Lack of Relationship of Glycated Haemoglobin with Lipids in Non-Insulin Dependent Diabetics with Proteinuria*

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ABSTRACT

Cardiovascular morbidity and morality is higher in diabetics with proteinuria. The causative factors for such incidence are as yet not known. We studied lipid profiles among non-insulin-dependent diabetics with proteinuria who otherwise have normal blood urea and serum creatinine concentrations and compared the results with those who did not have proteinuria. Twenty four-hour protein estimations were done in the proteinuric group. Glycated haemoglobin (GHb) was estimated in all subjects and the Pearson's correlation coefficients were calculated between GHb and the lipid fractions. Total cholesterol (TC) and triglycerides (TG) were higher and HDL cholesterol concentrations were lower in diabetics with proteinuria but the statistical significance was obtained only for TG in women on sexwise analysis. Diabetics without proteinuria (n=53): TC= 222.6 ± 6.5, TG = 196.1 ± 15.7, HDLC : 52.6 ± 2.3; diabetics with proteinuria (n = 36) : TC = 230.8 ± 7.4; TG = 218.4 ± 16.0; HDLC = 48.0 ± 2.6 (all data are mean ± S.E. and mg/dl). The correlations of GHb to lipid fractions were not significant in diabetics with proteinuria. Neither GHb nor lipids showed significant association with 24-hr protein excreted in the proteinuria group. We conclude that the lipid profiles do not bear a relationship to the glycaemic control once proteinuria manifests.

INTRODUCTION

Cardiovascular morbidity and mortality are higher in diabetics than in non diabetics [1]. An accelerated atherogenic process and hyperlipidaemias accompany diabetes [2]. Coronary heart disease is an important cause of death in diabetics with proteinuria even before the onset of end stage renal disease [3]. Microalbuminuria and proteinuria predict mortality in diabetes [4,5]. The underlying pathogenetic mechanisms for such high mortality are not clear. Recent studies have reported hyper lipoproteinaemia in insulin-dependent diabetics with proteinuria [6,7]. Although NIDDM is the commonest form of diabetes in our country, the effects of early nephropathy on lipids in non insulin-dependent diabetics is much less studied. A few studies on renal function in NIDDM focused mainly on the renal albumin excretion [8,9]. To our knowledge there are no studies on lipid components in NIDDM with proteinuria.

Glycated haemoglobin (GHb) is a chronobiochemical record of the glycaemic status of the preceding 8-12 weeks. With progression of renal impairment, hypoalbuminaemia takes precedence in causing hyperlipidaemias [10]. We, therefore postulate that the relationships of GHb with lipids may be different in those with proteinuria compared to those without proteinuria. With this background, we studied, the glycaemic control and lipid profiles in NIDDs with proteinuria who otherwise had normal renal function.

MATERIAL AND METHODS

Eighty nine NIDDs above the age of 30 years, confirmed diabetics after an oral glucose tolerance test according to the diagnostic criteria of WHO formed the subjects for this study [11]. The selection criteria were as follows: The sampling was done at random and included only those diabetics who had proteinuria with normal blood urea and serum creatinine concentrations, no clinical evidence of any other renal disease and a normal body mass. 24-hr urine protein was also estimated in them. Subjects with disorders like liver disease, renal failure and endocrine disease known to interfere with lipid levels were excluded. Another 53 NIDDs without proteinuria and fulfilling all the other criteria served as controls.

Fasting venous blood samples were collected in the morning after an overnight fast. Blood glucose and urea concentrations were estimated on the autoanalyser (Technicon instruments, Tarrytowen, NY). GHb was estimated using the method of Fluckiger and winterhalter [12]. Total Cholesterol was measured by the procedure of Zlatkis

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et al [13] and triglycerides by the method of Mendez et al [14]. High density lipoprotein cholesterol (HDLC) was estimated by polyanion precipitation method [15]. Low density liprprotein cholesterol (LDLC) was calculated using the formula of Freidwald et al [16]: LDLC = TC – (HDLC + TG/5). Proteinuria was confirmed using sulphosalicylic acid test. Twenty four-hour urinary protein was estimated in the proteinuric group using turbidimetirc method and serum creatinine was measured on the basis of Jaffe's reaction [17]. Body mass index (BMI) was calculated as weight in Kg/height in square meters. Blood pressure was recorded in the left upper arm in the sitting position and hypertension was diagnosed if systolic blood pressure was ≥ 160 mm of Hg and/or diastolic BP ≥ 95 mm of Hg. Ophthalmic examination was done after full dilation of the pupils and retinopathy was recorded according the criteria described elsewhere [18].

RESULTS

The demographic data and BMI of diabetics without and with proteinuria are shown in Table 1. The diabetic women with proteinuria had higher BMI than those without proteinuria ($t = 6.08$, $P < .01$), while the all other general characteristics were similar.

Table 2 shows the fasting blood glucose, GHb and lipid profiles in diabetics without and with proteinuria. Statistical comparisons between the diabetics without proteinuria and with proteinuria did not show significant difference except in the women with proteinuria . The women with proteinuria (n = 15) had significantly higher TG concentrations, compared to women without proteinuria (n = 22, 249.2 ± 26.6 vs 182.3 ± 19.9 mg/dl, $P = 2.11$, $p < .05$).

Table 1
Demographic data and BMI

	Without Protenuria			With Protenuria		
	Male	Female	Total	Male	Female	Total
Age	44.6 ± 2.2 (n=31)	47.0 ± 1.2 (n=22)	45.6 ± 1.0 (n=53)	48.8 ± 1.6 (n=21)	45.5 ± 1.9 (n=15)	47.4 ± 1.3 (n=36)
B.M.I	22.4 ± 0.4 (n=31)	21.4 ± 0.5 (n=22)	22.0 ± 0.3 (n=53)	21.6 ± 0.6 (n=21)	26.4 ± 0.7 (n=15)	23.6 ± 0.6 (n=36)

Table 2
Lipids in NIDDs with proteinuria*

Group & Gender	Fasting blood Glucose	GHb	Total Cholesterol	triglycerides	High density lipoprotein cholesterol	Low density lipoprotein cholesterol
(n)	mg%	%	mg%	mg%	mg%	mg%
Without Proteinuria (n=53)	205.5 ± 10.3	12.5 ± 0.55	222.6 ± 6.52	196.1 ± 15.67	52.6 ± 2.31	129.7 ± 5.99
men (n=31)	188.6 ± 16.59	12.6 ± 0.79	219.1 ± 8.79	205.9 ± 23.02	52.9 ± 3.09	123.2 ± 7.68
women (n=22)	229.4 ± 16.39	12.3 ± 0.72	227.6 ± 9.65	182.3 ± 19.89	52.2 ± 3.49	138.9 ± 9.40
With proteinuria (n = 36)	197.6 ± 10.09	12.0 ± 0.47	230.8 ± 7.36	218.44 ± 16.04	48.0 ± 2.61	137.4 ± 7.7
men (n = 21)	200.6 ± 13.19	12.9 ± 0.68	225.5 ± 9.66	196.4 ± 18.93	44.0 ± 3.69	140.2 ± 9.14
women (n=15)	193.6 ± 16.09	10.7 ± 0.44	238.3 ± 11.39	249.2 ± 26.59	53.1 ± 3.26	131.3 ± 10.89

* all data expresses as mean ± S.E.M. and comparisons made between diabetics without proteinuria and with proteinuria.
** compared tto women with proteinuria, $t = 2.11$, $p < 0.05$.

The coefficients of correlation [19] between GHb and lipids are shown in Table 3. Strong relationship was found between GHb and FBG and between TC and LDLC in both the groups. In

diabetics with proteinuria, the following correlations between the parameters were significant in men: FBG vs TG ($p < 0.001$), GHb vs TC ($p < 0.05$), GHb vs TG ($p < 0.001$), TV vs TG ($p < 0.05$); and in women :FBG vs HDLC ($p < 0.01$), GHb vs TC ($p < 0.02$). In the group with proteinuria none of the correlations to lipids were significant except the one between TC and HDLC (0.5) in men .

The 24 hr urinary protein in mg in the 36 diabetics with proteinuria was 499.6 ± 66.4 (468.8 ± 63.5 in men (n = 21) and 542.8 ± 134.7 in women (n = 15). In the group without proteinuria , blood urea (in mg/dl) was 25.3 ± .7 (n =53); 25.4 ± 0.9 in men (n=31) and 25.0 ± 1.0 in women (n=22). Serum, creatinine (in mg/dl) was 1.1 ± .05 (n=53); 1.1 ± .35 in men (n = 31) am d1.1 ± .08 in women (n=22). In diabetics with proteinuria blood urea was 23 ± 1.3 in men (n=21) and 21.9 ± 1.2 (n=15) in women. Serum creatinine was 0.9 ± 0.4 (n=36); 0.9 ± 0.6in men (n=21) and 0.9 ± 0.3 in women (n = 15). The concentrations of blood urea and serum creatinine were not different between the groups without and with proteinuria. Thirty and 21% of those without it were detected to have hypertension. Diabetic retinopathy was found in 19% of the subjects with proteinuria and 4% of those without protenuria.

Table 3
Correlation of GHb to lipids in NIDDs with proteinuria

Without Proteinuria (n=53) / With Proteinuria (n=36)	Fasting Blood Glucose (mg/dl)	GHb %	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	High Density Lipoprotein Cholesterol (mg/dl)	Low Density Lipoprotein Cholesterol (mg/dl)
FBG						
Men		0.842(0.001)	0.478(0.01)	0.587(0.001)	0.42(NS)	0.145(NS)
Women		0.760(0.001)	0.336(NS)	0.026(NS)	0.541(01)	0.133(NS)
Both		0.740(0.001)	0.425(0.01)	0.335(0.02)	0.236(NS)	0.185(NS)
GHb						
Men	0.626(0.001)		0.391(0.05)	0.524(NS)	0.06(NS)	0.1(NS)
Women	0.587(0.02)		0.492(0.02)	0.069(NS)	0.419(NS)	0.379(NS)
Both	0.566(0.001)		0.417(0.01)	0.361(0.01)	0.179(ns)	0.186(NS)
TC						
Men	0.393(NS)	0.236(NS)		0.431(0.05)	0.223(NS)	0.761(0.001)
Women	0.383(NS)	0.598(0.01)		0.03(NS)	0.392(NS)	0.868(0.001)
Both	0.376(0.05)	0.241(NS)		0.29(0.05)	0.284(05)	0.802(0.001)
TG						
Men	-0.005(NS)	0.335(NS)	0.136(NS)		-0.27(NS)	-0.107(NS)
Women	-0.059(NS)	0.003(NS)	0.094(NS)		-0.146(NS)	-0.339(NS)
Both	-0.039(NS)	0.087(NS)	0.150(NS)		0.027(NS)	-0.199(NS)
HDLC						
Men	0.113(NS)	-0.129(NS)	0.455(0.05)	0.092(NS)		-0.165(NS)
Women	0.188(NS)	-0.007(NS)	-0.04(NS)	-0.173(NS)		-0.092(NS)
Both	0.093(NS)	-0.194(NS)	0.31(NS)	0.183(NS)		-0.063(NS)
LDLC						
Men	0.393(NS)	0.212(NS)	0.8(0.001)	-0.288(NS)	-0.022(NS)	
Women	0.166(NS)	0.076(NS)	0.665(0.001)	0.326(NS)	-0.074(NS)	
Both	0.246(NS)	0.228(NS)	0.724(0.001)	-0.337(NS)	-0.128(NS)	

NS= non significant; the value are coefficients of correlation and those in the parentheses represent statistical significance (p value)

None of the Pearson's coefficients calculated between the 24 hr protein and each of the other parameters were significant (n = 36; men = 21, women = 15). The values were: Protein vs FBG: 0.139; 0.155 in men, 0.148 in women; Protein vs GHb: - 0.158; -0.053 in men, -0.146 in women; Protein vs TC: 0.086; 0.47 in men , -0.101 in women; Protein vs TG: 0.258;0.098 in men, 0.349 in women; Protein vs HDLC : 0.067; 0.319 in men , -0.24 in women; Protein vs LDLC : 0.059; -0.116

in men and -.034 in women respectively. All data expressed in results are mean ± SEM.

DISCUSSION

Hypertriglyceridaemia is an important lipoprotein abnormality at the early stage of renal impairment (20). In the present study, we did not observe any differences in the concentrations of TC,HDLC and LDLC between diabetics with and without proteinuria. TG concentrations are higher in diabetics with proteinuria and they were statistically significant in women. This may be due to the higher body mass of women. This may be due to the higher body mass of women with proteinuria. Alternately, proteiniuria has an effect on TG metabolism. There are no similar studies in NIDDs for comparing our observations. The present study differs from many others in that it was done in those without evidence of renal failure. Winocour et al [6] reported higher TC and lower HDLC concentration in insulin-dependent diabetics with proteinuria. A study by Vanini et al [7] in IDDM with albuminuria and with normal creatinine clearance noted increased TG, TC and LDLC levels. Elkeles et al found lower HDLC/LDLC ratio in women with proteinuria in insulin requiring diabetics [21]. The earliest clinical sign of nephropathy is proteinuria [22]. Higher protein excretion is associated with higher cardiovascular morbidity after accounting for the effects of BP in the diabetic [8]. Even in non-diabetics, proteinuria poses a risk of early mortality [23]. Relative morality due to the underlying cardiovascular disease is higher in women than in men. Borch-Johnsen and Kreiner found sex as an important variable in diabetics with proteinuria and the relative mortality as 2.6 times higher in women [4]. Since we aimed to look at the lipid profile in diabetics, it was not possible to look at the morbidity and mortality patterns. Further studies in large sample data are required for this problem. However our results and those of Borch-Johnson and Kreiner's corroborative evidence support that proteinuria is an important factor to mediate the alterations in lipids as observed here. This is furthermore supported by the correlations between GHb and lipids which was examined to determine whether the alterations in lipids were secondary to glycaemia or are of different origin (vide infra).

There have been several studies on the relationships [24-44] between metabolic control and lipids. Majority of the studies observed a positive association of GHb with TC and TG [25,27,30,31]. The observations on the association of GHb and with HDLC is not clear with reports of positive [33], negative [29,32] and no association [26].

The results in the present study in NIDDM without proteinuria showed relationships between GHb and TC and TG and no relationship with HDLC. This is in accordance with many previously published reports. In diabetics with protenuria. GHb did not correlate with TC, TG and HDLC, i.e., the relationships of GHb to lipids are not similar in diabetics with and without proteinuria. As sex differences in lipid profiles is documented [34], we analysed the coefficients sex wise. Except GHb versus TC, in both groups of women with and without proteinuria, all other correlations were non significant. Thus, overall a lack of relationships of GHb and lipids, in diabetics with proteinuria, is found. Usitupa et al [24] found a lack of correlation between glycaemia and TG in femal NIDDs. Lack of a significant relationship of GHb to the concentrations of TC, TG and HDLC was noted by Semenkovich et al [35] in black women with IDDM in the lipid profiles in women. All these observations lend much support to our postulate that glycaemia is not the any key factor in deranging lipoprotein metabolism.

Surprisingly, none of the parameters of carbohydrate and lipid derangements correlated with the amount of protein excreted in 24 hrs in diabetics with proteinuria. Vannini et al [7] observed that in IDDS there were no significant correlations between serum lipids and the amount of proteinuria. They also found higher TC, TG, LDLC and lower HDLC in patients with albuminuria and without renal failure. Our results in NIDDS are consonant with their findings. Bending et al [37] observed that long term improvement in glycaemia had no influence on clinically mainifested proteinuria in diabetics . Our results should not be interpreted to conclude that glycaemic control is unimportant in early nephropathy, as it is increasingly appreciated now, that the benefits of good metabolic control and management are manifold.

REFERENCES

1. Kannel WB, Mc Gee DL. Diabetes and cardiovascualr disease. The Farmingham study. J Am. Med. Assoc. 1979;24: 2035-38.
2. Robertson WB, Strong JP. Atherosclerosis in persons with hypertension and diabetes mellitus. Lab. Inv. 1968;18: 531-51.
3. Moloney A, Tunbridge WMG, Ireland JT, Watkins PJ. Mortality from diabetic nephropathy in the United Kingdom. Diabetologia 1983;25: 26-30.

4. Borch-Johnsen K, Kreiner S. Proteinuria : value are predictor of cardiovascular mortality in insulin dependent diabetes mellitus. Br.Med. J. 1987;294: 1651-54.

5. Jarret RJ, Viberti GC, Argyropoulous A, Hill RD, Mahmud U, Murrels TJ, Microalbuminuria predicts mortality in non insulin dependent diabetes. Diabetic Med. 1984;1: 17-19.

6. Winocour PH, Durrington PN, Ishola M, Anderson DC, Cohen H. Influence of proteinuria on vascular disease, blood pressure and lipoproteins in insulin-dependent diabetes mellitus. Br. Med .J. 1987;1: 1648-51.
7. Vanini P, Ciavarella A, Flammini M, et al Lipid abnormalities in insulin-dependent diabetic patients with albnuminuria. Diab. Care 1984;7:151-4.

8. Mattock MB, Keen H, Viberti GC, el Gohari Mr, Murrels TJ, Scott GS, Wing JR, Jackson PG. Coronary heart disease and urinary albumin excretion rate in type 2 (non-insulin dependent) diabetic patients. Diabetologia 1988; 31: 82-7.

9. Jensen T, Stender S, Deckert T, Abnormalities in Plasma concentrations of lipoproteins and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased albumin excretion. Diabetologia 1988; 31: 142-5.
10. Lewis B. (ed) The hyperlipidemias: Clinical and laboratory practice, Blackwell Scientific Publications, Oxford 1976;292.

11. World Health Organisation Expert Committee on diabetes mellitus, Third report, WHO Tech Rep Ser No. 727, 1985.

12. Fluckiger R, Winterhalter KH. Glycosylated hemoglobins. In Biochemical and Clinical aspects of hemoglobin abnormalities. Caughey WS (ed). Academy Press, New York 1978.

13. Zlatiks A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Med 1953; 4: 486-92.
14. Mendez J, Fankin B, Gahagan H. Simple manual procedure for determination of serum triglycerides. Clin Chem 1978; 21: 768-70.
15. Burstein M, Scholnicko HR, Morfin HR, Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions, J Lip Res 1970;11 : 583-95.
16. Friedwald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density cholesterol in Plasma without use of the preparatory ultra centrifuge. Clin Chem 1972; 18: 499-502.

17. Varley AH, Gowelock aH, Bell M. (eds). Practical Clinical Biochemistry Vol. I. William Heineman Publishers, London 1980; 596.
18. Jarret RJ, Keen H, Grabauskas V. The WHO multinational study of vascular disease in diabetes: General description . Diab Care 1979; 2: 175.
19. Snedector GW, Cochran WG. (eds). Statistical methods. Sixth edition, Oxford and IBH Publ. New Delhi, 1967.
20. Norbeck HE, Carlson LA. The uremic dyisipoproteinemia: its characteristics and relations to clinical factors. Acta Med Scand 1981; 209: 489-503.

21. Elkeles RS, Wu J, Hambley J. Hemoglobin A_1, blood glucose and high density lipoprotein cholesterol in insulin requiring diabetics. Lancet 1978; 2: 547-8.

22. Kussman MJ, Goldstein HH, Cleason PE. The clinical course of diabetic nephropathy. J Am Med Assoc 1976; 236: 1861-1863.
23. Abuelo JG. Protrinuria: Diagnostic principles and procedures. Ann Intern Med 1983; 98: 186-91.

24. Uusitupa M, Siitonen O, Voutilainen E, et al serum lipids and lipoprotein in newly diagnosed non insulin dependent (Type II diabetic patients, with special reference to factors influencing HDL cholesterol and Triglyceride levels. Diab Care 1986; 9: 17-22.
25. Peterson CM, Koenig RJ, Jones RL, Saudek CD, Cerami A. Correlation of serum triglyceride levels and hemoglobin A1C concentrations in Diabetes mellitus. Diabetes 1977; 26: 507-9.
26. Kennedy AL, Lappin TRJ, Avery TD, Hadden DR, Weaver JA, Montgomery DAD. Relation of high density lipoprotein cholesterol concentration to type of diabetes and its control Br. Med J 1978; 2: 1191-94.
27. Lisch HJ, Sailer S. Lipoprotein patterns in diet, in sulphonylurea and insulin treated diabetics. Diabetologia 1981; 20: 118-22.

28. Glasgow AM, August GP, Hung W. Relationship between control and serum lipids in Jyvenile onset diabetes. Diab Care 1981; 4: 76-80.

29. Calvert GD,Graham JJ, Mannik T, Wise PH, Yeates RA. Effects of therapy on Plasma high density lipoprotein cholesterol concentration in diabetes mellitus. Lancet 1978; 2: 66-8.
30. Aleyassine H, Gardiner RJ, Tonks DB, Koch P. Glycosylated hemoglobin in diabetes mellitus: Correlations with fasting plasma glucose, serum lipids and glycosuria. Diab care 1980; 3: 508-14.

31. Simpson RW, Mann JI, Hockaday TDR, Hockaday JM, Turner RC, Jelfs R. Lipid abnormalities in untreated maturity – onset diabetics and the effects of treatment . 1979; 16: 101-6.

32. Lopes-Virella MFL, Stone PG, Clowell JA. Serum high density lipoprotein in diabetic patients , Diabetologia 1977; 13: 285-91.

33. Klujber L, Malnar D, Kardos M, Jaszai V, Soltesz G, Mestyan J. Metabolic control , glycosylated hemoglobin and high density lipoprotein cholesterol in diabetic children. Eur J Pediatr 1979; 132: 289-97.

34. Walden CE, Knopp RH, Wahl PW, Beach KW, Strandness E. Sex differences in the effect of diabetes mellitus on lipoprotein, triglyceride and cholesterol concentrations. N.Engl. J. Med. 1984;311: 953-9.
35. Semenkovich CF, Ostlund RE, Schechtman KB. Plasma lipids in patientsx with type l diabetes mellitus. Arch Int Med 1989;149: 51-6.

36. Kodali VE. Lipid correlations wtihhemoglobin A1 in Asian Indian diabetics. Arhc Int Med 1991;151:1227.

37. Bending JJ, Viberti GC, Watkins PH, Keen H. Intermittent clinical proteinuria and renal function in diabetes: Br Med J 1986; 292: 83-6.