CHARLS Pilot: Blood-based Biomarker Documentation

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Biomarker Data in CHARLS

Data collection in CHARLS was done using a medically trained staff. Each data collection team had two recent graduates of a nursing school who collected the biological measurements. These nurses were trained in collection techniques by staff of the Public Health program at Peking University. They also watched the videos of biomarker and performance data collection provided by HRS and used for training of HRS interviewers. Nurses collected information on blood pressure, pulse, measured height, weight, waist circumference, lower leg length, and arm length. Performance tests were given to provide information on lung peak flow rate, grip strength and a timed five repetitions of sit to stand. Cardiochek-PA meters were used in the field to provide information on total and HDL cholesterol. Like HRS, CHARLS used dried blood spot (DBS) technology to provide blood samples for biochemical assay. Two dried blood spots were collected from each respondent onto Whatman 903 Protein Saver Cards. After the spots were collected, the cards were dried on a special, covered rack for at least 4 hours, then inserted into a ziplock bag along with a desiccant and then mailed from the field to our headquarters at Peking University. There they were stored in deep freezers at -20C at the Peking University laboratory.

About 25% of sample individuals did not participate in the biomarker data collection. The number of people with data differs for each marker. Analysis of who did not participate and associated sample weight corrections are described in the CHARLS User's Guide.

Cardiochek Meter Readings of Cholesterol

Cholesterol was measured at the time of interview without any request to fast prior to blood drop collection. The meter range for total cholesterol is 100 mg/dL as the bottom value and 400 mg/dL for the top value. For HDL, the meter range is from 15 mg/dL to 100 mg/dL. One value of total cholesterol was recorded as 40 mg/dL in the field and has been coded as missing.

Method of DBS-based C-reactive protein (CRP)

CRP concentrations in DBS specimens were measured using validated ELISA methods (McDade, 2004). The detection limit of this CRP assay is reported to be 0.028 mg/L. (There is an upper limit on the initial reading of the concentration off the OD curve such that the maximum is 10; on reanalysis with a diluted sample result could be multiplied by 2, so the upper limit would be 20) . The intra-assay coefficient of variation (CV) is 5.8% and the inter-assay CV is 8.2% (McDade, 2004).

In a number of cases where the coefficient of variation was above 10% a retest was done. In some cases these retests resulted in an indication of the level of concentration as below minimal value as redoing the assay often meant using a substandard blood spot. For these cases (N=40) we have entered the original CRP concentration value in a revised CRP variable. We have indicated these cases with a flag variable. A large number of cases are reported to be below minimum value (681). Initially cases without a detectable limit were retested but because the concentration was almost always still below the minimum level, this practice was stopped. The number of cases with undetectable levels of CRP was 28% in Gansu province and 44% in Zhejiang. Field reports indicate that spot quality may have been worse in Zhejiang province

because of the quality of the medical teams doing the collection. A test of whether the lab results changed over time indicated that this was not the case in Gansu province where we had variation in timing of assays (i.e. they were done from January to May in 2010 and then again from October through December in the same year). Assays were done in two time periods because of the need to acquire new reagent supplies. The first resupply was with defective material and several months passed before assays were started again with a new reagent supply. Assays on samples from Gansu were done in both periods but virtually all of the Zhejiang samples were measured in the later period.

The CRP values produced with the DBS assays are quite different from those based on whole blood values; values are on a much lower scale. For this reason we do not recommend the use of conventional cutoffs for high CRP based on whole-blood values.

Method of Hemoglobin (Hb) assays

Hemoglobin (Hb) level was measured using an ELISA protocol developed by Dr. Thomas McDade at Northwestern University, based on the method by O'Broin and Gunter (O'Broin, 1999). Across the range of the assay, the between-assay CVs for low, normal, and high control concentrations are 7.7%, 4.8%, and 6.2%, respectively.

Quality Control for DBS bioassays

CRP assay

Optic densities (OD) of all samples were measured using an automated ELISA reader at wave length of 490 nm. Every plate had 8 standard samples that were used to generate a standard curve to relate OD and standard CPR concentration. A standard curve for each microplate was generated by computer software program, based on 4-parameter method. On each plate there were also 3 control samples to ensure the stability of standard curves. All CHARLS samples, and CRP standards and controls were measured in duplicate. Individual samples with duplicate CV above 10% were rerun, unless both concentrations were less than 0.2 mg/L. When inter-assay variability exceeded 10%, which was based on control values, the entire microwell plate would be rerun.

Hb assay

OD of all samples were measured using an automated ELISA reader at wave length of 540 nm. All samples, standards, and controls were measured in duplicate. Individual samples with duplicate CV above 10% were rerun. Control samples (normal, low, and high levels) were used to ensure the stability of standard curves. When inter-assay variability exceeded 10%, the entire microwell plate would be rerun. In addition, all samples with initial hemoglobin concentrations below 8 g/dL or above 18 g/dL were also measured again to ensure the results were accurate. When low values were retested, all were fairly close to the original value except two where the difference was approximately 1 g/dL. In the cases where they were similar the retest value was used. In the two cases where there was some difference, the average was used. There was one high case which was retested twice, all readings were high (from 18 to 23 g/dL) and the average value of 21.6 g/dL was assigned.

CHARLS Validation Study

To examine the validity of collection and lab procedures in China we performed validation tests on DBS samples from community volunteers (non-CHARLS participants) collected at the UCLA Alhambra Research Center during the June through December 2010 period. Participants in the validation tests were adults 50 years of age or older. Participants provided signed consent to be part of the study and were paid $25 to participate. Participants provided a venous whole blood sample, a number of DBS, cholesterol measurements from a point of service Cardiochek PA meter, and hemoglobin readings from a point of service HemoCue meter (range 0-25.6 g/dL). The Cardiochek meter was the same model used in the field in China and the HemoCue Hb 301 meter was the same as that used in the field in Indonesia.

Validation involved a calibration of DBS results collected in Los Angeles and then assayed in the lab in China against whole blood values of CRP assayed at the University of Vermont by Russell Tracy and DBS results assayed in the lab of Thomas McDade at Northwestern University. McDade provided the training in DBS assays for the CHARLS lab staff. The whole blood was separated and frozen in the UCLA lab and frozen serum was sent to the University of Vermont. The DBS collected in Los Angeles were frozen after drying and sent frozen to China and Northwestern for analysis.

The validation study allows comparison of values of hemoglobin assayed from DBS in China with those collected using the Hemocue meter. Cholesterol values from Cardiochek meters collected in Los Angeles were compared to values collected from whole blood in Los Angeles.

Below we provide information on paired assays for CRP, hemoglobin, total cholesterol and HDL cholesterol. For each pair of matched assays we provide descriptive statistics; a simple regression linking the two values and providing an R^2; a scatterplot of the two values plotted against each other; and a Bland-Altman plot which indicates the size of the differences against the average values of the two assays.

Descriptive Information on Assays for Matched Samples

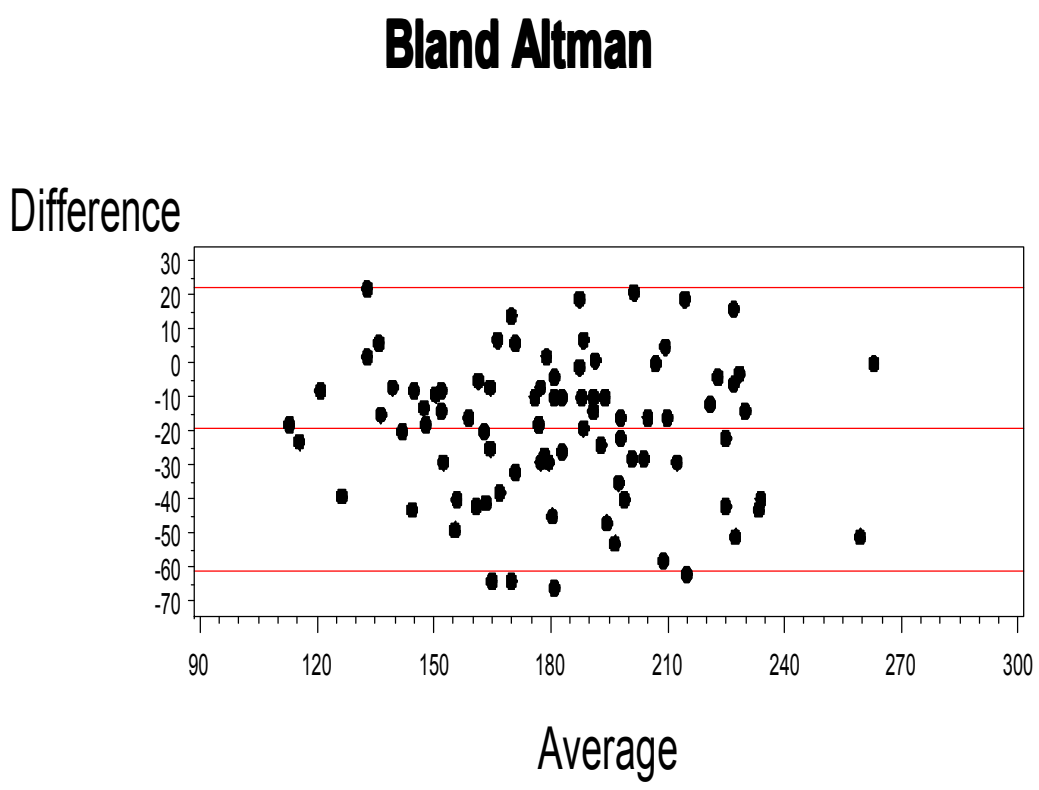
Cholesterol

Total Cholesterol (TC) was assessed using the Cardiocheck PA meter and blood serum in a sample of 88 persons. The average for the sample using the Cardiocheck meter (172 mg/dL) is about 20 points less than that based on the venous blood (192 mg/dL) (Table 1). The differences between two assays are fairly similar across the range as indicated in the scatter plots and the Bland-Altman plot (Figure 1). The R^2 between the 2 assays is .65.

Table 1. Total Cholesterol—Cardiocheck Meter vs. Vermont Blood Serum

Total Cholesterol	Average	Range	#Cases	Median	Interquartile Range
Cardiocheck	172.49 (32.72)	104.0-263.0	88	174.0	145.5-192.0
Vermont	191.73 (34.17)	122.0-285.0		191.0	167.5-215.0
Equation	Vermont = 46.43 + 0.84 Cardiocheck (11.68) (0.07)				
R^2	.65				

Figure 1: Validation Study of Cardiochek Meter: Comparison of Total Cholesterol Measured Using Cardiochek Meter and from Blood Serum at the University of Vermont



HDL Cholesterol (HDL) was assessed for 89 people using the two approaches. Again, the Cardiocheck meter provides somewhat lower values than the venous blood sample: means of 45 mg/dL and 53 mg/dL respectively (Table 2). The R^2 between these two values (.76) is higher than that for total cholesterol. The scatterplots and the Bland-Altman plots indicate the measurement differences are relatively similar across the assay range (Figure 2).

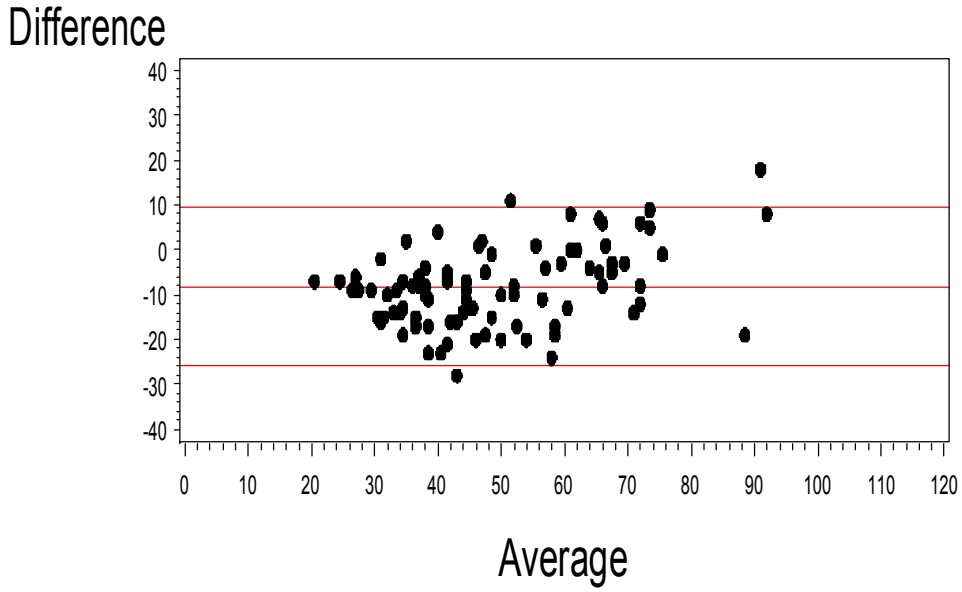
Table 2. HDL—Cardiocheck Meter vs. Vermont Blood Serum

HDL	Average	Range	#Cases	Median	Interquartile Range
Cardiocheck	44.79 (17.97)	17.0-100.0	89	40.00	31.00-58.00
Vermont	53.06 (14.62)	24.00-98.00		51.00	41.00-63.00
Equation	Vermont = 21.28 + 0.71 Cardiocheck (2.06) (0.04)				
R^2	.76				

Figure 2: Validation Study of Cardiochek Meter: Comparison of HDL Cholesterol Measured Using Cardiochek Meter and from Blood Serum at the University of Vermont

Bland Altman

Bland Altman



Difference (Cardiocheck HDL - UVSerum HDL)

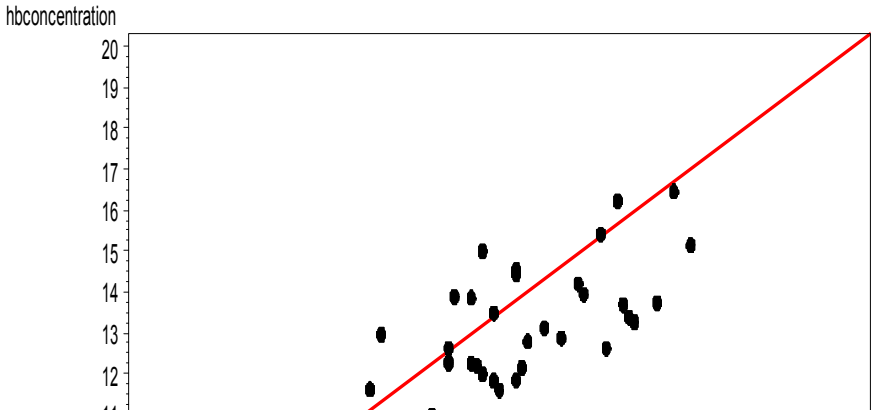
Hemoglobin

Hemoglobin levels were compared for 33 cases where hemoglobin was assessed with a DBS assay done in China and a reading on a Hemocue meter in Los Angeles. The average Hemocue meter value (14.02 g/dL) exceeded that from the DBS (13.30 g/dL) (Table 3). The higher level of the Hemocue meter reading is seen throughout the range (Figure 3). The R^2 from the equation linking the China DBS and the HemoCue reading is .36. Because the cutoff values for low hemoglobin (< 12 for women and < 13 for men) are close to the median and the mean in both distributions, cutoff values should be used with caution.

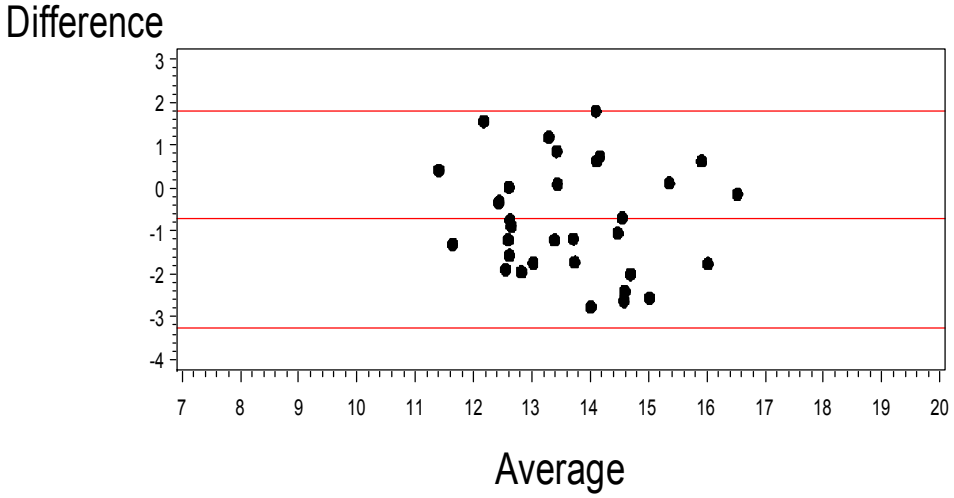
Table 3. Hemoglobin—CHARLS DBS vs. Hemocue Meter

Hemoglobin	Average	Range	#Cases	Median	Interquartile Range
China	13.30 (1.35)	11.00-16.46	33	13.13	12.26-13.95
Hemocue	14.02 (1.47)	11.20-16.90		13.80	13.00-15.30
Equation	Hemocue= 5.35 + 0.65 CHARLS (2.08) (0.16)				
R^2	.36				

CRP: McDade and China



CRP: McDade and China



Difference (CHARLS HB - Hemocue HB)

CRP

Comparison of CRP DBS Values from CHARLS and McDade Assays –

As indicated above, there are two CRP comparisons in the validation sample, first we consider the differences between assays done for CHARLS in China and assays done in McDade's lab for 34 cases (Table 1). Because the distribution of CRP is not normal – but highly skewed toward low values, we also examine logged values of CRP. The average value of the DBS assayed for CHARLS (1.12 mg/L) was lower than that reported from the McDade assay (1.42 mg/L), largely because the CHARLS assay did not have any values above 4.16 mg/L. McDade had only two values above 4.16 mg/L but these weight the mean. The median value was actually higher in the CHARLS data (.71 mg/L versus .59 mg/L) and the interquartile range was slightly greater. The R^2 of the equation linking the two is .43. The differences between the two assays are greater at higher values. The plots of the logged values of CRP are shown in Figure 5.

Table 4. CRP—CHARLS DBS Assay vs. McDade DBS Assay

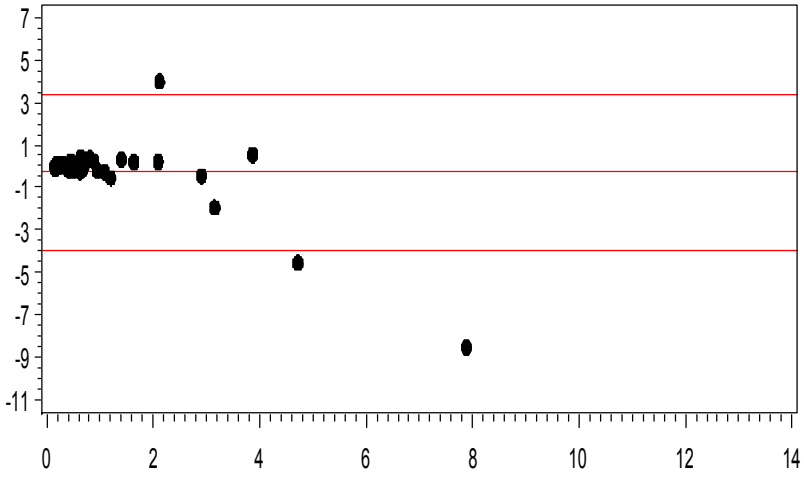
CRP	Average	Range	#Cases	Median	Interquartile Range
China	1.12 (1.14)	0.12-4.16	34	0.71	0.34-1.58
McDade	1.42(2.38)	0.12-12.16		0.59	0.26-1.24
Equation	McDade = -.10945 (0.44)	+ 1.36577 CHARLS CRP (0.28)			
R^2	.43				

Bland-Altman Analysis

CHARLS

CRP: McDade and China

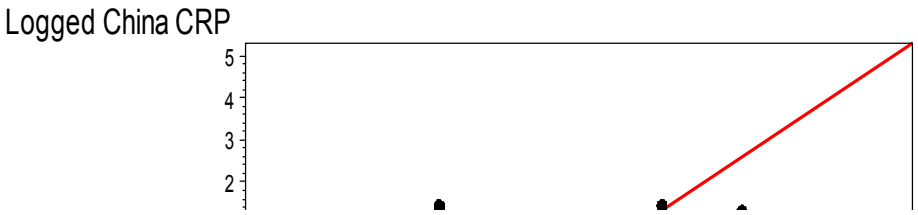
Difference



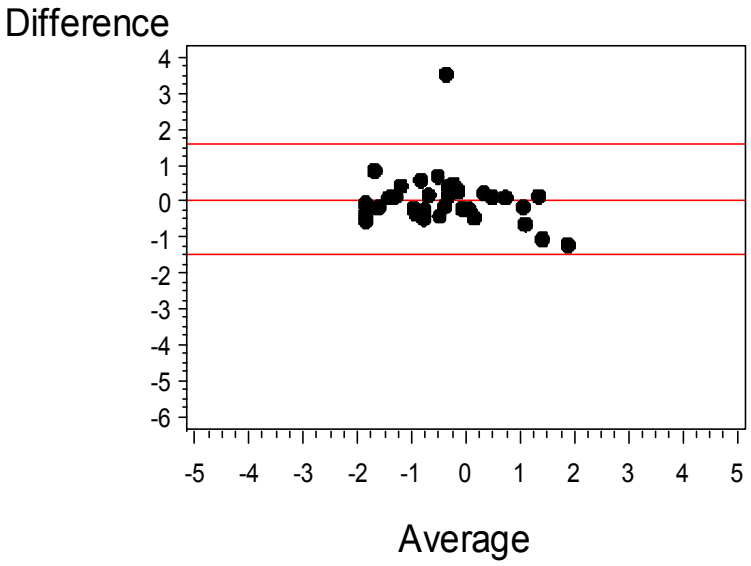
Average

Difference (CHARLS CRP - McDade CRP)

CRP: McDade and CHina



CRP: McDade and China



Difference(logged CHARLS CRP-logged McDade CRP))

CHARLS DBS Assay and Vermont Blood Serum Assay

The average CRP level from the venous blood is considerably higher than that from the DBS sample (2.39 mg/L versus 1.30 mg/L). The upper bound of the range is considerably higher (7.00 mg/L– 20.10 mg/L), the middle and lower ranges much less so (Figure 6A), as a consequence the difference between the medians is not as great as for the means. In logs, however, the differences are much more stable across the range of CRP values (Figure 6B). The R^2 from the equation linking the two measures of CRP is .30, somewhat lower than the value for the McDade value.

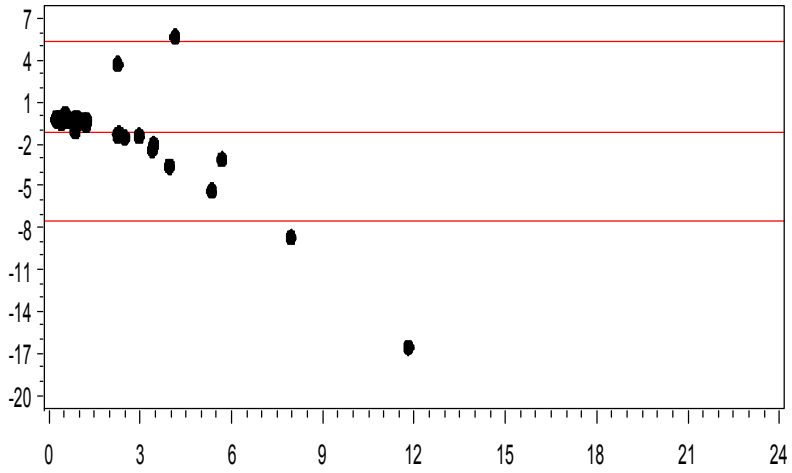
Table 5. CRP—CHARLS DBS Assay vs. Vermont Serum Assay

CRP	Average	Range	#Cases	Median	Interquartile Range
CHARLS	1.30 (1.44)	0.12-7.00	42	0.79	0.34-1.74
Vermont	2.39 (3.74)	0.33-20.10		1.05	0.51-2.93
Equation	Vermont Serum = 0.557 + 1.411 CHARLS CRP (0.66) (0.34)				
R^2	.30				

Bland-Altman Analysis

CRP: McDade and China

Difference

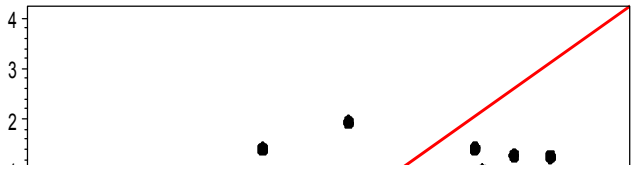


Average

Difference (CHARLS CRP - UVSerum CRP)

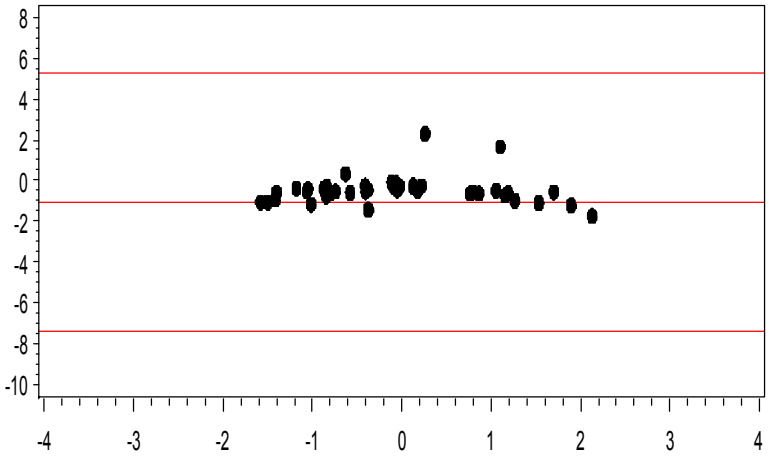
CRP: McDade and CHina

Logged China CRP



CRP: McDade and China

Difference



Average

Difference (Logged CHARLS CRP - Logged UVSerum CRP)

CHARLS data description

Next we describe the CHARLS biomarker data. We start with a table of descriptive statistics, also showing population frequencies below and above standard cutoffs. We then plot empirical densities together with histograms.

Systolic blood pressure has a mean of 135, with the median just below that. Some 36% of the CHARLS pilot population has systolic above the cutoff of 140. The diastolic mean is 77 (median almost the same) and only 15% has a reading over the cutoff of 90. Mean BMI is 23, but there are 33% of women and 19% of men with BMI 25 and over, indicating overweight. On the other hand only a trivial proportion has BMI 30 and over, so obesity is low. Underweight (under 18.5) is also low, 6-7%. Mean hemoglobin measure is 12.6, and the median is just under. Using the standard cutoffs of 12.0 for women and 13.0 for men, 51% of the sample have low hemoglobin levels. Note, however, that because a large part of the density is near the cutoffs, the fraction below is quite sensitive to where one sets the cutoff. Mean total cholesterol is 215 and for HDL it is 50. Some 33% of the sample has total cholesterol above the standard cutoff of 240, while 31% has HDL below the cutoff of 40. Finally, the mean CRP is 0.87, and the median 0.52. As seen above, in the validation analysis, the DBS measurements for CRP are lower than the venous blood measures. As a result, it is not a good idea to use the standard cutoff of above 3.0, as indicating high risk levels of CRP. However, the distribution of CRP, and log CRP look fairly standard, as indicated by the density plots below. We recommend using the data as a continuous variable.

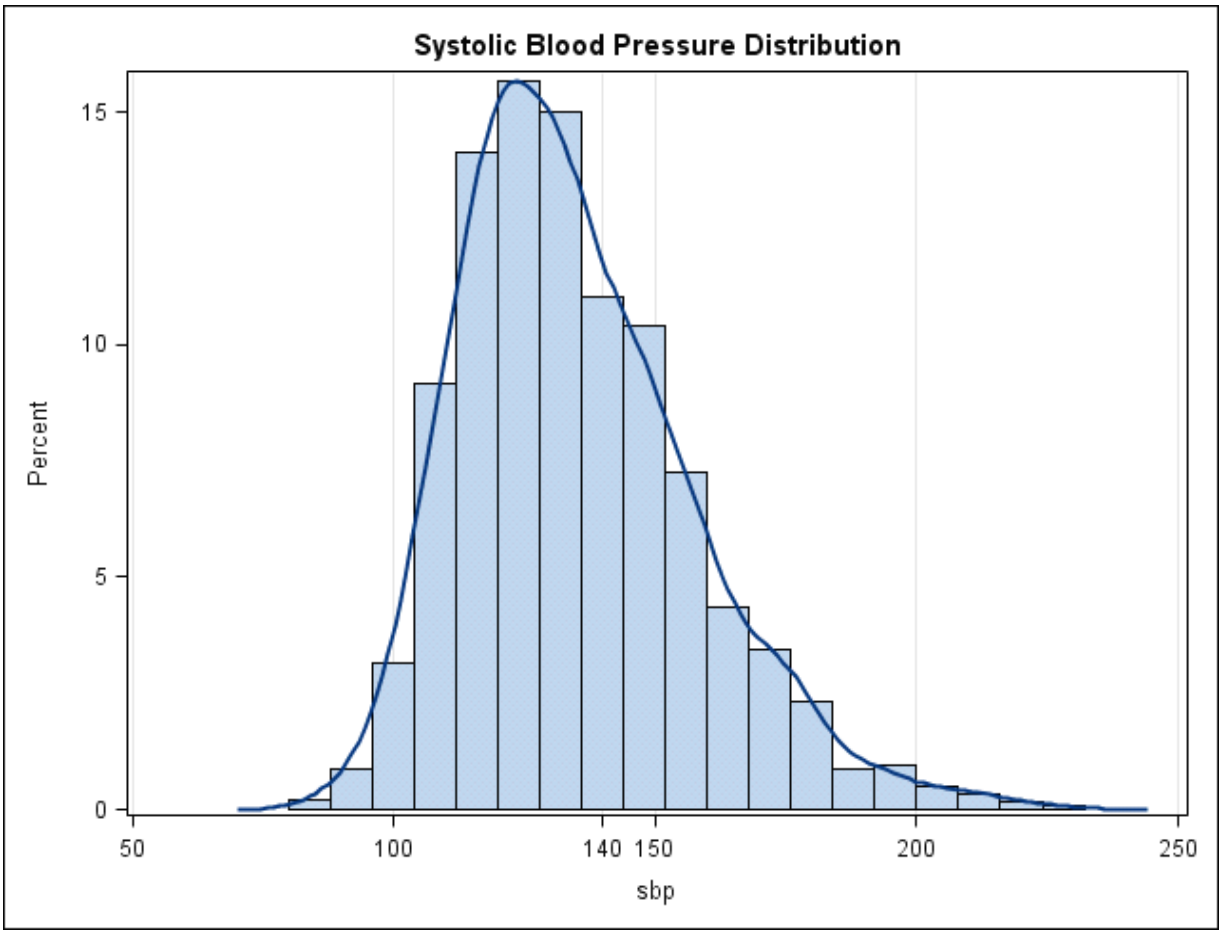
The density plots show considerable variation in the measures. Most of the distributions look reasonable, with very few outliers. The log of CRP looks far more symmetric than levels of CRP. For total and HDL cholesterol one can see the measured limits of the CariochekPA meter.

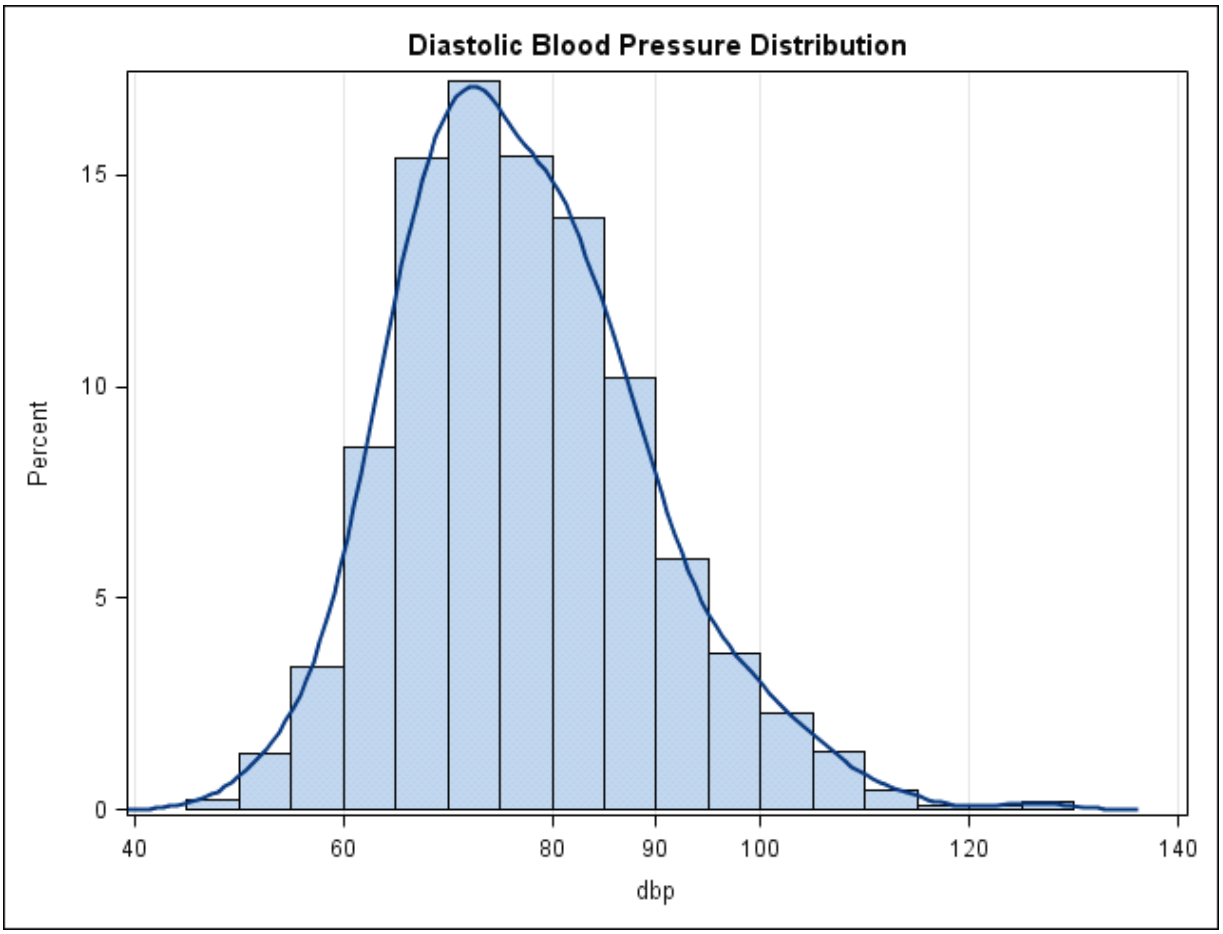
Table 6. Descriptive Statistics of Biomarkers in CHARLS

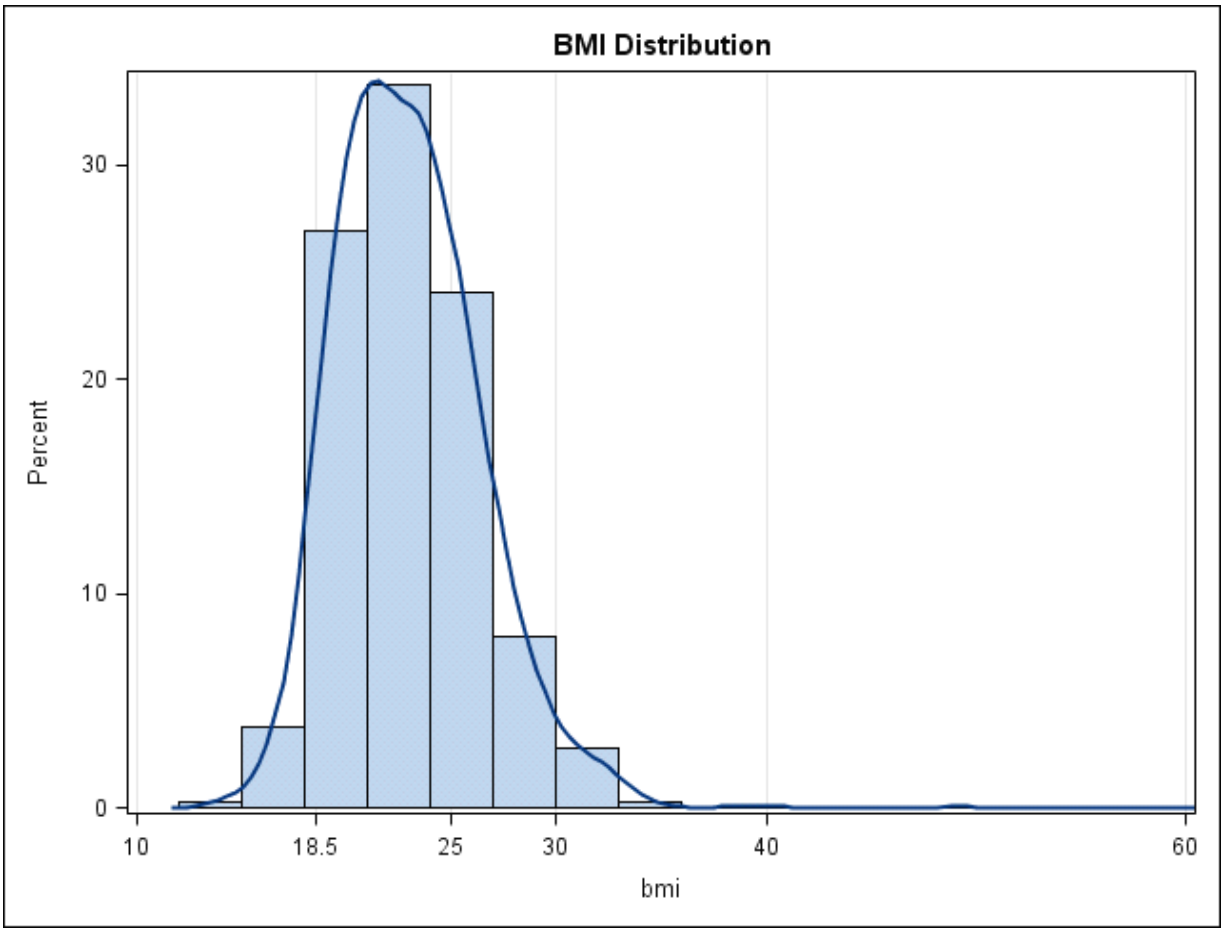
	N	Mean (SD)	Median	Interquartile Range	Ranges
Systolic Blood Pressure (mmHg)	1967	134.83 (22.40)	131.33	29.00	82.66-231.33
Diastolic Blood Pressure (mmHg)	1967	77.25 (11.96)	76.00	16.00	45.33-129.00
BMI (kg/m^2)	1946	22.99 (3.53)	22.70	4.60	13.93-62.50
CRP (mg/L)	1151	0.87 (1.25)	0.52	0.70	0.0007-13.97
Hemoglobin (g/dL)	1829	12.62 (1.74)	12.43	2.07	5.60-21.62
Total cholesterol (mg/dL)	1850	215.05 (66.80)	208.00	95.00	100.00-400.00
HDL cholesterol (mg/dL)	1842	50.22(18.70)	48.00	25.00	15.00-100.00

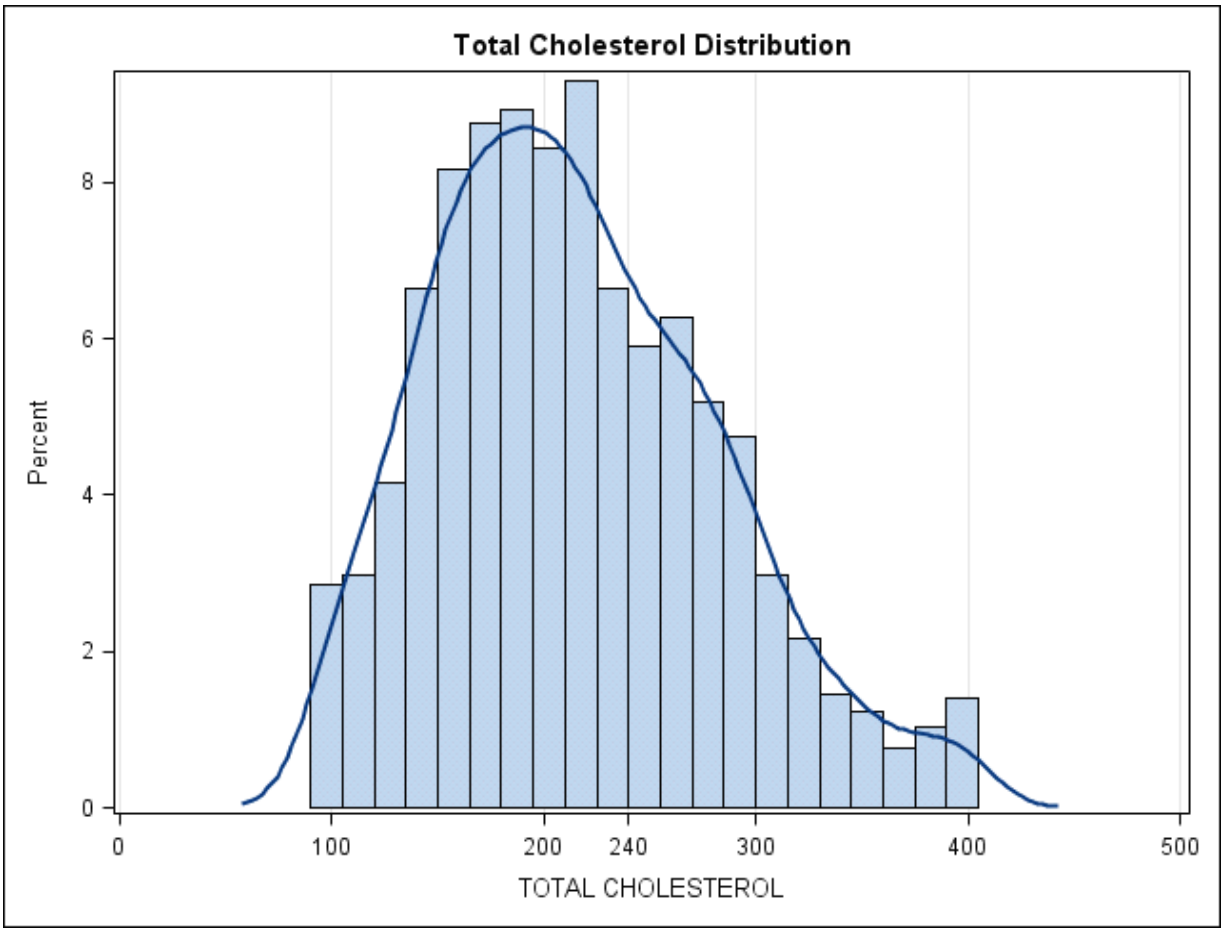
Table 7. %High Risk Biomarkers in CHARLS

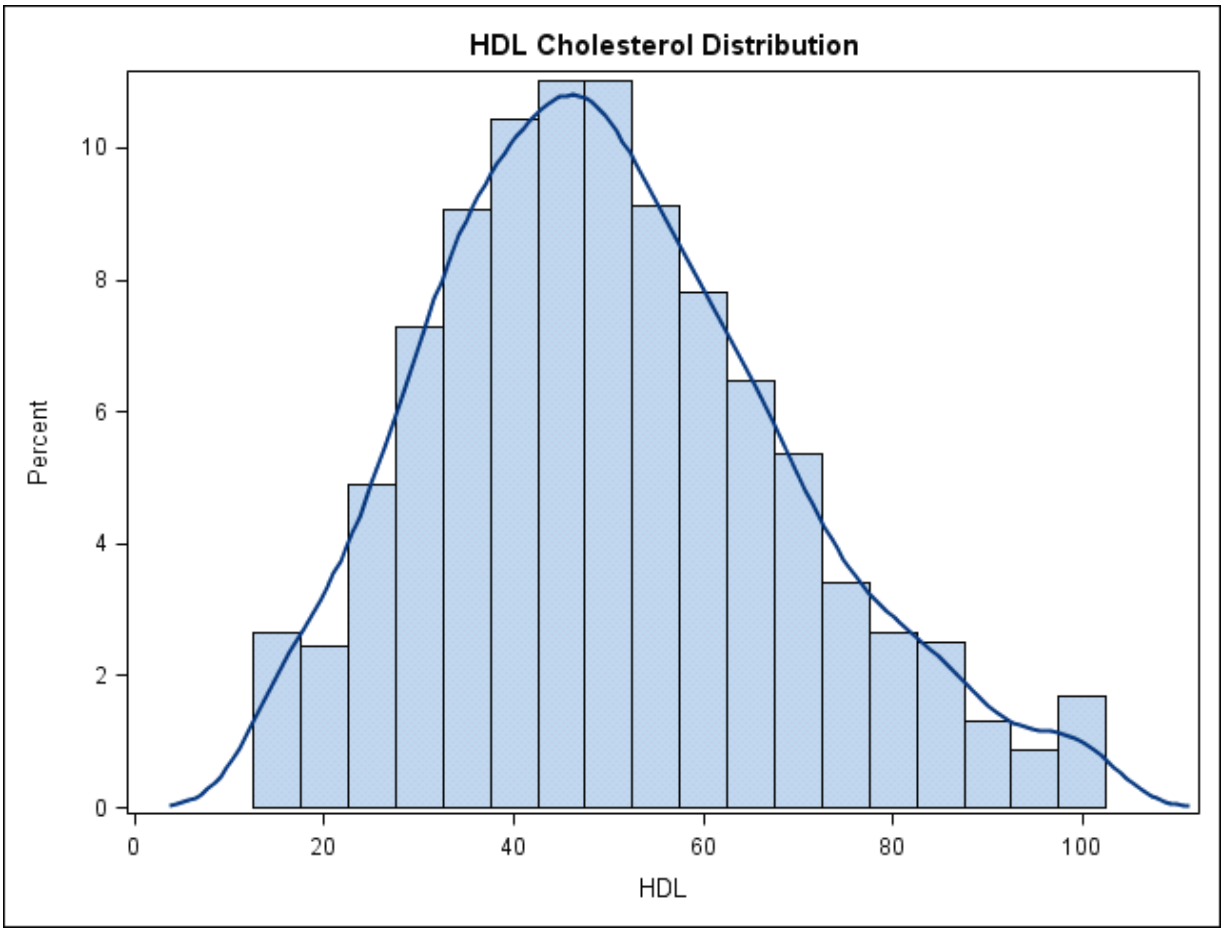
	Cutoff	Percent
Systolic Blood Pressure (mmHg)	>=140 mmHg	35.79%
Diastolic Blood Pressure (mmHg)	>=90 mmHg	14.18%
Underweight (kg/m^2)	<=18.5 kg/m^2	M: 6.39%
		F: 7.05%
Overweight (kg/m^2)	>=25 kg/m^2	M: 19.07%
		F: 32.77%
Obese (kg/m^2)	>=30 kg/m^2	M: 1.52%
		F: 4.87%
Hemoglobin (g/dL)	<13 g/dl (male)	50.55%
	<12 g/dl (female)	
Total cholesterol (mg/dL)	>=240 mg/dL	33.14%
HDL cholesterol (mg/dL)	<40 mg/dL	30.67%


Systolic Blood Pressure Distribution
Percent
15
10
5
0
50
100
140
150
200
250
sbp

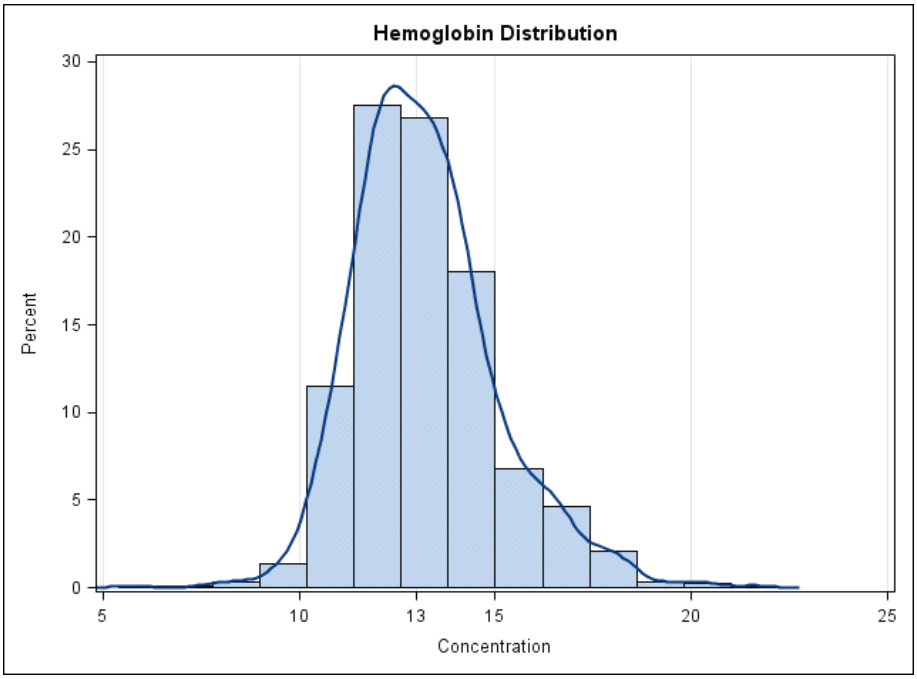

Diastolic Blood Pressure Distribution
Percent
0
5
10
15
40
60
80
90
100
120
140
dbp


BMI Distribution
Percent
30
20
10
0
10
18.5
25
30
40
60
bmi

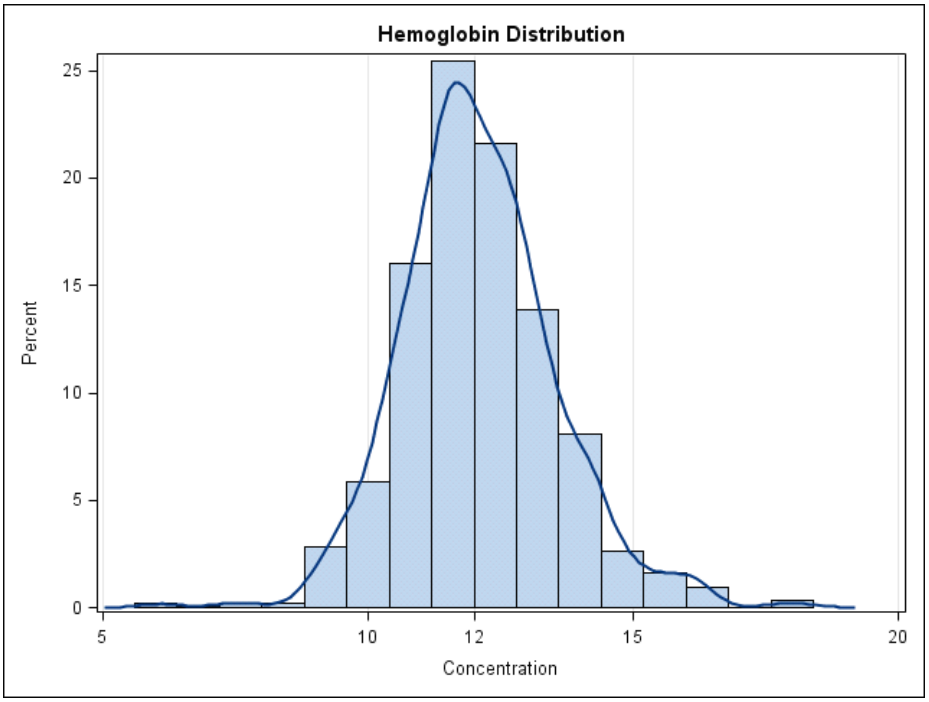

Total Cholesterol Distribution
Percent
0
2
4
6
8
0
100
200
240
300
400
500
TOTAL CHOLESTEROL

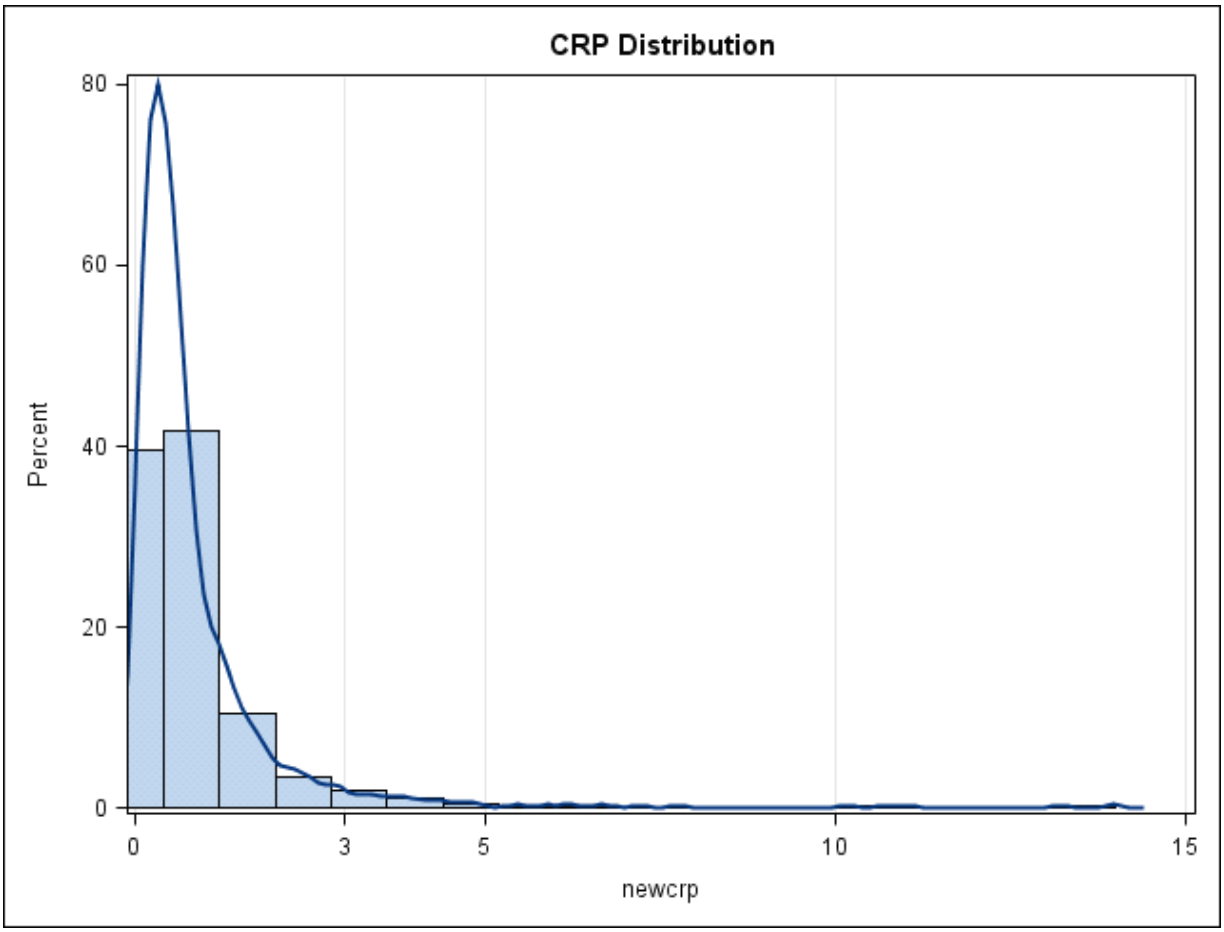

HDL Cholesterol Distribution
Percent
0
2
4
6
8
10
0
20
40
60
80
100
HDL

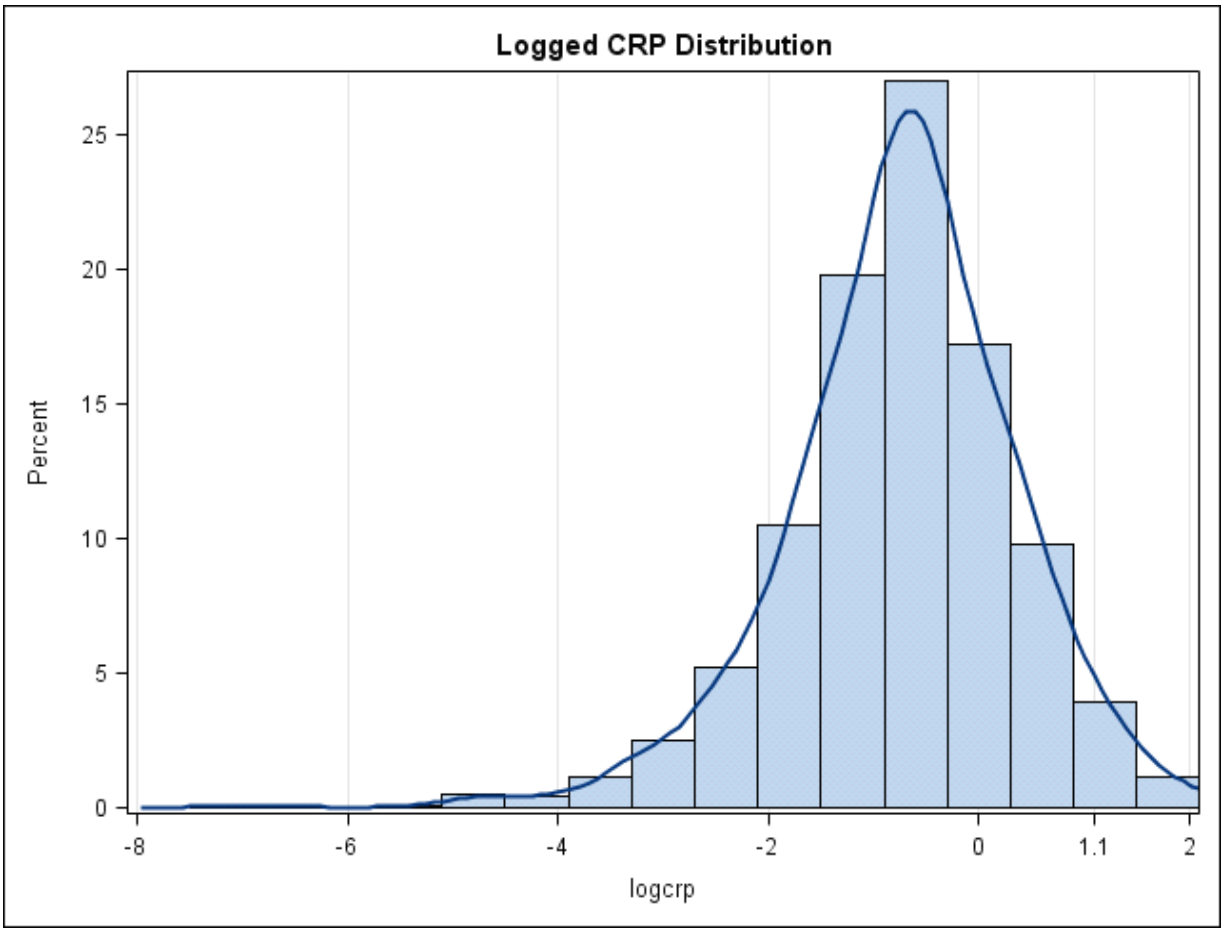
Males



Females




CRP Distribution
Percent
80
60
40
20
0
0
3
5
10
15
newcrp


Logged CRP Distribution
Percent
25
20
15
10
5
0
-8
-6
-4
-2
0
1.1
2
logcrp

Analysis of undetectable values of CRP

A large fraction of CRP cases had undetectable levels, 36% overall, higher in Zhejiang province. HRS as a comparison had about 50% undetectable. It is of interest to explore the correlates of respondents who had undectable values. Table 9 shows logit regression results for the Gansu sample with age, gender and whether the assay was done after September 2010. The date is of interest because the reaction antibody for the CRP assay had to be reordered in May 2010 and a good batch did not arrive until October. Because different production runs of the reagent may have small random differences it is of interest to see if the probability of an undetectable level is correlated with the batch. However since all of the Zhejiang samples were assayed after October 2010 (see Table 8), it only makes sense to explore this with the Gansu samples, for which 22% were assayed after October and the rest before May. As one can see in the logit regression the time dummy is not significant at standard levels, indicating that this potential concern is not an issue. Also a regression of the CRP levels on age, gender and the time dummy shows no impact of the time dummy. Hence the difference between undetectable levels in Gansu and Zhejiang is due to other factors. One may be smaller blood spots for Zhejiang, which apparently was true, but there may be other reasons as well.

Table 8. CRP levels by province

	Total % freq	*Gansu % freq*	*Zhejiang % freq*
Undetectable CRP	N= 1873	N= 870	N= 1003
Detectable	63.64% (n=1192)	72.18% (n=628)	56.23% (n=564)
Undetectable	36.36% (n=681)	27.82% (n=242)	43.77% (n=439)
Time of Detection	N= 1831	N= 857	N= 974
Before Sept 2010	36.21% (n=663)	77.25% (n=662)	0.10% (n=1)
After Sept 2010	63.79% (n=1168)	22.75% (n=195)	99.90% (n=973)

Table 9. Logistic regression on undetectable CRP (Gansu only)

	Estimate	*Standard Error*
Intercept	1.54	0.51
Age	-0.05	0.01
Male	0.37	0.16
After Sept 2010	0.11	0.19

Table 10. Regression on CRP (N=1083)

	Parameter Estimate	*Standard Error*
Intercept	-0.01	0.24
Age	0.02	0.00
Male	-0.00	0.08
After Sept 2010	-0.08	0.08
$R^2 = 0.02$		

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