A Correlation Between Serum Cholesterol and Glycosylated Hemoglobin in Nondiabetic Humans

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The relationship of serum cholesterol and average blood glucose as glycosylated hemoglobin was investigated in 43 nondiabetic subjects. Glycosylated hemoglobin correlated strongly with total cholesterol ($r = .63$, $P < .001$). A significant negative partial correlation ($r = -.25$, $P = .05$) was seen between high-density lipoprotein cholesterol and glycosylated hemoglobin if total cholesterol was held constant. This negative partial correlation was much stronger when the analysis was limited to subjects with total cholesterol greater than 5.20 mmol/L (200 mg/dL, $r = -.51$, $P = .006$). Subjects at high risk for atherosclerosis with total cholesterol greater than 7.25 mmol/L (280 mg/dL) have a glycosylated hemoglobin (6.32 percent) that is in the range of well-controlled diabetics. Subjects with moderately elevated cholesterol have moderately elevated glycosylated hemoglobin (5.92 percent) compared with normocholesterolemic subjects (5.53 percent). These results support the hypothesis that elevated blood glucose that is associated with hypercholesterolemia is responsible for some of the atherogenesis associated with high cholesterol levels.

The epidemiological relationship between elevated serum cholesterol and atherosclerotic heart disease has been well documented in the Framingham study[1] and the Seven Countries study.[2] The mechanism by which cholesterol causes heart disease, however, remains obscure.

The relationship of diabetes mellitus with atherosclerotic heart disease is also well known and has been documented in the Framingham study.[1] Diabetic men were found to have twice the rate of myocardial infarction as nondiabetic men, and diabetic women had six times the rate of myocardial infarction as nondiabetic women. A relationship between abnormal glucose metabolism and atherosclerotic heart disease in nondiabetic subjects was found in the Honolulu Heart Program.[3] Japanese men living on the island of Oahu were given a 50-g glucose challenge and the one-hour plasma glucose was measured. A linear relationship was found between the one-hour plasma glucose and the incidence of coronary heart disease within 12 years. Wahlberg[4] and Sloan et al[5] reported an increased incidence of glucose tolerance abnormalities in patients with atherosclerotic heart disease.

Elevated blood glucose in diabetics has been shown to lead to nonenzymatic glycosylation of body proteins such as collagen. Advanced glycosylation products of collagen are able to bind low-density lipoprotein (LDL) cholesterol particles, leading to the accumulation of cholesterol in the intimal and medial layers of arteries, the precurser of atherosclerotic heart disease.[6] Elevated cholesterol levels are also known to be associated with diabetes mellitus, and good diabetic control is associated with lowering of serum cholesterol levels.[7] These findings demonstrate that there is a relationship between elevated serum glucose levels and serum cholesterol levels in diabetic patients. Elevated glucose levels are also found in nondiabetic patients with atherosclerotic heart disease, and elevated serum glucose levels cause atherosclerosis in humans. If elevated cholesterol levels are also associated with elevated serum glucose levels in nondiabetic subjects, then it is reasonable to suggest that some of the atherosclerosis associated with elevated cholesterol levels may be due to concomitant elevations in serum glucose levels in these subjects.

The present study was done to determine whether elevated cholesterol levels are associated with elevated blood glucose levels as measured by glycosylated hemoglobin levels in normal and hypercholesterolemic nondiabetic subjects.

METHODS

Subjects were nondiabetic adults (fasting blood glucose between 1.65 and 2.65 mmol/L) (64 and 102 mg/dL) who

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TABLE 1. MEANS, STANDARD DEVIATIONS, AND RANGES FOR GLYCOSYLATED HEMOGLOBIN, FASTING GLUCOSE, CHOLESTEROL, AND HIGH-DENSITY LIPOPROTEIN CHOLESTEROL FOR 43 SUBJECTS

Variable	Mean	Standard Deviation	Range
Glycosylated hemoglobin	5.85%	0.61%	4.8–7.5%
Fasting blood glucose	4.59 mmol/L (82.7 mg/dL)	0.52 mmol/L (9.29 mg/dL)	3.55–5.66 mmol/L (64–102 mg/dL)
Cholesterol	5.90 mmol/L (227.5 mg/dL)	1.55 mmol/L (59.2 mg/dL)	3.35–9.25 mmol/L (129–357 mg/dL)
High-density lipoprotein	1.50 mmol/L (58.3 mg/dL)	0.35 mmol/L (14.1 mg/dL)	0.95–2.55 mmol/L (36–98 mg/dL)

TABLE 2. MEANS AND STANDARD DEVIATIONS FOR GLYCOSYLATED HEMOGLOBIN, FASTING GLUCOSE, AND HIGH-DENSITY LIPOPROTEIN CHOLESTEROL IN SUBJECTS WITH LOW, MODERATE, AND HIGH TOTAL CHOLESTEROL

Cholesterol Range	Variable	Mean	Standard Deviation
5.20 mmol/L (<200 mg/dL) (n = 19)	Glycosylated hemoglobin	5.53%	0.45%
	Glucose	4.38 mmol/L (78.7 mg/dL)	0.49 mmol/L (8.8 mg/dL)
	High-density lipoprotein	1.55 mmol/L (59.3 mg/dL)	0.38 mmol/L (14.5 mg/dL)
5.20 mmol/L, <7.28 mmol/L (>200 mg/dL, <280 mg/dL) (n = 14)	Glycosylated hemoglobin	5.92%	0.63%
	Glucose	4.73 mmol/L (85.2 mg/dL)	0.47 mmol/L (8.41 mg/dL)
	High-density lipoprotein	1.35 mmol/L (51.35 mg/dL)	0.30 mmol/L (12.0 mg/dL)
7.28 mmol/L (>280 mg/dL) (n = 10)	Glycosylated hemoglobin	6.32%	0.54%
	Glucose	4.80 mmol/L (86.6 mg/dL)	0.51 mmol/L (9.18 mg/dL)
	High-density lipoprotein	1.70 mmol/L (66.0 mg/dL)	0.30 mmol/L (12.5 mg/dL)

Glycosylated hemoglobin is higher in group 3 than group 2 (P = .05), and higher in group 2 than group 1 (P = .05). Group 3 is higher than group 1 (P = .005). Glucose is higher in group 3 than group 1 (P = .025), and higher in group 2 than group 1 (P = .025). High-density lipoprotein is higher in group 3 than in group 2 (P = .005) (one-way t test).

were in generally good health, and who were not taking any cholesterol-lowering medications. The 19 men and 24 women ranged in age from 27 to 75 years. Subjects who had any evidence of atherosclerotic heart disease were eliminated from the study. Some were taking part in ongoing studies of hypercholesterolemia, while others were patients at the Family Practice Center, office personnel, and nurses. An effort was made to include both normal and hypercholesterolemic subjects in the study. The means, standard deviations, and ranges for fasting glucose, glycosylated hemoglobin, and total and high-density lipoprotein (HDL) cholesterol for the sample of 43 subjects is given in Table 1.

Fasting blood was drawn from each subject and analyzed for glucose, cholesterol, HDL cholesterol and glycosylated hemoglobin. Cholesterol and HDL cholesterol were measured by the cholesterol oxidase method using the Kodak Ectachem automated technique. All measurements were performed in a laboratory that subscribes to the quality control services of the College of American Pathologists.

The measurement of average blood glucose levels in

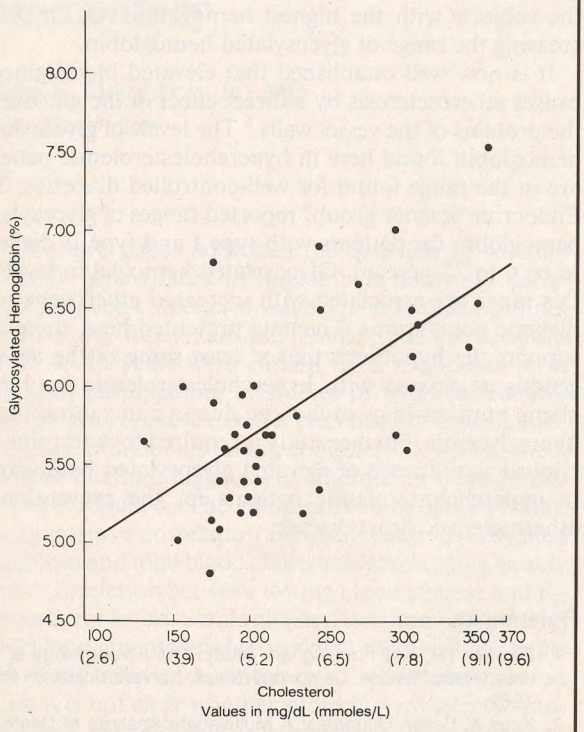

Figure 1. Correlation between total cholesterol and glycosylated hemoglobin in 44 human subjects. Pearson's *r* = .63, P < .001

humans has been facilitated by the discovery of glycosylated hemoglobin.[8] Recently, boronate resin affinity chromatography has been shown to measure only the stable glycosylated hemoglobin, which is linearly related to the average blood glucose (glucose = .886 × glycosylated hemoglobin + 0.3 mmol/L).[9,10] This method is reproducible and technically easy to do, giving results much more reliable than the older cation exchange methods that measured hemoglobin A_{1c} or "fast hemoglobin." Glycosylated hemoglobin was measured by the boronate resin affinity chromatography technique using the Endocrine Sciences kit.[9]

This study met the approval of the Human Subjects Committee of Wayne State University.

RESULTS

For analysis the subjects were divided into three groups. Subjects with total cholesterol below 5.20 mmol/L (200 mg/dL) are not at risk for atherosclerosis according to the Framingham study.[1] Subjects with total cholesterol between 5.20 and 7.25 mmol/L (200 and 280 mg/dL) are at increased risk for atherosclerotic heart disease but are not at greater risk for death from all causes, according to the Seven Countries study.[2] Subjects with total cholesterol over 7.25 mmol/L (280 mg/dL) are at greater risk for death from heart diseases and from all causes. The means and standard deviations for glucose, glycosylated hemoglobin, and HDL cholesterol are given in Table 2 for the three groups. Glycosylated hemoglobin is lowest in the group with normal cholesterol levels and is significantly increased in the moderate and in the high-cholesterol groups. No consistent pattern is seen in the other variables between groups.

Correlation analysis was done between glycosylated hemoglobin, fasting blood glucose, total cholesterol, and HDL cholesterol for the total group and for the subset of subjects with cholesterol greater than 5.20 mmol/L (200 mg/dL), ie, those identified as being at risk for atherosclerosis. The scatter plot and regression line for the correlation between total cholesterol and glycosylated he-

TABLE 3. CORRELATIONS AMONG GLYCOSYLATED HEMOGLOBIN, FASTING GLUCOSE, AND TOTAL AND HIGH-DENSITY LIPOPROTEIN CHOLESTEROL FOR ALL SUBJECTS AND SUBJECTS WITH TOTAL CHOLESTEROL > 5.20 mmol/L

Variable	Glucose	Cholesterol	High-density Lipoprotein
All subjects			
Glycosylated hemoglobin			
Pearson's *r* =	.33	.63	−.10
(significance)	(.015)	(.001)	(.25)
Glucose		.36	.05
		(.008)	(.38)
Cholesterol			.15
			(.17)
Partial correlation glycosylated hemoglobin (cholesterol constant)			−.26 (.05)
Subjects with cholesterol *>5.20 mmol/L (200 mg/dL)*			
Glycosylated hemoglobin	.09 (.34)	.60 (.001)	−.14 (.25)
Glucose		.10	.33
		(.32)	(.06)
Cholesterol			.38
			(.03)
Partial correlation glycosylated hemoglobin (cholesterol constant)			−.51 (.006)

moglobin is shown in Figure 1. Total cholesterol is strongly correlated with glycosylated hemoglobin in both the total group and the high-cholesterol group. The negative correlation between HDL cholesterol and glycosylated hemoglobin is confounded with the positive correlation between total and HDL cholesterol in these subjects. Partial correlation analysis was done holding total cholesterol constant and revealed the expected negative partial correlation between HDL cholesterol and glycosylated hemoglobin in the total sample. This partial correlation was even stronger in the high-cholesterol group. These results are summarized in Table 3.

DISCUSSION

The data reported here demonstrate a clear and statistically significant relationship between cholesterol and glycosylated hemoglobin levels in nondiabetic subjects. Subjects with cholesterol levels above 7.25 mmol/L (280 mg/dL) have an average glycosylated hemoglobin level (6.32 percent), which is higher than the mean of 136 normal subjects (5.5 percent SD = 0.5 percent) reported by the endocrine science group,[9] the mean of 107 normal subjects at the Mayo Clinic[10] (4.96 percent SD = 0.65), and the mean of 5.53 percent for the population of 19 normocholesterolemic subjects reported here. Subjects with moderately elevated cholesterol levels have intermediate levels of glycosylated hemoglobin as expected. HDL cholesterol is seen to be negatively correlated with glycosylated hemoglobin, but this correlation becomes apparent only when total cholesterol levels are controlled. The relationship between glycosylated hemoglobin and plasma lipids is, thus, quite similar to the relationship between atherosclerotic heart disease risk and plasma lipids.[12]

The only previous report of this effect in the literature was by Susenko et al,[11] who found a significant negative correlation between hemoglobin A_{1c} and HDL cholesterol, as seen here, in 137 female subjects ($r = -.25$, $P < .01$), but not in 111 male subjects ($r = -.12$, NS). They also reported no significant correlation between hemoglobin A_{1c} and total cholesterol in their sample. The means and standard deviations of total cholesterol for subjects in their study, 5.10 mmol/L, SD = 1.01 (197 mg/dL, SD = 39 mg/dL) for women and 5.10 mmol/L, SD = 1.06 (198 mg/dL, SD = 41 mg/dL) for men, are lower and narrower than those reported here, which would tend to decrease the magnitude of the cholesterol-glycosylated hemoglobin correlation by decreasing the range of total cholesterol. They measured hemoglobin A_{1c} by a column chromatography method, combining both stable glycosylated hemoglobin and labile Shiff Base components of "fast hemoglobin" in their results. Hemoglobin A_{1c} measurements are known to underestimate glycosylated hemoglobin at high levels. Susenko et al also eliminated from their study the subjects with the highest hemoglobin A_{1c}, thus decreasing the range of glycosylated hemoglobin.

It is now well established that elevated blood glucose causes atherosclerosis by a direct effect of the glucose on the proteins of the vessel walls.[6] The levels of glycosylated hemoglobin found here in hypercholesterolemic patients are in the range found for well-controlled diabetics. The Endocrine Science group[9] reported ranges of glycosylated hemoglobin for patients with type I and type II diabetes to be 6 to 22 percent. Glycosylated hemoglobin levels in this range are associated with increased atherogenesis in diabetic populations. The data presented here, therefore, support the hypothesis that at least some of the atherogenesis associated with hypercholesterolemia in nondiabetic humans may, in fact, be due to concomitant mild hyperglycemia. Further study is required to determine the clinical significance of elevated glycosylated hemoglobin in hypercholesterolemic patients for the prevention of atherosclerotic heart disease.

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Commentary

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The above paper addresses the question of whether stable glycosylated hemoglobin is positively correlated with blood cholesterol values in individuals without diabetes. Forty-three male and female nondiabetic adults aged 27 to 75 years were chosen from a population of individuals participating in studies of hypercholesterolemia and family practice center patients, office personnel, and nurses. Individuals taking cholesterol-lowering medications or having a diagnosis of atherosclerotic heart disease were excluded. The findings include a significant, strongly positive correlation between stable glycosylated hemoglobin and total blood cholesterol levels and a weakly positive correlation between fasting blood glucose and total cholesterol levels. Stable glycosylated hemoglobin and fasting blood glucose levels correlated weakly. The results of the study need to be interpreted with some caution because it is not clear whether subjects were selected randomly, and the population from which the sample was selected is not clearly described. Nevertheless, the findings are of interest. This commentary will review the association between impaired glucose metabolism and risk of coronary heart disease (CHD), the evidence for a causal relationship between impaired glucose metabolism and hypercholesterolemia, and the mechanisms by which impaired glucose metabolism may adversely affect lipid metabolism without increasing total cholesterol levels. Impaired glucose metabolism is defined as *abnormal glucose metabolism without evidence of diabetes mellitus.* I use this wording to avoid using the term *impaired glucose tolerance,* which refers to a specific entity diagnosed by specific criteria.

While diabetes mellitus is an established risk factor for CHD, impaired glucose metabolism, as manifested by mildly abnormal fasting blood glucose levels, has not consistently been found to be an independent risk factor for CHD.[1] The lack of a relationship could be due to fasting blood glucose levels being a poor marker of mild abnormalities of glucose metabolism. Another marker of impaired glucose metabolism, hyperinsulinemia, has been independently related to CHD endpoints in the Paris Prospective Study and two other prospective studies.[1] Insulin levels increase with age and are strongly correlated with body mass index in both sexes.[2] Post-challenge blood glucose levels have also been associated with subsequent CHD endpoints independent of body mass, total cholesterol levels, hypertension, left ventricular hypertrophy, and hematocrit.[3] Elevated stable glycosylated hemoglobin levels, like hyperinsulinemia and post-challenge blood glucose measurements, may be a sensitive and possible early marker of impaired glucose metabolism. Future studies of the relationship of stable glycosylated hemoglobin to subsequent CHD endpoints will be of interest.

Regardless of its independent effects on CHD endpoints, as suggested by the foregoing paper, impaired glucose metabolism measured by stable glycosylated hemoglobin may influence CHD risk through its effects on lipid metabolism. The finding that stable glycosylated hemoglobin is positively associated with total blood cholesterol values in nondiabetic individuals raises the question of whether it is a causal association. A causal association would be supported by a consistent, strong, graded association, the finding that modification of elevated stable glycosylated hemoglobin levels results in a change in total cholesterol values, and an accepted mechanistic explanation. To what extent are these criteria successfully met?

The correlation between stable glycosylated hemoglobin and total cholesterol levels in nondiabetic individuals has, to my knowledge and to the authors' knowledge, been previously examined only in the study by Susenko et al.[4] The absence of a significant association in that study may have been due to inadequate power to detect an association, if one existed. Nevertheless, other markers of impaired glucose metabolism, such as fasting blood glucose levels and blood insulin levels, have not been consistently associated with increased blood cholesterol (though these markers are associated with elevated triglycerides).[2] The present study does find a moderately strong and graded association. Indeed, in extreme impairment of glucose metabolism, that is, diabetes mellitus, there is an association between blood glucose and total cholesterol.[5,6]

There have been no studies of the effect of treatment of impaired glucose metabolism on blood cholesterol. Even in diabetes, improvement of blood glucose control has often not resulted in decreased blood cholesterol values.[7]

Current evidence for a biologically plausible mechanism for elevated blood cholesterol levels in impaired glucose metabolism is controversial. Insulin stimulates cholesterol synthesis in the gut; however, it also suppresses cholesterolgenesis in the liver. The differential effects may ac-

count for the observed variable relationship between blood glucose and cholesterol.[8] Even in diabetes mellitus, there is disagreement concerning the mechanisms of hypercholesterolemia.[6]

Hence, there is at present a paucity of evidence for a causal association between impaired glucose metabolism and blood cholesterol. This lack of evidence is primarily due to a lack of study rather than a preponderance of negative evidence.

Even if impaired glucose metabolism does not affect total cholesterol levels, it could influence CHD risk through changes in lipid metabolism not reflected in total cholesterol measurement. Some evidence supports this statement. First, elevated insulin levels, which, as discussed earlier, are probably a sensitive and early marker of impaired glucose metabolism, increase fatty acid synthesis, which in turn inhibits cholesterol ester hydrolysis. Since cholesterol esters cannot leave the cell, cholesterol efflux from cells is decreased.[9] Second, elevated blood glucose levels result in decreased 2,3 diphosphoglycerate and phosphate levels and increased glycosylated hemoglobin and sorbitol levels, all of which may contribute to tissue hypoxia, which in turn promotes smooth muscle cell proliferation and accumulation of cholesterol.[10] Third, as pointed out by the authors, elevated blood glucose levels result in glycosylation of collagen and increased low-density lipoprotein (LDL) binding to collagen. Fourth, diabetic plasma contains an abnormal LDL particle that may mediate the impaired cholesterol transport in diabetes mellitus.[11] The abnormal LDL competes with normal LDL at the apoprotein B, E receptor and may stimulate cholesterol ester accumulation. Although not found in nondiabetic individuals, this abnormal lipoprotein could be present in individuals with impaired glucose metabolism. Thus, hyperglycemia and hyperinsulinemia may result in increased tissue deposition of cholesterol without necessarily affecting total blood cholesterol levels. Whether this process occurs in nondiabetic individuals with evidence of impaired glucose metabolism is not clear.

In sum, a causal relationship between stable glycosylated hemoglobin and total cholesterol levels has not been clearly proven, though the preceding study lends credence to this hypothesis. Nevertheless, impaired glucose metabolism may adversely affect lipid metabolism without affecting total cholesterol measurement. Finally, recent prospective studies do support the relationship of impaired glucose tolerance to CHD risk and raise the question of whether other markers are more sensitive to impaired glucose metabolism than is fasting blood glucose. There appears to be renewed interest in abnormal glucose metabolism in individuals who do not meet the criteria for diabetes mellitus.

What are the clinical implications? Specifically, should physicians check for evidence of impaired glucose metabolism in individuals with elevated blood cholesterol levels? The evidence at this time would not support this action. The majority of the dietary modifications utilized in lowering blood cholesterol levels and controlling elevated body weight in these individuals will probably favorably modify carbohydrate metabolism.

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