APPENDIX-G

Any Goodness in "Good Cholesterol" ?

• Part 1. Some Background: Controversy over the Goodness of "Good Cholesterol"

We shall use the term HDL(2+3) to signify the combination of the true High-Density Lipoproteins, HDL-2 and HDL-3 (Chapter 44, Part 3e).

During the 1950s, it became evident that plasma levels of HDL(2+3) are inversely related to plasma levels of the Sf 0-400 lipoproteins, in clinically healthy populations (details in Gofman 1954-a, + DeLalla 1958, + DeLalla 1961). Additionally, HDL(2+3) levels are depressed in a number of clinical entities where there is a marked elevation of lipoproteins of the Std Sf 0-400 classes --- for instance, Xanthoma Tendinosum, Active Nephrotic Syndrome, Chronic Biliary Obstruction, Glycogen Storage Disease (details in Gofman 1954-a), and Acute Hepatitis (Pierce 1954-b, p.235). During that same period, evidence was accumulating that the cholesterol-rich lipoproteins (Sf 0-20) and the triglyceride-rich lipoproteins (Sf 20-400) are each independently atherogenic (Appendix-E).

In the 1965 Lyman Duff Memorial Lecture (Gofman 1966), we presented results from two prospective studies: Framingham at about 12 years of follow-up, and Livermore at about 10 years of follow-up (details in Appendix E, Part 12c). The Livermore Study, which included measurements of HDL-2 and HDL-3, provided the first PROSPECTIVE confirmation that their plasma concentrations might be inversely related to de novo cases of Ischemic Heart Disease (Gofman 1966, pp.686-687). By contrast, the HDL-1 concentrations were virtually identical in the base population and in the de novo IHD cases. The various Livermore findings were based on 38 de novo IHD cases which grew out of a base-population of 1,961 men, with average age of 43.7 years at entry to the study. We wrote (Gofman 1966, p.687):

"From these data, it is not possible to conclude whether or not the observed lowerings of HDL-2 and HDL-3 in Ischemic Heart Disease are in excess of those anticipated from the inverse correlations [with levels of Sf 0-400]. This, again, would ultimately be desirable information, since if there is any lowering beyond that expected from interclass correlations, the possibility of a protective role of High-Density Lipoproteins would require consideration."

The possibility, of an anti-atherogenic effect from the HDL(2+3) lipoproteins, was a topic of numerous studies in the 1960s and 1970s. In 1978 (Gofman 1978, pp.14-18), I explained my skepticism that the existing evidence supported an independent anti-atherogenic role for HDL(2+3). And some 20 years later, I am still a skeptic. This appendix describes, in Part 5, the kind of testing which I believe would be required to settle the issue.

We have not been alone in our doubts.

Is there any goodness in "good cholesterol"? The terms "good cholesterol" and "bad cholesterol" are everywhere, now. This suggests that the cholesterol transported by the purportedly protective High-Density Lipoproteins is "good," and the cholesterol transported by the atherogenic

Low-Density Lipoproteins is "bad" --- a concept which was explicitly challenged in the New England Journal of Medicine during 1989 by Gordon + Rifkind (Part 2).

• Part 2. Important Issues Raised by Gordon and Rifkind

David J. Gordon and Basil M. Rifkind (Gordon 1989) are the authors of "High-Density Lipoprotein --- the Clinical Implications of Recent Studies," in the NEJM (Gordon 1989). We can associate ourselves with several of their doubts and comments. For instance, they state (Gordon 1989, p.1314):

"The association, of lower HDL levels with higher rates of coronary disease within populations in observational epidemiologic studies, has given rise to the hypothesis that interventions that raise low levels of HDL cholesterol will reduce coronary disease rates. However, neither our present understanding of lipid metabolism nor these epidemiologic observations can provide assurance that low levels of HDL cholesterol are a causative rather than a coincidental factor in coronary disease, or that intervention would be beneficial."

Gordon and Rifkind point out (at p.1312) that "It has been hypothesized that HDL is involved in the 'reverse transport' of cholesterol from peripheral tissues to the liver." About this idea, Gordon and Rifkind have the following relevant observations (Gordon 1989, p.1312):

"At least three caveats should be kept in mind. First, the relevance of these reverse-transport pathways to the rate of deposition (or removal) of cholesterol in atherosclerotic plaques has yet to be established. Second, the complex interrelation of cholesterol and triglyceride metabolism and the many lipoproteins involved may make it misleading to consider any single component of this system in isolation. Low plasma levels of HDL are often found in conjunction with high plasma levels of atherogenic, triglyceride-rich lipoproteins, and it is difficult to determine whether low levels of HDL cholesterol have a direct etiologic role in atherogenesis or serve only as a marker of a more fundamental disorder. Finally, the popular designation of HDL as 'the good cholesterol' is misleading, because the anti-atherogenic role that has been hypothesized for it pertains not to any unique property of its cholesterol but to the direction in which it transports that cholesterol."

Emphasis Added --- By the Explicit Data in Our Figure G-1

From the preceding paragraph, we shall repeat, underline, and comment upon the following sentence:

"... Low plasma levels of HDL are often found in conjunction with high plasma levels of atherogenic, triglyceride-rich lipoproteins, and it is difficult to determine whether low levels of HDL cholesterol have a direct etiologic role in atherogenesis or serve only as a marker of a more fundamental disorder ..."

These words in 1989 suggest that little progress had occurred on the problem we described in 1966 (Part 1, above): The need to determine whether LOW levels of HDL(2+3) are an independent cause of Ischemic Heart Disease, or whether such levels are "automatically" low when the levels of the atherogenic Sf 0-400 lipoproteins are high.

Not only are low plasma levels of HDL(2+3) "often" found in conjunction with high plasma levels of atherogenic, triglyceride-rich lipoproteins, but we can show that this relationship is PROMINENT in a sample of 891 American males, ages 30-39, whose lipoproteins were measured in our Livermore Lipoprotein Study (Appendix-E). I would be extremely surprised if a similar inverse relationship failed to exist in other (non-Livermore-Lab) institutions in the United States. Our Figure G-1 depicts the strong inverse relationship between HDL(2+3) and the combined Sf 0-400 lipoproteins --- details in Part 3.

• Part 3. Data from a Livermore Population: Inverse Correlations

In our Livermore Lipoprotein Study, 891 male participants were in the age-band 30-39 years old when we enrolled them into the database and measured their plasma lipoproteins, during the years 1954-1957. With 891 persons, this age-band constituted over half of the 1,961 males in the study.

Figure G-1: HDL(2+3) Regressed on Std. Sf 0-400 Lipoproteins

To prepare Figure G-1, we sorted the 891 records in ascending order by their plasma concentrations (milligrams per deciliter) of the combined Std Sf 0-400 lipoproteins. Then we divided the database into deciles, with each of the first nine having 89 persons and with the tenth having 90 persons. For each decile, we calculated the average concentrations of the Std Sf 0-400 and the HDL(2+3) lipoproteins. The ten resulting pairs of Observed Values are tabulated in Figure G-1, and shown as boxy symbols within the graph.

The Observed HDL(2+3) values are regressed linearly on the Observed Std Sf 0-400 values. The regression output is shown to the right, in Figure G-1. Then, following the steps described in Chapter 6, Part 3, we write the Equation of Best Fit and calculate the third column of values --- the Calculated Best-Fit HDL(2+3) values, including the two "extensions." The Line of Best Fit in the graph reflects the pairing of the Observed Std Sf 0-400 values with the Calculated Best-Fit HDL(2+3) values.

Next, we examine each of the four major segments, within the atherogenic band of Std Sf 0-400 lipoproteins. Each is in a demonstrably inverse relationship with the HDL(2+3) in this population sample. However, the following point deserves emphasis:

By themselves, these inverse relationships with the atherogenic lipoproteins are NOT evidence that High-Density Lipoproteins are anti-atherogenic --- as Part 5 shows.

Figures G-2, G-3, + G-4: HDL vs. Segments of the Std Sf 0-400 Lipoproteins

Figures G-2, G-3, and G-4 are prepared in the manner described for Figure G-1, except that the 891 records were sorted by the indicated SEGMENTS of the Std Sf 0-400 spectrum. Additionally, when there are no low values on the horizontal axis, the scale of that axis does not start at zero. This can cause the mistaken impression that the y-intercept would not match the Constant --- an illusion which vanishes if one widens those graphs so that the scale begins at zero.

All segments of the Std Sf 0-400 lipoproteins are in an inverse relationship with HDL(2+3). The relationships for the Std Sf 20-100 and 100-400 segments of the spectrum have a steep component and a flatter component, making their relationships less linear and more complex than the overall relationship in Figure G-1. (We note that observations in Rubins 1995 appear consistent with our 1957 data.)

• Part 4. Data from a Framingham Population: Effects of Selective Pressure

Part 4 illustrates the effects of selective pressure in an epidemiologic study --- the development of an "outgrowth" population from a base population.

During the 1950s, our group at the Donner Laboratory measured the Std Sf 0-12, 12-20, 20-100, and 100-400 lipoproteins on several thousand entrants to the Framingham Heart Study (Appendix-E, Part 12). These Framingham entrants included 687 men in the 30-39 year age-band --- which is the same age-band evaluated in Part 3 above from a Livermore population. For the Livermore population (but not the Framingham population), the HDL-1, HDL-2, and HDL-3 measurements were made in addition to the Std Sf 0-12, 12-20, 20-100, and 100-400 lipoproteins.

In 1965, Dr. Thomas R. Dawber (then Director of the Framingham Study) provided a listing of the 319 de novo cases of IHD which had occurred during the intervening years among the entrants measured about 12 years earlier by Donner, and we reported the results in our Lyman Duff Memorial Lecture (Gofman 1966). Below are the results for the 687 males, ages 30-39 when measured, from Gofman 1966 (p.683, Table 3, which includes standard deviations of the means). All lipoprotein and cholesterol measurements are in mg/dl.

Measures (Mean)	De Novo IHD	Base Population	Difference	Significance test
Std Sf 0-12	390.2	341.9	48.3	p=0.001
Std Sf 12-20	75.4	62.1	13.3	p=0.01
Std Sf 20-100	139.7	102.3	37.4	p<0.001

Std Sf 100-400	145.8	77.3	68.5	p<0.001
Std Sf 0-400	751.1	583.6	167.5	p<0.001
Atherogenic Index	102.1	76.6	25.5	p<0.001
Cholesterol	267.7	222.6	45.1	p<0.001
Systolic B.P.	136.2	130.3	5.9	p~0.05
Diastolic B.P.	91.6	84.6	7.0	p=0.001
Relative Weight	119.3	111.9	7.4	p<0.01

These Framingham results illustrate the role of SELECTIVE PRESSURES in an epidemiological study. What grows out of this base population (of 687 males at Framingham) depends upon such pressures. Since the four Standard Sf classes are atherogens, we know that their levels will --- as a result of the selective pressure --- be elevated in the group which develops de novo ISCHEMIC HEART DISEASE, compared with levels in the base population from which the cases grew out. And that is precisely what is observed in the results which are tabulated above.

Outgrowth of De Novo IHD from a Base Population-Sample

The base population-samples, in the prospective Framingham and Livermore Studies, are not assumed to be "risk-free" with respect to future manifestation of clinical Ischemic Heart Disease. Rather, these base populations are assumed to have a distribution of persons with various risk-factors for future manifestation of clinical IHD. If a particular biochemical variable is suspected of contributing to that risk, a prospective study (having adequate size of sample and duration of follow-up time) is expected to show that the variable is indeed associated with IHD evolution in some members of the base population.

The Framingham results tabulated above show that plasma lipoprotein levels in each segment of the Sf 0-400 lipoprotein spectrum were significantly higher in the "outgrowth" population (the 32 persons who later developed overt de novo IHD) than in the base population. Such findings are consistent with a causal relationship of those lipoproteins with the development of IHD --- and with the Lipid Hypothesis of Atherosclerosis.

HDL(2+3) in the IHD Cases Above vs. the Base Population

Although the Framingham base population above does not have HDL(2+3) measurements, it has the same age-band and gender as the Livermore base population which shows HDL(2+3) concentration having a strong, inverse relationship with the concentration of Sf 0-400 lipoproteins (Figure G-1). Therefore, in the absence of contrary evidence, it seems likely that the outgrowth sample (above) in the Framingham Study would have shown lower mean concentrations of HDL(2+3) than its base population --- because the outgrowth sample showed higher mean concentrations of Sf 0-400 lipoproteins than its base population.

Using the entire base population of males in the Livermore Study, our 10-year follow-up showed statistically significant reductions in mean HDL-2 and HDL-3 concentrations in the outgrowth sample of 38 de novo IHD cases, compared with the base population's mean concentrations (Gofman 1966, p.686, Table 14). The same outgrowth sample of de novo IHD cases also showed statistically significant elevations in mean values of Sf 0-12, Sf 12-20, and Sf 20-100 lipoproteins (Gofman 1966, p.684, Table 10) --- as reported in Appendix-E.

• Part 5. Testing a "Protective" Effect for HDL(2+3): Three Possibilities

Three possibilities exist for HDL(2+3):

Case 1: The HDLs themselves are neutral with respect to atherogenesis and IHD development.
Case 2: The HDLs themselves are independent anti-atherogens (protective against IHD).
Case 3: The HDLs themselves are independent atherogens.

5a. Case 1: Outgrowth De Novo Sample and Base Population on Same Figure

Suppose HDL(2+3) themselves are neutral with respect to atherogenesis and IHD development. And suppose we did a prospective study with a very large number of persons in the base population and

a large outgrowth of de novo IHD cases. Then what would we expect to see if we made a new and expanded Figure G-1 from the results?

On the new Figure G-1, we would plot the values and best-fit line (or curve, if curvature exists) not only for the base population, but also separately for the outgrowth de-novo-IHD population sample.

Neutrality of HDL(2+3) with respect to atherogenesis and IHD would mean that there would be NO SELECTIVE PRESSURE for or against HDL(2+3) in the cohort of de novo IHD cases which would grow out of the base population. Therefore, we would expect to find that the line of best fit for the de novo IHD cases would lie directly over (within experimental error) the line of best fit for the base population.

At EQUAL concentrations of Sf 0-400 lipoproteins, the concentrations of HDL(2+3) would not differ significantly between the base and the outgrowth populations. Such a result would be consistent, of course, with higher MEAN Sf 0-400 and lower MEAN HDL(2+3) levels among the IHD cases, than among the base population.

5b. Case 2: The HDLs Themselves Are Independently Protective against IHD

If we would go through the same exercise for Case 2 as we did for Case 1, we would find that the line (or curve) for the de novo IHD cases would lie BENEATH the line for the base population in a revised Figure G-1 --- if HDL(2+3) have an independent anti-atherogenic effect.

Why BENEATH? If HDL(2+3) have an independent protective effect against IHD, above and beyond their inverse relationship with the Sf 0-400 lipoproteins, then there would be SELECTIVE PRESSURE to prevent the HDLs from getting into the IHD outgrowth group. This is the expectation for an anti-atherogen which protects against IHD development. The de novo IHD sample would grow out of the base population partly BECAUSE it is impoverished in the protective HDL(2+3). So, the protective HDL would necessarily "stay behind," and the de novo IHD cohort would be LESS RICH in HDL(2+3) than the base population. For each value of Std Sf 0-400 among the de novo IHD cases, the HDL (2+3) would lie below the value which would have obtained for "neutrality." This is the implication of claims that HDL(2+3) have a protective effect against IHD.

5c. Case 3: The HDLs Themselves Are Independent Atherogens

If we would go through the same exercise for Case 3 as we did for Case 1, we would find that the line (or curve) for the de novo IHD cases would lie ABOVE the line for the base population in a revised Figure G-1 --- if HDL(2+3) are independent atherogens.

Why ABOVE? If HDL(2+3) are independent atherogens, the SELECTIVE PRESSURE would make the IHD outgrowth cohort ENRICHED in the HDL(2+3) compared with the base population. Therefore, at each point along the Std Sf 0-400 line (or curve), the HDL(2+3) values would be higher in the IHD cohort than in the base population.

• Part 6. Does the Existing Evidence Pass the Test in Part 5b?

We do not rule out the existence of HDL anti-atherogens. Either the existing evidence can pass the test for Case 2 described above (or an equivalent test), or it can not pass the test. Unless one becomes convinced that existing evidence has already passed such a test, there seems to be little basis for considering that any HDL entity truly merits to be called "protective" or "the good cholesterol."

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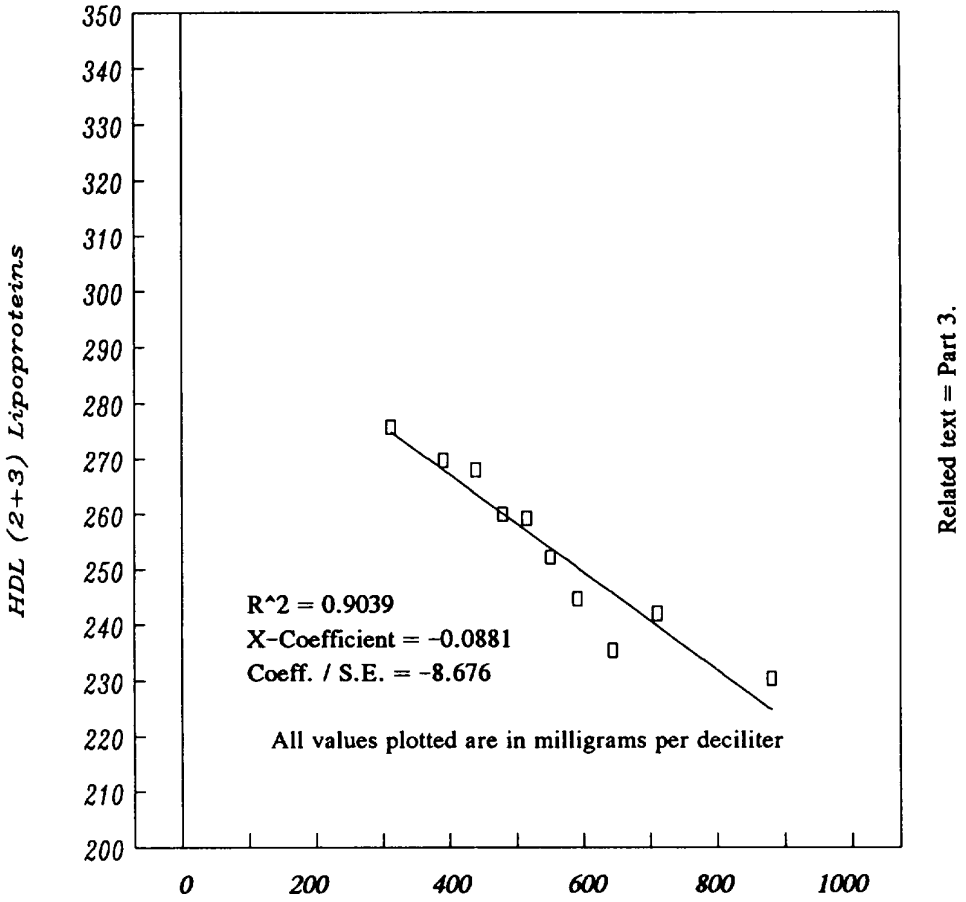
Figure G-1

Regression of HDL (2+3) on Std Sf 0-400



Std Sf 0-400 Lipoproteins

Data for Plotting (HDL(2+3) vs. Std Sf 0-400

Deciles	STD Sf 0-400	HDL(2+3) Obs.	HDL(2+3) Calc.
Decile 1	312.3	275.7	274.8
Decile 2	390.8	269.7	267.9
Decile 3	439.3	267.9	263.6
Decile 4	479.1	260.0	260.1
Decile 5	515.7	259.2	256.9
Decile 6	551.1	252.1	253.7
Decile 7	591.3	244.5	250.2
Decile 8	643.8	235.5	245.6
Decile 9	710.8	242.0	239.7
Decile 10	880.3	230.4	224.8
Extension	900.0		223.0
Extension	925.0		220.8

Regression of HDL(2+3) on Std Sf0-400

Regression Output:

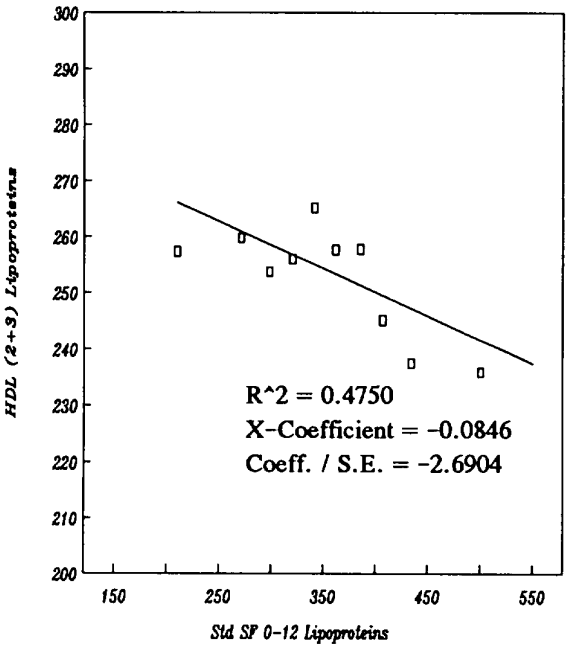
Constant	302.2803
Std Err of Y Est	5.0322
R Squared	0.9039
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	-0.0881
Std Err of Coef.	0.0102
Coeff. / S.E.	-8.6764

Equation of best fit:

HDL(2+3) = (-0.0881 * Std Sf 0-400) + 302.2803

Figure G-2

Regression of HDL(2+3) on Std Sf 0-12



All values plotted are in milligrams per deciliter

Data for Plotting (HDL(2+3) vs. Std Sf 0-12

Deciles	STD Sf 0-12	HDL(2+3) Obs.	HDL(2+3) Calc.
Decile 1	210.0	257.4	266.1
Decile 2	271.5	259.8	260.9
Decile 3	298.3	256.8	258.7
Decile 4	320.3	258.4	256.8
Decile 5	341.2	265.2	255.0
Decile 6	361.6	257.7	253.3
Decile 7	385.3	264.2	251.3
Decile 8	406.8	247.7	249.5
Decile 9	434.1	237.4	247.2
Decile 10	500.4	235.8	241.6
Extension	525.0		239.5
Extension	550.0		237.4

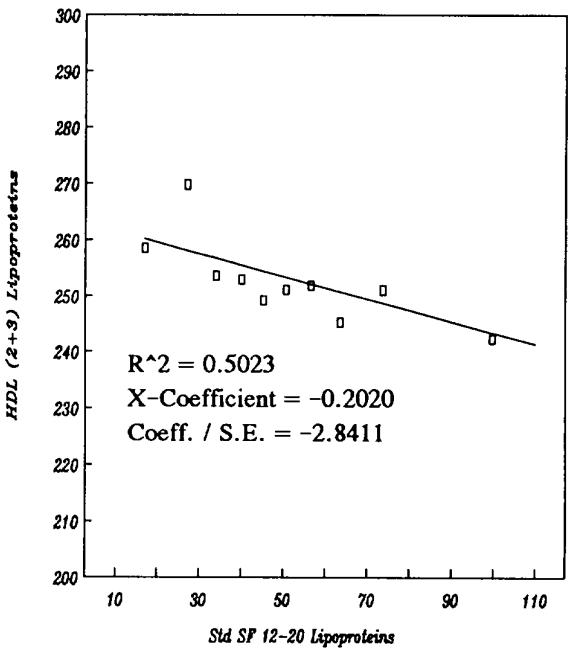
Regression Output:

Constant	283.9044
Std Err of Y Est	7.9335
R Squared	0.4750
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	-0.0846
Std Err of Coef.	0.0315
Coeff./ S.E.	-2.6904

Equation of best fit:

HDL(2+3) = (-0.0846 * Std Sf 0-12) + 283.904

Regression of HDL (2+3) on Std Sf 12-20



All values plotted are in milligrams per deciliter

Data for Plotting (HDL(2+3) vs. Std Sf 12-20

Deciles	STD Sf 12-20	HDL(2+3) Obs.	HDL(2+3) Calc.
Decile 1	16.9	255.3	260.2
Decile 2	27.1	269.8	258.1
Decile 3	34.0	253.6	256.7
Decile 4	40.0	257.7	255.5
Decile 5	45.2	251.1	254.5
Decile 6	50.7	251.1	253.4
Decile 7	56.6	251.9	252.2
Decile 8	63.5	247.1	250.8
Decile 9	73.7	251.1	248.7
Decile 10	99.8	244.6	243.4
Extension	100.0		243.4
Extension	110.0		241.4

Regression Output:

Constant	263.5890
Std Err of Y Est	5.1742
R Squared	0.5023
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	-0.2020
Std Err of Coef.	0.0711
Coeff./ S.E.	-2.8411

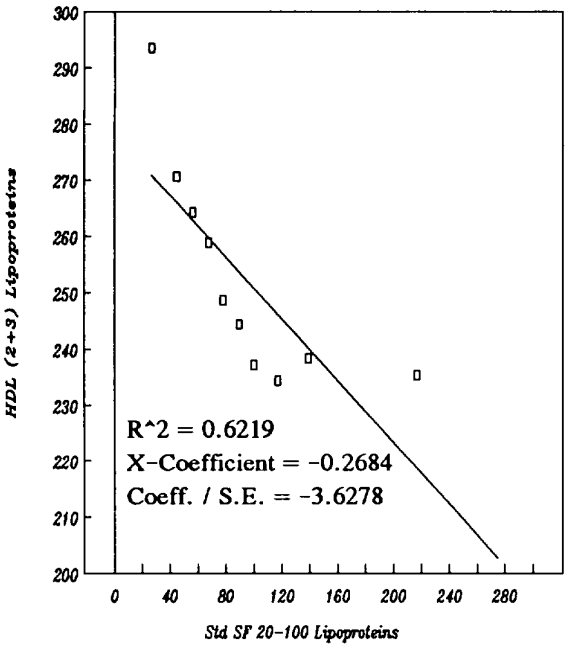
Equation of best fit:

HDL(2+3) = (-0.2020 * Std Sf 12-20) + 263.589

Related text = Part 3.

Figure G-3

Regression of HDL (2+3) on Std Sf 20-100



All values plotted are in milligrams per deciliter

Data for Plotting (HDL(2+3) vs. Std Sf 20-100

Deciles	STD Sf 20-100	HDL(2+3) Obs.	HDL(2+3) Calc.
Decile 1	26.6	293.6	271.7
Decile 2	44.6	270.7	266.8
Decile 3	55.9	266.2	263.8
Decile 4	67.6	258.8	260.7
Decile 5	77.7	250.9	258.0
Decile 6	89.1	244.9	254.9
Decile 7	100.0	239.7	252.0
Decile 8	117.2	237.1	247.3
Decile 9	138.9	238.3	241.5
Decile 10	216.8	237.0	220.6
Extension	250.0		211.7
Extension	275.0		205.0

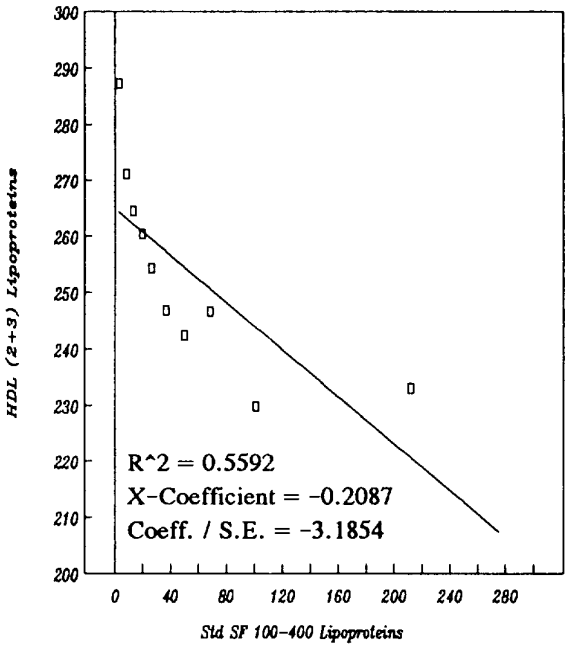
Regression Output:

Constant	278.7990
Std Err of Y Est	12.1795
R Squared	0.6219
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	-0.2684
Std Err of Coef.	0.0740
Coeff./ S.E.	-3.6278

Equation of best fit:

HDL(2+3) = (-0.2684 * Std Sf 20-100) + 278.799

Regression of HDL (2+3) on Std Sf 100-400



All values plotted are in milligrams per deciliter

Data for Plotting (HDL(2+3) vs. Std Sf 100-400

Deciles	STD Sf 100-400	HDL(2+3) Obs.	HDL(2+3) Calc.
Decile 1	2.9	287.2	264.3
Decile 2	8.3	271.2	263.1
Decile 3	12.9	264.4	262.2
Decile 4	19.5	260.3	260.8
Decile 5	26.2	254.3	259.4
Decile 6	36.7	246.9	257.2
Decile 7	49.8	242.4	254.5
Decile 8	68.1	246.7	250.6
Decile 9	101.2	229.8	243.7
Decile 10	212.2	233.0	220.6
Extension	250.0		212.7
Extension	275.0		207.5

Regression Output:

Constant	264.8587
Std Err of Y Est	12.4490
R Squared	0.5592
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	-0.2087
Std Err of Coef.	0.0655
Coeff. / S.E.	-3.1854

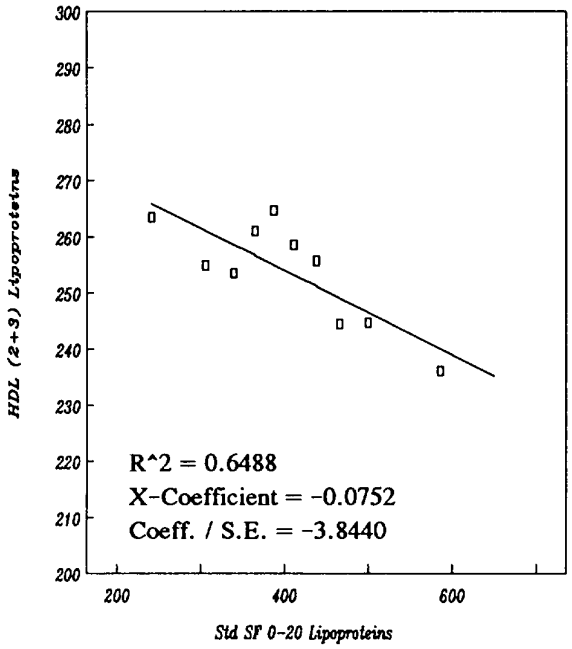
Equation of best fit:

HDL(2+3) = (-0.2087 * Std Sf 100-400) + 264.8587

Related text = Part 3.

Figure G-4

Regression of HDL (2+3) on Std Sf 0-20



All values plotted are in milligrams per deciliter

Data for Plotting (HDL(2+3) vs. Std Sf 0-20

Deciles	STD Sf 0-20	HDL(2+3) Obs.	HDL(2+3) Calc.
Decile 1	241.4	263.5	265.9
Decile 2	305.6	254.9	261.1
Decile 3	339.6	253.5	258.5
Decile 4	364.5	261.0	256.6
Decile 5	386.9	264.7	254.9
Decile 6	410.7	258.6	253.1
Decile 7	438.2	255.7	251.1
Decile 8	465.3	244.4	249.0
Decile 9	499.7	244.6	246.5
Decile 10	585.7	236.1	240.0
Extension	600.0		238.9
Extension	650.0		235.2

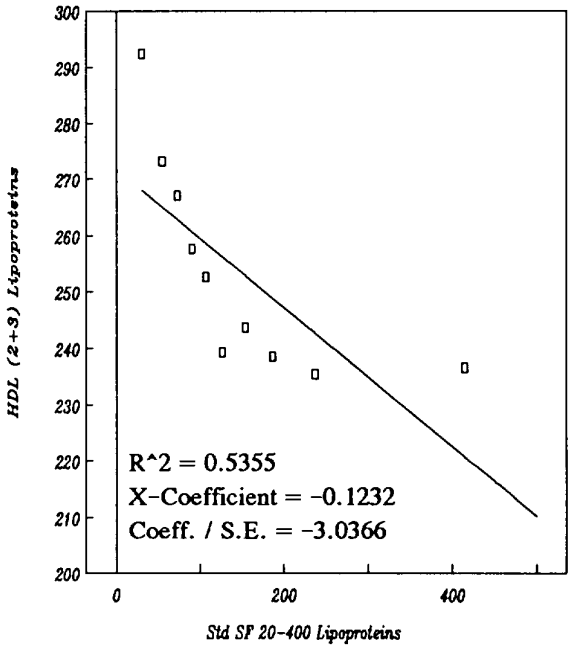
Regression Output:

Constant	284.0316
Std Err of Y Est	5.8424
R Squared	0.6488
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	-0.0752
Std Err of Coef.	0.0196
Coeff. /S.E.	-3.8440

Equation of best fit:

HDL(2+3) = (-0.0752 * Std Sf 0-20) + 284.0316

Regression of HDL (2+3) on Std Sf 20-400



All values plotted are in milligrams per deciliter

Data for Plotting (HDL(2+3) vs. Std Sf 20-400

Deciles	STD Sf 20-400	HDL(2+3) Obs.	HDL(2+3) Calc.
Decile 1	30.5	292.4	268.0
Decile 2	54.8	273.2	265.0
Decile 3	72.4	267.1	262.8
Decile 4	89.8	257.7	260.7
Decile 5	106.8	252.7	258.6
Decile 6	126.3	239.2	256.2
Decile 7	154.0	243.6	252.8
Decile 8	186.2	238.5	248.8
Decile 9	236.8	235.4	242.6
Decile 10	415.0	236.5	220.6
Extension	450.0		216.3
Extension	500.0		210.2

Regression Output:

Constant	271.7754
Std Err of Y Est	13.7250
R Squared	0.5355
No. of Observations	10
Degrees of Freedom	8
X Coefficient(s)	-0.1232
Std Err of Coef.	0.0406
Coeff. /S.E.	-3.0366

Equation of best fit:

HDL(2+3) = (-0.1232 * Std Sf 20-400) + 271.7754