	481	25.2 (0.7)	41.69	2.8	0.74		19	35.1	305	33 (0.84)	36.39	2.6	0.97	
INFLAMMATORY MEASURES																
High-sensitivity C-Reactive Protein (mg/l)	31	48.2	481	4.4 (0.2)	123.87	4.5	0.39		18	22.4	302	2.6 (0.1)	129.87	3.8	0.17	
HEMATOLOGICAL MEASURES																
Red Blood Count (×10e12)	36	2.4	549	4.5 (0.08)	7.13	1.8	0.42	0.37	20	2.7	371	5 (0.08)	6.88	1.6	0.46	0.36
White Blood Count (×10e9)	34	8.9	509	6.5 (0.34)	27.23	5.3	0.38	0.50	19	15.8	332	6.4 (0.4)	30.69	6.3	0.55	0.55

	FEMALES								MALES							
	HCHS/SOL Within-Individual Variation Study		HC HS/SOL Sample Handling Study						HC HS/SOL Within-Individual Variation Study		HCHS/SOL Sample Handling Study					
	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]
Platelet Count (×10e9)	36	6.3	546	267.3 (9)	25.56	3.4	0.28	0.37	20	8.3	371	228.1 (9.75)	23.32	4.3	0.4	0.36
Hemoglobin (g/dl)	35	2.5	549	12.9 (0.26)	8.81	2	0.36	0.34	20	2.9	371	14.9 (0.25)	6.95	1.7	0.48	0.31
% Hematocrit	36	3.6	550	39.8 (0.8)	7.62	2	0.54	0.36	20	3	370	44.6 (0.76)	6.71	1.7	0.52	0.32
Mean Corpuscular Volume (fl)	35	2.6	549	88.6 (0.6)	6.84	0.7	0.39	0.06	19	1	371	89.4 (0.6)	5.83	0.7	0.21	0.06
Mean Corpuscular Hemoglobin (pg)	35	1.2	550	28.7 (0.28)	7.78	1	0.19		20	1.2	369	29.7 (0.27)	5.42	0.9	0.27	
Mean Corpuscular Hemoglobin Concentration (g/dl)	35	2.8	552	32.5 (0.36)	4.1	1.1	0.72		20	2.3	372	33.3 (0.36)	3.94	1.1	0.64	
% Red Cell Distribution Width	36	3.1	552	13.8 (0.09)	9.89	0.7	0.32	0.16	19	1.4	374	13.5 (0.12)	7.98	0.9	0.21	0.14
% Neutrophils	34	11	508	55.6 (3.29)	18.13	5.9	0.69	0.55	20	15.4	326	53 (3.57)	20.74	6.7	0.81	0.59
% Lymphocytes	34	17.4	507	33.5 (2.78)	25.03	8.3	0.77	0.53	20	17.8	326	33.6 (2.69)	26.29	8	0.74	0.55
% Monocytes	33	15.9	512	7.5 (1)	25.47	13.4	0.82	0.52	20	19.7	327	9 (1.15)	26.74	12.8	0.88	0.53
% Eosinophils	33	39.4	513	2.7 (0.51)	81.59	19.1	0.54	0.39	20	27.9	325	3.4 (0.55)	101.24	16.4	0.32	0.39
% Basophils	33	73.8	508	0.5 (0.37)	73.99	72.1	1.4		20	61.2	324	0.6 (0.37)	73.32	66.4	1.23	
Neutrophil Count (×10e9)	34	15.2	505	3.7 (0.34)	37.66	9.3	0.47	0.55	20	38.3	329	3.5 (0.37)	42.37	10.8	0.94	0.62
Lymphocyte Count (×10e9)	33	12.1	508	2.1 (0.16)	26.94	7.4	0.53	0.53	19	12.4	323	2.1 (0.16)	30.29	7.6	0.48	0.50
Monocyte Count (×10e9)	33	16.4	515	0.5 (0.07)	30.51	13.7	0.7	0.51	20	21	331	0.6 (0.08)	30.98	15.1	0.83	0.55

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	FEMALES								MALES							
	HCHS/SOL Within-Individual Variation Study		HC HS/SOL Sample Handling Study						HC HS/SOL Within-Individual Variation Study		HCHS/SOL Sample Handling Study					
	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]	N	CV_I	N	Mean (SD)	CV_G	CV (P+ A)	II	NHANES II[18, 19]
Eosinophil Count (×10e9)	33	30.3	515	0.2 (0.04)	89.78	20.4	0.41		20	23.8	330	0.2 (0.04)	108.52	19.1	0.28	
Basophil Count (×10e9)	34	168.3	508	0 (0.03)	153.06	134.7	1.41		20	90.4	332	0 (0.03)	136.5	121.7	1.11	

CV_I = coefficient of variation for within-individual variability

CV_G = coefficient of variation for between-individual variability. Mean values of the blinded duplicate $CV_{(P+A)}$ = coefficient of variation for process and analytical variability

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The sample size reported excluded outliers defined as paired differences in absolute value greater than three standard deviations within sex. Hence, the overall sample size is not identical to the sum of the sample sizes for females and males.

Table 4

Age group specific estimates for within individual and between individual variability in HCHS/SOL by age group

	Age Group: 18–44 y							Age Group: 45+ y						
	N	CV_I	N	Mean (SD)	CV_G	$CV_{(P+A)}$	II	N	CV_I	N	Mean (SD)	CV_G	$CV_{(P+A)}$	II
LIVER ENZYME MEASURES														
Alanine aminotransferase (U/l)	25	23.3	333	25.6 (1.42)	81.19	5.5	0.29	25	24.9	456	26.1 (1.76)	63.93	6.7	0.4
Aspartate aminotransferase (U/l)	25	17.1	334	23.1 (1.59)	48.66	6.9	0.38	25	11.4	453	24.4 (1.83)	49.15	7.5	0.28
GGT (U/l)	24	18.8	332	29 (1.26)	102.56	4.3	0.19	24	16.3	442	34 (2.73)	90	8	0.2
KIDNEY FUNCTION MEASURES														
Cystatin C (mg/l)	25	5.3	332	0.71 (0.03)	18.47	3.6	0.35	24	5.1	438	0.84 (0.03)	26.89	3.5	0.23
Creatinine (mg/dl)	24	7.5	338	0.81 (0.05)	24.36	6.7	0.41	25	7	455	0.86 (0.06)	46.37	6.5	0.21
Urine creatinine, random (mg/dl)	25	43.9	399	164 (6.09)	53.59	3.7	0.82	26	38.7	519	133.5 (3.6)	54.22	2.7	0.72
Urine microalbumin, random (mg/dl)	23	67.2	389	23 (2.66)	353.52	11.6	0.19	21	38.2	490	34.6 (2.38)	442.52	6.9	0.09
Log Albumin/creatinine ratio (mg/g)	24	29.4	390	1.9 (0.14)	46.17	7.5	0.66	22	12.8	487	2.3 (0.08)	48.25	3.6	0.27
LIPID MEASURES														
Total cholesterol (mg/dl)	25	7.6	336	185.5 (5.73)	19.97	3.1	0.41	26	6.1	451	207.1 (6.07)	18.95	2.9	0.36
Triglycerides (mg/dl)	25	22.3	336	120.9 (4.5)	72	3.7	0.31	26	18.4	453	143.6 (6.51)	62.6	4.5	0.3
HDL-cholesterol (mg/dl)	25	8.5	334	49.3 (1.45)	26.52	2.9	0.34	26	5.5	455	50.3 (1.68)	25.12	3.3	0.26
LDL-cholesterol (mg/dl)	25	9.3	329	112 (4.49)	26.93	4	0.37	26	10	439	127.8 (4.57)	26.41	3.6	0.4
DIABETES RELATED MEASURES														
Insulin, fasting (mU/l)	25	34.3	338	12.7 (1.7)	64.64	134	0.57	25	22.2	429	13.1 (1.58)	83.09	12	0.3
Insulin, post OGTT (mU/l)	21	33.5	479	70.9 (6.45)	99.67	9.1	0.35	16	29.4	439	104.1 (7.91)	88.93	7.6	0.34
Glucose, fasting (mg/dl)	24	3.4	343	97.9 (2.79)	31.88	2.8	0.14	26	6.5	433	110.9 (2.95)	37.88	2.7	0.18

	Age Group: 18–44 y							Age Group: 45+ y						
	N	CV_I	N	Mean (SD)	CV_G	$CV_{(P+A)}$	II	N	CV_I	N	Mean (SD)	CV_G	$CV_{(P+A)}$	II
Glucose, post OGTT (mg/dl)	21	23.8	486	108.3 (4.12)	30.26	3.8	0.8	16	15.2	441	137.6 (4.06)	36.06	3	0.43
% Glycosylated Hemoglobin	27	2	421	5.5 (0.05)	16.62	0.9	0.14	30	2.4	505	6.1 (0.06)	21.49	1	0.12
IRON RELATED MEASURES														
Ferritin (ug/l)	24	20.2	325	95.9 (3.84)	100.98	4	0.2	24	16.5	435	130.1 (6.99)	96.21	5.4	0.18
Iron (ug/dl)	25	34.7	332	87.9 (2.66)	38.59	3	0.9	25	23.5	451	88.7 (2.71)	36.77	3.1	0.64
Total Iron Binding Capacity (TIBC) (ug/dl)	25	5.2	336	327.4 (10.62)	14.94	3.2	0.41	26	4.9	453	318.9 (10.22)	14.58	3.2	0.4
% Transferrin saturation	25	35.1	333	27.7 (0.72)	42.94	2.6	0.82	25	29	452	28.7 (0.78)	40.62	2.7	0.72
INFLAMMATION MEASURES														
High-sensitivity C-Reactive Protein (mg/l)	24	26.1	335	3.2 (0.16)	125.86	5.1	0.21	25	58.6	453	4.2 (0.19)	138.4	4.5	0.42
HEMATOLOGICAL MEASURES														
Red Blood Count (×10e12)	26	2.8	421	4.8 (0.08)	8.42	1.7	0.39	30	2.3	500	4.7 (0.08)	8.84	1.7	0.33
White Blood Count (×10e9)	24	12.5	385	6.4 (0.36)	27.23	5.5	0.5	29	11	454	6.5 (0.36)	29.84	5.6	0.41
Platelet Count (×10e9)	26	6.7	421	255.5 (9.28)	24.87	3.6	0.31	30	7.3	496	248.3 (9.35)	27.04	3.8	0.3
Hemoglobin (g/dl)	26	3.2	420	13.8 (0.26)	10.82	1.9	0.34	30	2.7	500	13.6 (0.24)	10.21	1.8	0.32
% Hematocrit	26	3.3	419	41.9 (0.8)	9.41	1.9	0.41	30	3.4	501	41.6 (0.77)	8.94	1.9	0.43
Mean Corpuscular Volume (fl)	25	2.1	420	88.3 (0.61)	6.13	0.7	0.37	28	1.3	499	89.4 (0.6)	6.68	0.7	0.21
Mean Corpuscular Hemoglobin (pg)	25	1.2	421	29.1 (0.29)	7.12	1	0.21	30	1.2	500	29.1 (0.27)	7.04	0.9	0.21
Mean Corpuscular Hemoglobin Concentration (g/dl)	25	2.4	420	33 (0.38)	4.12	1.1	0.66	29	2.1	501	32.7 (0.33)	4.24	1	0.55
% Red Cell Distribution Width	26	3.2	423	13.5 (0.11)	9.02	0.8	0.37	29	2.1	499	13.8 (0.09)	9.07	0.6	0.24
% Neutrophils	24	10.2	382	55.4 (2.71)	18.21	4.9	0.62	30	14.3	451	53.9 (3.84)	20.1	7.1	0.8

	Age Group: 18–44 y							Age Group: 45+ y						
	N	CV_I	N	Mean (SD)	CV_G	$CV_{(P+A)}$	II	N	CV_I	N	Mean (SD)	CV_G	$CV_{(P+A)}$	II
% Lymphocytes	24	16.7	385	33.1 (2.32)	24.42	7	0.74	30	18.3	451	34 (3.28)	26.18	9.6	0.79
% Monocytes	23	9	385	8.1 (0.96)	26.47	12	0.57	29	21.1	450	8.1 (1.06)	28.38	13.2	0.88
% Eosinophils	24	28	387	2.8 (0.53)	92.67	18.6	0.36	29	37.7	451	3 (0.52)	93.66	17.3	0.44
% Basophils	24	66.4	385	0.5 (0.36)	79.72	75.7	1.26	30	75.2	452	0.6 (0.4)	79.33	67.8	1.28
Neutrophil Count (×10e9)	23	14.6	382	3.6 (0.32)	39.32	8.7	0.43	29	18.7	450	3.6 (0.37)	39.76	10.5	0.54
Lymphocyte Count (×10e9)	23	12.6	384	2.1 (0.14)	26.05	6.9	0.55	29	11.9	445	2.1 (0.16)	29.65	7.7	0.48
Monocyte Count (×10e9)	23	17.4	387	0.5 (0.07)	30.3	13.1	0.72	29	15.6	457	0.5 (0.08)	33.54	14.6	0.64
Eosinophil Count (×10e9)	24	28.8	389	0.2 (0.04)	96.31	20.8	0.37	29	26.5	456	0.2 (0.04)	104.13	19.2	0.31
Basophil Count (×10e9)	24	119.2	388	0 (0.03)	156.76	131.3	1.13	30	133.2	452	0 (0.03)	137.93	126.9	1.33

CV_I = coefficient of variation for within-individual variability

CV_G = coefficient of variation for between-individual variability. Mean values of the blinded duplicate

$CV_{(c+m)}$ = coefficient of variation for process and analytical variability

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