Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes

David R McCance, Robert L Hanson, Marie-Aline Charles, Lennart T H Jacobsson, David J Pettitt, Peter H Bennett, William C Knowler

Abstract

***Objective*—To compare the ability of tests measuring two hour plasma glucose, fasting plasma glucose, and glycated haemoglobin concentrations in predicting the specific microvascular complications of non-insulin dependent diabetes mellitus.**

***Design*—Cross sectional and longitudinal analysis of the relation between complications and concomitant results of the three tests.**

***Setting*—Gila River Indian Community, Arizona.**

***Subjects*—Pima Indians (cross sectional, n=960), aged 25 years or above who were not receiving insulin or oral hypoglycaemic treatment at the baseline examination.**

***Main outcome measures*—Development of retinopathy and nephropathy.**

***Results*—Cross sectionally, frequency distributions of logarithms of the three sets of results were bimodal, with the prevalence of retinopathy and nephropathy being, respectively, 12·0-26·7 and 3·9-4·2 times as high above as below cut off points which minimised overlap (two hour plasma glucose concentration 12·6 mmol/l; fasting plasma glucose concentration 9·3 mmol/l; glycated haemoglobin (HbA_{1c}) concentration 7·8%). Longitudinally, each of the three measures of glycaemia significantly predicted the development of retinopathy ($P<0·0001$) and nephropathy ($P<0·05$). Receiver operating characteristic curves showed that two hour plasma glucose concentration was superior to fasting plasma glucose concentration ($P<0·05$) for prevalent cases of retinopathy, but otherwise no variable had a significant advantage for detecting incident or prevalent cases of either complication.**

***Conclusions*—These findings suggest that determination of glycated haemoglobin or fasting plasma glucose concentrations alone may be acceptable alternatives to measuring glucose concentration two hours after challenge with 75 g glucose for the diagnosis of diabetes.**

Diabetes and Arthritis Epidemiology Section, Phoenix Epidemiology and Clinical Research Branch, National Institute of Diabetes and Digestive and Kidney Diseases and National Institute of Arthritis and Musculoskeletal and Skin Diseases, Phoenix, Arizona 85014, United States
David R McCance, *visiting associate*
Robert L Hanson, *medical staff fellow*
Marie-Aline Charles, *visiting associate*
Lennart T H Jacobsson, *visiting associate*
David J Pettitt, *assistant chief of section*
Peter H Bennett, *branch chief*
William C Knowler, *section chief*

Correspondence to:
Dr D R McCance, Sir George E Clark Metabolic Unit, Royal Victoria Hospital, Belfast BT12 6BA.

BMJ 1994;308:1323-8

Introduction

Hyperglycaemia, fasting and as assessed by the oral glucose tolerance test, is the accepted means of defining diabetes mellitus.[1-6] The present consensus on diagnostic criteria was reached about a decade ago.[4-6] Discovery of the bimodal distribution of fasting and two hour plasma glucose concentrations and later glycated haemoglobin concentration in populations with a high prevalence of diabetes[7-13] strengthened the concept of the diabetic state (defined as the upper component of these distributions) as a distinct entity. The clear increase in prevalence[7 11 14-16] and incidence[17] of microvascular complications at about the glucose concentration corresponding to the separation of the two components of the frequency distributions gave clinical and prognostic relevance to the diagnostic criteria currently used to define diabetes.

Given the inconvenience, variability, and non-physiological nature of the oral glucose tolerance test,[18 19] various alternative screening tests for the detection of diabetes have been suggested.[7 20-32] Evaluation of the superiority of a screening test, however, depends on a reference or standard by which to assess the alternative test. Ultimately such tests can be judged only in terms of their ability to predict a relevant clinical end point, such as the specific complications of diabetes. A fundamental problem in the evaluation is the high degree to which measures of glycaemia correlate with each other, and few studies have compared the different tests directly.

We examined the risk of microvascular complications in relation to concomitant measurements of plasma glucose concentration (fasting and two hours after 75 g load) and glycated haemoglobin concentration in Pima Indians, a population in which about half of those over 35 years of age have the disease.[33] Alternatives to current diagnostic methods for diabetes are discussed.

Methods

A longitudinal study of diabetes and its complications has been conducted among the Pima Indian residents of the Gila River Indian Community in Arizona since 1965. About every two years all residents of the community aged 5 years and over regardless of health are asked to participate in a standardised medical examination including a medical history, a physical examination, and laboratory tests.

Direct ophthalmoscopic examination through dilated pupils was performed by a physician who was unaware of the glucose or glycated haemoglobin concentrations. For this analysis, diabetic retinopathy was defined by the presence of at least one microaneurysm or haemorrhage or proliferative retinopathy. Laboratory tests performed at each examination include measurement of glycated haemoglobin (HbA_1 or HbA_{1c}) and urinary protein and creatinine concentrations and a modified oral glucose tolerance test with determination of the plasma glucose concentration in fasting subjects and after they had ingested 75 g carbohydrate (Glucola, Ames, Elkhart, Indiana). Plasma glucose concentration was measured by the potassium ferricyanide method with an autoanalyser.[34] The stable fraction of total glycated haemoglobin (HbA_1) was measured by electrophoresis[35 36] and haemoglobin A_{1c} by high performance liquid chromatography.[37] Haemoglobin A_1 was estimated from 1 January 1982 until 1 January 1990 after which haemoglobin A_{1c} has been measured.

Subjects were asked to void at the beginning of the oral glucose tolerance test, and a urine specimen was collected two hours later. The presence of protein was determined by dipstick (Labstix, Ames). In urine specimens containing a trace or more of protein on dipstick, protein was measured quantitatively by the method of Shevky and Stafford,[38] and the urine creatinine concentration was determined. For this analysis nephropathy was defined as a protein to creatinine ratio of $\geq 1{\cdot}0$ g:g (113 mg protein:mmol creatinine); a ratio equivalent to a total protein excretion rate of about 1 g/day.[39]

The present study includes both longitudinal and cross sectional analyses among subjects aged 25 years and over. Subjects receiving insulin or oral hypoglycaemic treatment at the baseline examination (longitudinal study) or at the last biennial examination (cross sectional study) were excluded from the analyses.

In the cross sectional analysis the prevalence of retinopathy or nephropathy was determined in 960 subjects (384 men and 576 women) with two hour and fasting plasma glucose and haemoglobin A_{1c} measurements at the last examination. Subjects were included regardless of whether they had had retinopathy or nephropathy at any time in the past. The prevalence of each complication was examined by tenths or fifths of the distribution of each test variable.

In the longitudinal analysis subjects who were free of retinopathy or nephropathy at baseline (their first biennial examination after 1 January 1982) with concomitant measurements of two hour and fasting plasma glucose and haemoglobin A_1 (rather than haemoglobin A_{1c}) concentrations were identified. These subjects were followed up until they developed the complication of interest or until 30 November 1991.

Statistical analysis—In all analyses logarithms of glycated haemoglobin and plasma glucose concentrations were used to reduce skewness.

Frequency distributions—In the cross sectional analysis frequency distributions of two hour and fasting plasma glucose and haemoglobin A_{1c} measurements at the last biennial examination were plotted. The parameters of a model of two overlapping Gaussian distributions with common variances were calculated from these distributions by maximum likelihood estimation (BMDP Statistical Software, program LE). For each variable the optimum cut off or antimodal point, which minimises the area of overlap, was determined algebraically from the model.[40] The prevalences of retinopathy and nephropathy were examined above and below these cut off points. The sensitivity and specificity of the optimum cut off points of each of the three test variables for the presence of retinopathy and nephropathy were estimated. In the longitudinal analysis the cumulative incidence of a complication above and below these cut off points was estimated from Kaplan-Meier survival curves, and the survival distributions were compared by using the log rank statistic.

Receiver operating characteristic analysis—In both cross sectional and longitudinal studies the two hour and fasting plasma glucose and glycated haemoglobin concentrations were compared by using receiver operating characteristic curves.[41,42] This method compares the diagnostic properties of a test by expressing sensitivity as a function of 1 − specificity. The areas under the curves represent the probability that a subject chosen at random who had or developed retinopathy or nephropathy had a higher test value than one who did not have or develop these complications. The significance of the differences between two areas was analysed by using a 1989 version of the CLABROC program (obtained from Dr Charles Metz, department of radiology, University of Chicago).

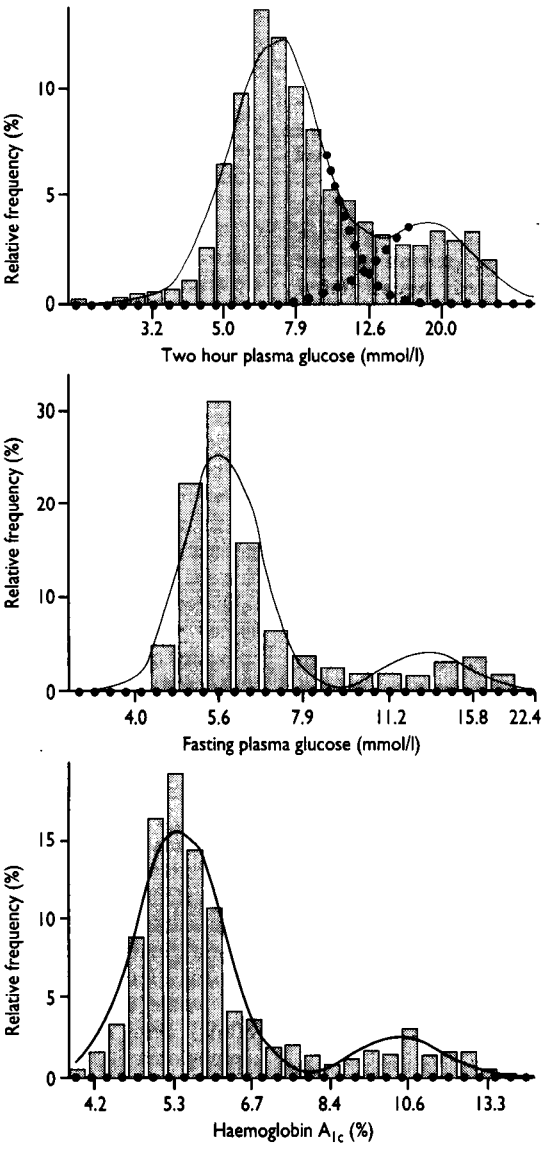

FIG 1—*Frequency distributions of two hour plasma glucose, fasting plasma glucose and glycated haemoglobin concentrations for subjects aged 25 years and over plotted on logarithmic scale. Smooth curves represent a mathematical model of two overlapping normal distributions which were fit by maximum likelihood*

Results

Figure 1 shows the frequency distributions of two hour and fasting plasma glucose and haemoglobin A_{1c} concentrations for subjects in the cross sectional study. The distribution of each test variable is bimodal. Antimodal cut off points, which divided the two components of the distributions with a minimum of overlap, were 12·6 (95% confidence interval 12·1 to 13·1) mmol/l, 9·3 (9·1 to 9·5) mmol/l, and 7·8% (7·7 to 7·9) for two hour plasma glucose, fasting plasma glucose, and glycated haemoglobin concentrations, respectively.

CROSS SECTIONAL AND PROSPECTIVE COMPARISONS

Table I shows the prevalence of retinopathy and nephropathy above and below the cut off points for two hour and fasting plasma glucose and glycated haemoglobin concentrations and their corresponding sensitivities and specificities. Two hour plasma glucose concentration was more sensitive than the two other variables but was less specific than glycated haemoglobin or fasting glucose concentration for both combinations.

Table II shows the five year incidence of each complication above and below antimodal cut off points of two hour and fasting plasma glucose and glycated haemoglobin concentrations. Of 927 subjects (313 men

TABLE I—*Prevalence of retinopathy and nephropathy above and below optimal antimodal cut off points for two hour plasma glucose, fasting plasma glucose, and glycated haemoglobin concentrations and their corresponding sensitivities and specificities*

Condition	Two hour plasma glucose (mmol/l)		Fasting plasma glucose (mmol/l)		Haemoglobin A_{1c} (%)	
	<12·6	≥12·6	<9·3	≥9·3	<7·8	≥7·8
Retinopathy*:						
No of subjects	743	210	818	135	818	135
Percentage with retinopathy	0·5	13·3	1·2	16·3	1·3	15·6
Sensitivity (%)	87·5		68·8		65·6	
Specificity (%)	80·2		87·7		87·6	
Nephropathy†:						
No of subjects	740	211	817	134	817	134
Percentage with nephropathy	1·6	6·2	1·8	7·5	1·8	7·5
Sensitivity (%)	52·0		40·0		40·0	
Specificity (%)	78·6		86·6		86·6	

*Values missing for seven subjects. †Values missing for nine subjects.

TABLE II—*Five year incidence (percentages) of retinopathy and nephropathy above and below antimodal cut off points for fasting and two hour plasma glucose and glycated haemoglobin concentrations. Figures are numbers of subjects at risk at baseline*

Condition	Two hour plasma glucose (mmol/l)		Fasting plasma glucose (mmol/l)		Haemoglobin A_1* (%)	
	<12·6	≥12·6	<9·3	≥9·3	<9·4	≥9·4
Retinopathy	769 (0·0)	158 (17·5)	822 (1·3)	105 (17·3)	842 (1·1)	85 (22·9)
Nephropathy	768 (1·2)	173 (3·6)	824 (1·3)	117 (4·1)	844 (1·4)	97 (3·8)

*Cut off value calculated from regression equation of two glycated haemoglobin variables.

and 614 women) free of retinopathy and 941 subjects (327 men and 614 women) free of nephropathy at the baseline examination, 33 (3·6%) and 25 (2·7%), respectively, developed these complications during a mean (range) follow up of 4·5 (1·4-8·3) years.

The incidence and prevalence of retinopathy were 13·3-∞ and 12·0-26·7 times as high above as below the cut off points. Corresponding ratios for incidence and prevalence of nephropathy were 2·7-3·2 and 3·9-4·2, respectively. Subjects with glycated haemoglobin concentrations above the cut off point had the highest five year incidence (22·9%) of retinopathy, whereas those with glycated haemoglobin (15·6%) and fasting plasma glucose (16·3%) concentrations above the cut off point had the highest prevalence of retinopathy. Subjects with the fasting plasma glucose and glycated haemoglobin concentrations above the cut off points also had a higher incidence and prevalence of nephropathy than those whose two hour plasma glucose values fell above the cut off point.

To assess the occurrence of retinopathy and nephropathy in relation to equivalent degrees of hyperglycaemia, the five year incidence and prevalence of each complication was examined by tenths or fifths of the distribution of two hour and fasting plasma glucose and glycated haemoglobin concentrations for retinopathy (fig 2) or nephropathy (fig 3) respectively. All three variables were predictive of retinopathy ($P<0·0001$; log rank statistic) and nephropathy (two hour plasma glucose $P<0·05$; fasting plasma glucose concentration $P<0·01$; haemoglobin A_1 $P<0·05$). Judged by the absence of retinopathy below the 80th centile the two hour glucose concentration was superior to fasting plasma glucose and glycated haemoglobin concentrations in predicting the incidence of retinopathy (fig 2, top), but the prevalence of retinopathy was similar for each of the three variables (fig 2, bottom). The figure provides clear evidence of a threshold between the 80th and 90th centiles, below which retinopathy is absent or rare and above which the incidence is considerably higher. The five year incidence and the prevalence of nephropathy (fig 3) were similar among the three variables and again were higher in the upper fifth of the distribution. The associations of glycaemia with nephropathy, however, were less strong than those with retinopathy.

RECEIVER OPERATING CHARACTERISTIC ANALYSES

Figure 4 shows the receiver operating characteristic analyses for the prevalence of retinopathy and nephropathy. The area under the curve for two hour plasma glucose concentration was significantly greater than that of fasting plasma glucose concentration ($P<0·05$) for retinopathy, but otherwise there were no significant differences between the individual areas for retinopathy or nephropathy. Similarly, for the data on incidence of each complication, two hour plasma glucose concentration had an area slightly but not significantly larger than that for fasting plasma glucose and haemoglobin A_1 concentrations for retinopathy, thus indicating slightly greater accuracy for predicting this complication. None of the areas, however, differed significantly for the prediction of incidence of retinopathy or nephropathy. For both cross sectional and longitudinal analyses the areas under the curves were greater for retinopathy than for nephropathy.

In addition to antimodal cut off points given above, the point which maximises the sum of sensitivity and specificity can be used to discriminate between groups of subjects who have a high risk for retinopathy. Alternatively, if one assumes certain costs attached to misclassification of subjects, equivalent cut off points to that of the two hour plasma glucose concentration (11·1 mmol/l)[6] for the other glycaemic variables may be derived from those points with the same slope on the receiver operating characteristic curve.[43,44] As shown in table III, these cut off points vary according to the measures and means chosen to define them. By using antimodal cut off points the prevalence of diabetes in this sample varies from 14% to 22%. On the other hand, cut off points defined by maximising the sensitivity and specificity to identify retinopathy in the

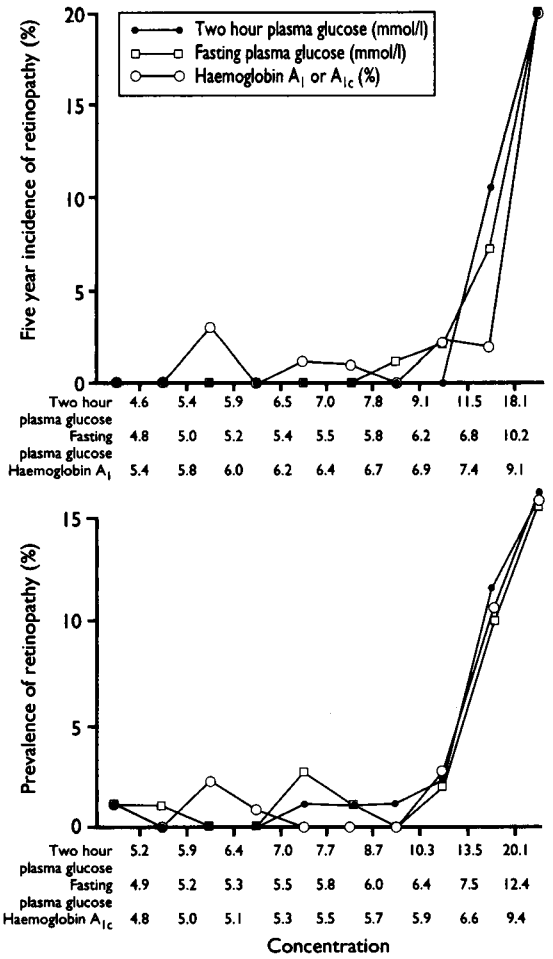

FIG 2—*Five year cumulative incidence (top) and prevalence (bottom) of retinopathy in relation to tenths of two hour plasma glucose, fasting plasma glucose, and glycated haemoglobin A_1 and A_{1c} concentrations*

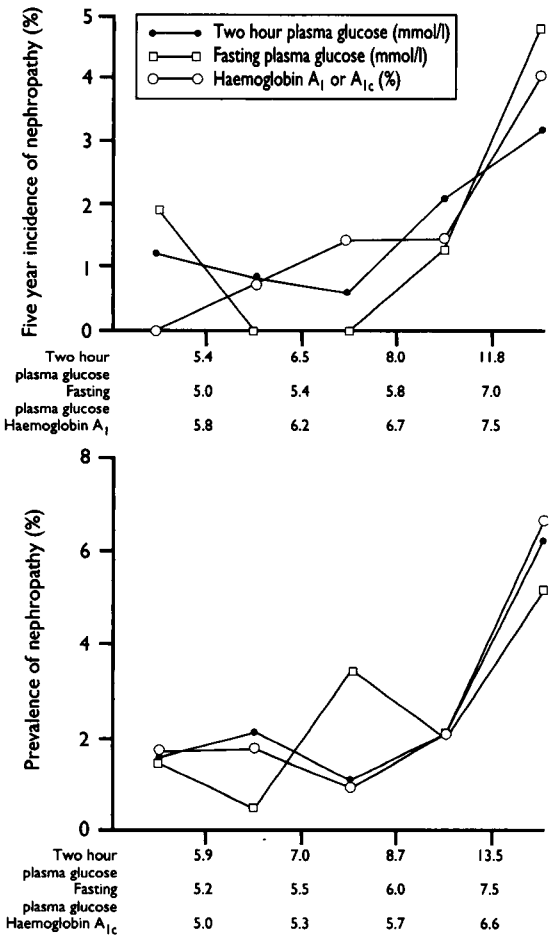

FIG 3—*Five year cumulative incidence (top) and prevalence (bottom) of nephropathy (urinary protein/creatinine ratio ≥ 1·0) according to fifths of two hour plasma glucose, fasting plasma glucose, and glycated haemoglobin A_1, A_{1c} concentrations*

TABLE III—*Sensitivities and specificities for retinopathy and percentages of subjects above various cut off points**

	Two hour plasma glucose test	Fasting plasma glucose test	Haemoglobin A_{1c} test
Antimodal:			
Cut off point	12·6 mmol/l	9·3 mmol/l	7·8%
Sensitivity (%)	87·5	68·8	65·6
Specificity (%)	80·2	87·7	87·6
Percentage of subjects above cut off point	22·0	14·2	14·0
WHO equivalent:			
Cut off point	11·1 mmol/l	6·8 mmol/l	6·1%
Sensitivity (%)	87·5	81·2	81·3
Specificity (%)	75·8	77·1	76·8
Percentage of subjects above cut off point	26·3	24·9	25·3
Maximum sensitivity plus specificity:			
Cut off point	13·0 mmol/l	7·2 mmol/l	7·0%
Sensitivity (%)	87·5	81·3	78·1
Specificity (%)	81·4	80·4	84·7
Percentage of subjects above cut off point	20·9	21·7	17·4

*For details see text.

population led to similar estimates of the prevalence of diabetes with the different glycaemic variables (20·9, 21·7, and 17·4 for two hour and fasting plasma glucose and glycated haemoglobin concentrations, respectively).

Discussion

The appropriate interpretation of any diagnostic test depends on knowledge of its prognostic importance. Even though the glucose tolerance test has been used for diagnostic purposes for diabetes for the past 40 years, data concerning the development of microvascular complications in relation to degrees of glucose tolerance, other than in subjects with diagnosed diabetes, have been largely confined to the two hour plasma glucose value.[15-17 45] Longitudinal follow up data for fasting plasma glucose and glycated haemoglobin concentrations are limited. We measured two hour and fasting plasma glucose and glycated haemoglobin concentrations concomitantly, and subjects were followed up over five years for the development of microvascular complications.

BIMODAL DISTRIBUTIONS SUGGEST CUT OFF POINTS

The frequency distributions of the three test variables were each bimodal and, as shown previously, membership in the upper component of the distribution can be equated with having diabetes. For each measure of glycaemia, antimodal cut off points had comparable sensitivity and specificity for the presence of retinopathy and for nephropathy, though haemoglobin A_{1c} concentration tended to be the most specific and two hour plasma glucose concentration the most sensitive (table I).

Although subjects were followed up for only five years, the development of complications within this time suggests that the degrees of glycaemia defined in this study also have long term prognostic relevance. Retinopathy and nephropathy have been shown to be strong predictors of the development of the more serious long term complications of diabetes such as proliferative retinopathy, end stage renal disease, cardiovascular disease, amputation, and even mortality. Moreover, the cross sectional analyses evaluate complications in relation to both shorter and longer durations of hyperglycaemia. The optimal cut off points derived from the frequency distributions seem to be points which distinguish subjects with a high prevalence of retinopathy and nephropathy and who have a high risk for developing these complications in the future. Thus the longitudinal and cross sectional analyses provide important complementary information and lead to similar conclusions.

The precision of the estimate of antimodal values derived from the parameters of a model of two overlapping distributions depends on sample size and the distribution model. We have adopted a model with

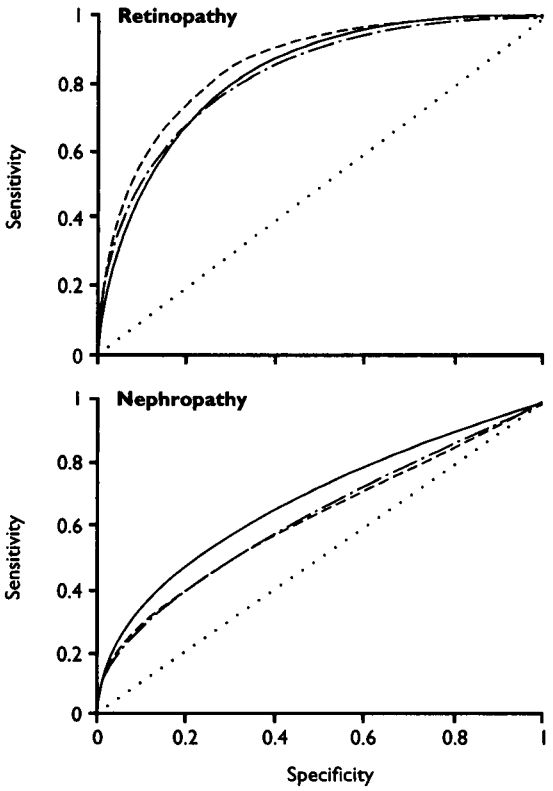

FIG 4—*Receiver operating curves for prevalence of retinopathy (top) and nephropathy (bottom) for two hour plasma glucose (----), fasting plasma glucose (·····), and glycated haemoglobin (——) concentrations, with values expected by chance (—·—)*

Clinical implications

- Hyperglycaemia, fasting and as assessed by the oral glucose tolerance test, is the standard method of defining diabetes mellitus
- The oral glucose tolerance test is cumbersome and not widely available
- Fasting plasma glucose and glycated haemoglobin concentrations have been suggested as alternative means of diagnosis
- Evaluation of the superiority of a diagnostic test ultimately can be judged only in terms of its ability to predict the specific long term microvascular complications of diabetes
- This study showed that two hour plasma glucose, glycated haemoglobin, and fasting plasma glucose concentrations are equivalent as predictors of the development of retinopathy and nephropathy
- Measuring glycated haemoglobin or fasting plasma glucose concentration may be acceptable and more convenient than measuring two hour plasma glucose concentration for the diagnosis of diabetes

a logarithmic transformation and common variance, which we think provides the best visual fit to the data. The use of models with separate variances or with transformations other than logarithmic would result in different cut off values. We therefore advise caution against placing undue emphasis on the absolute values of any of these variables; we have presented them because we think that they provide a means of comparing a dichotomy of each of three test variables against a relevant biological and clinical end point.

NO GLYCAEMIC MEASUREMENT CLEARLY SUPERIOR

A threshold was evident for both the incidence and the prevalence of retinopathy between the 80th and 90th centiles of each glucose and glycated haemoglobin variable, below which retinopathy was virtually absent and did not subsequently develop and above which a distinct increase in this complication occurred. Although the incidence and prevalence of nephropathy were higher in the upper fifth of the distribution, the association of the degree of glycaemia with nephropathy is weaker than with retinopathy.

Incidences suggested that two hour plasma glucose concentration was slightly superior to either of the two other variables in predicting retinopathy (fig 2), but this advantage, according to the receiver operating characteristic analysis, was not significant for retinopathy or nephropathy. We also found no significant difference among the three variables for the prediction of retinopathy among diabetic subjects.[46] The areas under the curves were much smaller for nephropathy than for retinopathy, indicating that the ability of any measure of hyperglycaemia to predict this complication is not as good. In general, the results are similar if nephropathy is defined by a lower protein to creatinine ratio (0·5 or above) or by dipstick proteinuria of 1+ or more (data not shown). These findings are consistent with the occurrence of proteinuria as a result of other diseases and with the selective occurrence of diabetic nephropathy, which, in contrast with retinopathy, affects a smaller subset of patients and in which the role of hyperglycaemia is less well defined.[47]

We also assessed whether a combined index of the three measures is superior to any one alone. By using receiver operating characteristic analysis the composite variable showed the greatest area, although it was not statistically superior to any of the individual measures of glycaemia for the prediction of retinopathy or nephropathy in either cross sectional or longitudinal analyses.

ORAL GLUCOSE TOLERANCE TESTS IN PERSPECTIVE

The oral glucose tolerance test has been the preferred test for diagnosing diabetes in epidemiological studies for over 40 years. This choice has persisted despite the widely recognised costs and inconvenience of the test. Our study shows that when two hour and fasting plasma glucose and glycated haemoglobin concentrations are examined in relation to what may be the most relevant clinical end point—that of the long term complications of diabetes—each of the three variables has a similar association with prevalence and five year incidence of these complications and has similar characteristics on receiver operating characteristic analysis. These findings suggest that the choice of a measure of glycaemia for diagnostic purposes might well favour glycated haemoglobin or fasting plasma glucose concentration. By using values of the glycaemic variables that maximise sensitivity plus specificity for the presence of retinopathy the prevalence of diabetes is similar for two hour (20·9%) and fasting plasma glucose (21·7%) and glycated haemoglobin (17·4%) concentrations, thus providing further evidence of the equivalence of these measures of glycaemia for classification and diagnostic purposes.

The selection of diagnostic points, based on any of these measures of glycaemia, depends on considerations which include the beneficial and adverse effects of diagnosis and treatment. Our results pertain to a population with a high prevalence of diabetes, which affects the analysis of distributions. We have no reason, however, to suspect that the equivalence of the three glycaemic measures is not generalisable to other populations, given that complications in the Pima Indians are qualitatively indistinguishable from those in other populations and risk factors for their development are similar.

CONCLUSIONS

Our results suggest that from considerations of the risk of microvascular complications measurement of glycated haemoglobin or fasting plasma glucose concentration may be just as useful as measurement of two hour plasma glucose concentration for diagnostic purposes.

We thank the members of the Gila River Indian Community who participated in the study and the laboratory staff who performed the laboratory tests.

1 John HJ. Glucose tolerance and its value in diagnosis. *Journal of Metabolic Research* 1922;1:497-548.
2 Mayer JH, Womack CR. Glucose tolerance 1. A comparison of four types of diagnostic tests in 103 control subjects and 26 patients with diabetes. *Am J Med Sci* 1950;219:161-73.
3 Fajans SS, Conn JW. The early recognition of diabetes mellitus. *Ann N Y Acad Sci* 1959;82:208-18.
4 National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979;28: 1039-57.
5 WHO Expert Committee on Diabetes Mellitus. Second report. *World Health Org Tech Rep Ser* 1980;646:9-14.
6 Report of a WHO Study Group. *Diabetes mellitus. World Health Org Tech Rep Ser* 1985;727:9-17.
7 Miller M, Bennett PH, Burch TA. *Hyperglycaemia in Pima Indians: a preliminary appraisal of its significance. Biomedical challenges presented by the American Indian.* Washington, DC: Pan American Health Organisation, 1968:89-103.
8 Rushforth NB, Bennett PH, Steinberg AG, Burch TA, Miller M. Diabetes in the Pima Indians: evidence of bimodality in glucose tolerance distributions. *Diabetes* 1971;20:756-65.
9 Rushforth NB, Bennett PH, Steinberg AG, Miller M. Comparison of the two- and one-hour glucose levels of the oral GTT in the diagnosis of diabetes in Pima Indians. *Diabetes* 1975;24:538-46.
10 Flock EV, Bennett PH, Savage PJ, Webner CJ, Howard BV, Rushforth NB, *et al.* Bimodality of glycosylated hemoglobin distribution in Pima Indians; relationship to fasting hyperglycemia. *Diabetes* 1979;28:984-9.
11 Zimmet P, Whitehouse S. Bimodality of fasting and two-hour glucose tolerance distributions in a Micronesian population. *Diabetes* 1978;27: 793-800.
12 Raper LR, Taylor R, Zimmet P, Milne B, Balkau B. Bimodality in glucose tolerance distributions in the urban Polynesian population of Western Samoa. *Diabetes Res* 1984;1:19-26.
13 Rosenthal M, McMahan CA, Stern MP, Eifler CW, Haffner SM, Hazuda HP, *et al.* Evidence of bimodality of two hour plasma glucose concentrations in Mexican Americans: results from the San Antonio heart study. *Journal of Chronic Disease* 1985;38:5-16.
14 Rushforth NB, Miller M, Bennett PH. Fasting and two-hour post-load glucose levels for the diagnosis of diabetes: the relationship between glucose levels and complications of diabetes in Pima Indians. *Diabetologia* 1979;16:373-9.
15 Al Sa Yegh H, Jarrett RJ. Oral glucose tolerance tests and the diagnosis of

diabetes: results of a prospective study based on the Whitehall Survey. *Lancet* 1979;ii:431-3.
16 Jarrett RJ, Keen H. Hyperglycaemia and diabetes mellitus. *Lancet* 1976;ii: 1009-12.
17 Pettitt DJ, Knowler WC, Lisse JR, Bennett PH. Development of retinopathy and proteinuria in relation to plasma glucose concentrations in Pima Indians. *Lancet* 1980;ii:1050-2.
18 Committee on Statistics of the American Diabetes Association. Standardization of the oral glucose tolerance test. *Diabetes* 1969;18:299-307.
19 Sherwin RS. Limitations of oral glucose tolerance test in diagnosis of early diabetes. *Primary Care* 1977;4:255-66.
20 Modan M, Halkin H, Karasik A, Lusky A. Effectiveness of glycosylated hemoglobin, fasting plasma glucose and a single post load plasma glucose level in population screening for glucose intolerance. *Am J Epidemiol* 1984;119:431-44.
21 Simon D, Coignet MC, Thibult N, Senan C, Eschwege E. Comparison of glycosylated hemoglobin and fasting plasma glucose with two-hour post-load plasma glucose in the detection of diabetes mellitus. *Am J Epidemiol* 1985;122:589-93.
22 Verillo AT, Golia R, Nunziata V. The relationship between glycosylated haemoglobin levels and various degrees of glucose intolerance. *Diabetologia* 1983;24:391-3.
23 Dods RF, Bolmey C. Glycosylated hemoglobin assay and oral glucose tolerance test compared for detection of diabetes mellitus. *Clin Chem* 1979;25:764-8.
24 Albutt EC, Nattrass M, Northam BE. Glucose tolerance and glycosylated haemoglobin measurement for diagnosis of diabetes mellitus—an assessment of the criteria of the WHO expert committee on diabetes mellitus. *Ann Clin Biochem* 1985;22:67-73.
25 Forrest RD, Jackson CA, Gould BJ, Casbum-Budd M, Taylor JE, Yudkin JS. Four assay methods for glycated hemoglobin compared as screening tests for diabetes mellitus: the Islington diabetes survey. *Clin Chem* 1988;34:145-8.
26 Santiago JV, Davis JE, Fisner F. Hemoglobin A_{1c} levels in a diabetes detection program. *J Clin Endocrinol Metab* 1978;47:578-80.
27 Hanson RL, Nelson RG, McCance DR, Beart J, Charles MA, Pettitt DJ, *et al.* Comparison of screening tests for non-insulin-dependent diabetes mellitus. *Arch Intern Med* 1993;153:2133-40.
28 Lester E, Frazer AD, Shepherd CA, Woodroffe FJ. Glycosylated haemoglobin as an alternative to the glucose tolerance test for the diagnosis of diabetes mellitus. *Ann Clin Biochem* 1985;22:74-8.
29 Bennett PH, Knowler WC. Early detection and intervention in diabetes mellitus: is it effective? *Journal of Chronic Disease* 1984;37:653-66.
30 Davidson JK. Screening for diabetes mellitus. In: Davidson JK, ed. *Clinical diabetes mellitus*. New York: Thieme, 1986:108-13.
31 Singer DE, Samet JH, Coley CM, Nathan DM. Screening for diabetes mellitus. *Ann Intern Med* 1988;109:639-49.
32 American Diabetes Association Position Statement. Screening for diabetes mellitus. *Diabetes Care* 1989;12:588-90.
33 Knowler WC, Pettitt DJ, Saad MF, Bennett PH. Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev* 1990;6:1-27.
34 Hoffman WS. A rapid photoelectric method for the determination of glucose in blood or urine. *J Biol Chem* 1937;120:51-5.
35 Menard L, Dempsey ME, Blankstein LA, Aleyassine H, Wacks M, Soeldner JS. Quantitative determination of glycosylated haemoglobin A_1 by agar gel electrophoresis. *Clin Chem* 1980;26:1598-602.
36 Nathan DM, Dunn BS, Francis TB. Two commercial methods evaluated for eliminating the labile fraction from the assay for glycated hemoglobin (glycohemoglobin). *Clin Chem* 1984;30:109-10.
37 Ellis G, Diamandis EP, Giesbrecht EE, Daneman D, Allen LC. An automated 'high pressure' liquid chromatographic assay for hemoglobin A_{1c}. *Clin Chem* 1984;30:1746-52.
38 Shevky NC, Stafford DD. A clinical method for the estimation of protein in urine and other body fluids. *Arch Intern Med* 1923;32:222-5.
39 Kunzelman CL, Knowler WC, Pettitt DJ, Bennett PH. Incidence of proteinuria in type 2 diabetes mellitus in the Pima Indians. *Kidney Int* 1989;35:681-7.
40 Steinberg AG, Rushforth NB, Bennett PH, Burch TA, Miller M. On the genetics of diabetes mellitus. In: Cerasi E, Luft R, eds. *Pathogenesis of diabetes mellitus*. Stockholm: Almqvist and Wiksell, 1970:237-64.
41 Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;143:29-36.
42 Metz CE, Wang PL, Kronman HB. A new approach for testing the significance of difference between ROC curves measured from correlated data. In: Deconick F, ed. *Proceedings of the VIIIth conference on information processing in medical imaging*. The Hague: Nijoff M, 1984:432-5.
43 Erdreich LS, Lee ET. Use of relative operating characteristic analysis in epidemiology. *Am J Epidemiol* 1982;114:649-62.
44 Zweig MH, Campbell G. Receiver operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;39: 561-77.
45 Bennett PH, Knowler WC, Pettitt DJ, Lisse JR. The prognostic significance of the glucose tolerance test. In: Cumming IA, Funder JW, Mendelsohn FAO, eds. *Proceedings VI international congress of endocrinology*. Amsterdam: Elsevier/North Holland Biomedical Press, 1980:711-4.
46 Liu QZ, Pettitt DJ, Hanson RL, Charles MA, Klein R, Bennett PH, *et al.* Glycated haemoglobin, plasma glucose and diabetic retinopathy: cross-sectional and longitudinal analyses. *Diabetologia* 1993;36:428-32.
47 Larkins RG, Dunlop ME. The link between hyperglycaemia and diabetic nephropathy. *Diabetologia* 1992;35:499-504.

(Accepted 22 February 1994)

Low cholesterol concentrations and severe depressive symptoms in elderly people

S Lori Brown, Marcel E Salive, Tamara B Harris, Eleanor M Simonsick, Jack M Guralnik, Frank J Kohout

Epidemiology, Demography, and Biometry Program, National Institute on Aging, Bethesda, MD 20892, USA
S Lori Brown, *epidemiologist*
Marcel E Salive, *epidemiologist*
Tamara B Harris, *chief of geriatric epidemiology office*
Eleanor M Simonsick, *epidemiologist*
Jack M Guralnik, *chief of epidemiology and demography office*

College of Dentistry, University of Iowa, Iowa City, IA 52242, USA
Frank J Kohout, *professor*

Correspondence to:
Dr S Lori Brown, Epidemiology, Demography, and Biometry Program, National Institute on Aging, 7201 Winsconsin Ave, Suite 3C309, Bethesda, MD 20892, USA.

BMJ 1994;308:1328-32

Abstract

***Objective*—To investigate the reported association between low serum cholestrol concentration and severe depressive symptoms in an elderly population.**

***Design*—Cross sectional analysis of pooled data from three communities of the established populations for epidemiologic studies of the elderly. Participants who completed their interview, including the Centers for Epidemiologic Studies' depression scale and consented to measurement of their cholesterol concentration were included in the study.**

***Subjects*—3939 men and women aged ≥ 71.**

***Methods*—χ^2 analysis, *t* tests, and multivariate regression analysis of the association between low cholesterol concentration and severe depressive symptoms. All analyses were stratified by sex, and multivariate analyses were adjusted for age, self reported health, physical function, number of drugs used, and weight loss.**

***Main outcome measure*—Score of depressive symptoms on the Centers for Epidemiologic Studies' depression scale.**

***Results*—Depressive symptoms, cholesterol concentration, weight, and use of drugs were all associated with age in men and women. The relative odds of severe depressive symptoms (score ≥ 16) for those with low cholesterol concentrations (< 4·14 mmol/l) were 1·9 (95% confidence interval, 1·1 to 3·3) for the older group of men and 1·8 (1·1 to 2·9) for the older group of women. This association was also observed when depressive symptoms were analysed as a continuous rather than a categorical variable. In multivariate models that adjusted for age, self reported health, physical function, number of drugs used, and weight loss, the association was substantially weakened.**

***Conclusions*—After several factors relating to health had been controlled for, no significant association between low cholesterol concentration and severe depressive symptoms was found.**

Introduction

Although a high serum cholesterol concentration is a well established risk factor for heart disease,[1] an analysis of results of cholesterol lowering clinical trials has suggested that the benefits of reducing heart disease by reducing cholesterol concentration are offset by an increase in deaths from external causes such as suicide, accidents, and murder.[2-4] This increase in deaths from causes unrelated to illness occurred regardless of whether the cholesterol lowering regimen was dietary or pharmacological, prompting the authors to suggest that lowering cholesterol concentration might have neurochemical consequences. One explanation for this may be that lowering cholesterol concentration causes changes in the cholesterol content of the synaptosomal membrane and a decrease in the number of serotonin receptors.[5] Because a low serotonin concentration has been associated with suicidal depres-