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Relation of Abnormal Composition of Lipoproteins to HbA_{1c} Levels in Non-Insulin Dependent Diabetes

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Plasma lipids and VLDL and HDL composition were studied in a control group of 20 non diabetic subjects and in 31 male middle-age patients with non-insulin dependent diabetes treated by oral hypoglycemic agent glibenclamide and a weight maintaining diet. Data for the diabetics were separated based on haemoglobin A_{1c} of less or greater than 7 %. VLDL composition abnormalities were more frequent in the diabetic patients with HbA_{1c} of > 7 %. VLDL-cholesterol, VLDL-triglycerides and VLDL-phospholipids were high in all diabetics whereas VLDL-apo B increased only in diabetics with HbA_{1c} > 7 %. Apo CII and apo CIII levels and also apo CII/apo CIII ratio were also reduced in the diabetic patients with HbA_{1c} levels of more than 7 %. Increases in the apo E and apo E/apo C ratio were also seen in the more hyperglycemic diabetics with HbA_{1c} levels > 7 %. In contrast apo CII and apo CIII levels and also Apo CII/Apo CIII ratio remained unaltered in diabetic patients with less than 7 % HbA_{1c} levels. In these patients increases in the apo E levels were found while the apo E/apo C ratio remained unaltered. All diabetic patients showed increases in HDL-triglycerides and triglyceride/total cholesterol ratio with respect to control. Decreases in HDL-apo AI were also seen in both groups of diabetics, but the HDL-apo AI/HDL-apo AII ratio did not differ from control.

Key words: HbA_{1c}, Lipoproteins, Apoproteins, Diabetes type II.

Abnormalities in serum lipoproteins are frequent and may contribute to the increased incidence of atherosclerosis in diabetic patients (3). Most commonly, triglyceride-rich lipoproteins are high (7) and HDL may decrease (2).

It is generally the rule that more moderate degrees of hypertriglyceridaemia due to accumulation of VLDL in plasma

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respond to a tightening of diabetic control (20). In addition in most published reports a good correlation of HDL composition with the control of diabetes was lacking, because levels of glucose are not often a valid indicator of metabolic control in adults due to the changes in renal threshold associated with diabetic nephropathy.

Glycated Hb is being used with increased frequency to monitor long-term blood glucose control in diabetes mellitus (8). This is the basis for the present report, which investigates the modifications of plasma lipids and VLDL and HDL lipoprotein composition male diabetic patients with $HbA_{1c} < 7$ % or $AbA_{1c} > 7$ % treated by the same oral hypoglycemic agent glibenclamide and a weight maintaining diet. Hence all the patients were well matched with regard to hypoglycemic agent and diet.

Materials and Methods

Thirty one diagnosed male patients from 50-60 years old with non-insulin dependent diabetes mellitus (Type II diabetes), selected by using a table of random numbers from a diabetic population (Instituto de Diabetología, Madrid, Spain), took part in this study. They all consented to participate voluntarily in this study. The diabetic patients were tested for factors which have an effect on lipid metabolism (5). Each person was interviewed by an experienced registered nurse about physical activity, diet, smoking habits, alcohol consumption and whether drugs affecting lipid metabolism were being taken. Age, height, weight and blood pressure were also recorded.

Table I shows that the three studied groups, normal subjects and both of the diabetic groups were well-matched with regard to age, % of light obese subjects according to their body max index (BMI) calculated as weight (kg) divided by height (m) (19); % of light smokers (10-15 cigarettes day) as described previously (5), % of light drinkers (daily ethanol intake lower than 40 g) according to WHO criteria (28) and % of subject with a moderate physical activity according to WHO criteria (27). Diastolic blood pressure was lower than 95 mm Hg (25).

Further the patients were divided in two groups according to their HbA_{1c} levels, less or more than 7 %. A 7 % of glycated haemoglobin as a cut off point was established according to the data reported in a previous work (23).

The patients were treated by diet and were administered 15 mg of the oral hypoglycemic agent glibenclamide, distributed in three doses per day.

Table I. *Percentage of men with moderate physical activity, consuming alcohol or cigarettes, age and Body Max Index (BMI) of control and diabetic patients.*
Values are mean ± SD; n = number of subjects.

		Diabetic patients	
	Control (n = 20)	$HbA_{1c} < 7$ % (n = 14)	$HbA_{1c} > 7$ % (n = 17)
Age	55.2 ± 0.5	54.8 ± 1.3	55.9 ± 0.7
BMI (Kg/m^2)	25.8 ± 0.3	26.0 ± 0.2	26.6 ± 0.6
Smokers (10-15 cigarettes/day)	42.0	47.4	45.4
Alcohol intake (< 40 g/day)	25.0	30.0	27.0

The diabetic patients received a weight maintaining diet providing 55 % of the calories as carbohydrate, 30 % as fat (10 % of polyunsaturated fatty acids, 10 % of monounsaturated fatty acids and 10 % of saturated fatty acids) 15 % as protein and 30 g/day as fibre.

Blood was collected after 10-12 h fasting in EDTA. VLDL and HDL lipoproteins were isolated from plasma according to procedures described by HAVEL *et al.* (10). Plasma samples were centrifuged in a Beckman model L8M ultracentrifuge using a type SW 50.1 rotor.

Triglyceride, phospholipid and total cholesterol levels in plasma and in the isolated VLDL and HDL lipoproteins fractions were quantified using Enzymatic Color Kits (Boehringer Mannheim, GmbH).

VLDL-Apoproteins levels were measured by polyacrilamide gel electrophoresis. Equal volumes of VLDL and tetramethylurea were mixed and centrifuged. After determination of the protein content in the supernatant (17), 0.1 mg of the soluble VLDL-Apoprotein were applied to polyacrilamide gel columns (c = 7.5 %) containing 8 mol/L urea and electrophoresis was carried out in order to determine the apoproteins (apo) CII, CIII and E according to the method of KANE (14). The resulting bands were stained with Coomassie R-250 Brilliant Blue, (0.25 % in acetic acid) and destained in 7 % acetic acid. The dye uptake was determined by scanner integrator 570 nm Preference Sebia Atom.

VLDL-apo B and HDL-apo AI, and HDL-apo AII levels were measured using the immunoturbidimetric automatized method standarized by Technicon Laboratory in an Autoanalizer Technicon Ra-500.

Glycosilated haemoglobin A_{1c} was separated by isoelectric focusing of erythrocyte haemolysates using the method of JEPPSON *et al.* (13), with light modifications (22). The relative concentration of HbA_{1c} was expressed as a percentage of the total haemoglobin.

Lipid and Apo quality control was carried out according to the Laboratory Manual of the Lipid Research Clinics Program (16). The interassay variation coefficients were: Total cholesterol 3 %, Triglycerides 3.4 %, Phospholipids 2.6 %, HDL-cholesterol 7.3 %, HDL_2-cholesterol 6.8 %, HDL_3-cholesterol 5.9 %, apo Al 7.2 %, apo AII 6.9 %, apo B 8.4 % and HbA_{1c} 7.2 %. The recovery rate of total cholesterol, triglycerides and phospholipids in the isolated VLDL, LDL and HDL particles, ranged from 99 % to 100 %. A recovery rate of apo B was also found in the isolated VLDL particles ranging from 99 % to 100 %.

Statistical analysis was performed using the Mann-Withney "U" test.

Results

Table II data demonstrate that triglyceride levels rose in both groups of diabetic patients with respect to control. In addition, triglyceride levels were also higher in diabetic with HbA_{1c} levels of > 7 % than in diabetics with HbA_{1c} < 7 %. Decreases in HDL_2-cholesterol and HDL_3-cholesterol levels were only seen in the diabetic patients with HbA_{1c} levels of more than 7 %.

Both groups of diabetic patients, table III, showed increases in VLDL-cholesterol, VLDL-triglycerides and VLDL-phospholipids with respect to control while VLDL-apo B and cholesterol/

Table II. *Glucose concentrations (mg/dl) HbA_{1c} levels (%) and Total cholesterol (TC), HDL2-cholesterol (HDL2-C), HDL3-cholesterol (HDL3-C), Triglycerides (Tg) and Phospholipids (Ph) concentrations (mmol/L) of the control and male diabetic patients with $HbA_{1c} < 7$ % or $HbA_{1c} > 7$ %.*
Data are mean values ± SEM. n = number of subjects. Statistical analysis was performed using the Mann Whitney "U" test.

		Diabetic patients	
	Control (n = 20)	$HbA_{1c} < 7$ % (n = 14)	$HbA_{1c} > 7$ % (n = 17)
Glucose	82.12 ± 1.15	140.27 ± 6.80[a]	192.20 ± 8.80[ab]
HbAlc	5.83 ± 0.12	6.52 ± 0.15[a]	8.10 ± 0.23[ab]
TC	5.27 ± 0.10	5.68 ± 0.16	5.73 ± 0.36
HDL2-C	0.56 ± 0.02	0.57 ± 0.03	0.40 ± 0.04[ab]
HDL3-C	0.80 ± 0.03	0.77 ± 0.05	0.57 ± 0.05[ab]
Tg	0.80 ± 0.03	1.23 ± 0.13[a]	1.57 ± 0.16[ab]
Ph	2.54 ± 0.05	2.60 ± 0.12	2.58 ± 0.21

[a] $p < 0.010$ comparing vs control.
[b] $p < 0.010$ comparing diabetic patients with $HbA_{1c} > 7$ % vs diabetic patients with $HbA_{1c} < 7$ %.

Table III. *Plasma VLDL composition of the control and male diabetic patients with $HbA_{1c} < 7$ % or $HbA_{1c} > 7$ %.*
VLDL-lipid concentration (mmol/L) VLDL-apos concentration (mg/dL). Data are mean ± SD. n = number of subjects. Statistical analysis was performed using the Mann Whitney "U" test.

		Diabetic patients	
	Control (n = 20)	$HbA_{1c} < 7$ % (n = 14)	$HbA_{1c} > 7$ % (n = 17)
VLDL-Cholesterol	0.62 ± 0.03	0.83 ± 0.14[a]	1.35 ± 0.28[ab]
VLDL-Tryglycerides	0.53 ± 0.04	0.67 ± 0.03[a]	0.71 ± 0.02[a]
VLDL-Phospholipids	0.31 ± 0.02	0.49 ± 0.05[a]	0.58 ± 0.17[a]
VLDL-Apoprotein B	4.25 ± 0.64	4.88 ± 0.35	9.62 ± 0.34[ab]
Cholesterol/Tiglycerides ratio	1.17 ± 0.20	1.24 ± 0.18	1.90 ± 0.10[bc]
Apoprotein CII	1.09 ± 0.26	1.12 ± 0.24	0.48 ± 0.02[bc]
Apoprotein CIII	4.51 ± 0.47	4.72 ± 0.15[a]	2.94 ± 0.28[ab]
Apoprotein E	1.38 ± 0.16	11.72 ± 0.15[a]	1.74 ± 0.22[a]
Apoprotein E/Apoprotein C ratio	0.23 ± 0.03	0.29 ± 0.04	0.50 ± 0.12[ab]
Apoprotein CII/Apoprotein CIII ratio	0.24 ± 0.05	0.24 ± 0.04	0.16 ± 0.02[ab]

[a] $p < 0.010$ comparing vs control.
[b] $p < 0.010$ comparing diabetic patients with $HbA_{1c} > 7$ % vs diabetic patients with $HbA_{1c} < 7$ %.

triglycerides ratio increased only in the diabetic patients with HbA_{1c} levels of more than 7 %.

These patients with $HbA_{1c} > 7$ % had also a high apo E/apo C ratio but a lowered apo CII/apo CIII ratio compared to control. In contrast, apo E/apo C and apo CII/apo CIII ratios from diabetic patients with HbA_{1c} levels of less than 7 % did not differ significantly from control.

Table IV. *Plasma HDL composition of the control and male diabetic patients with HbA_{1c} < 7 % or HbA_{1c} > 7 %.*
HDL-lipid concentration (mmol/L) HDL-apos concentration (mg/dL). Data are mean ± SD. n = Number of subjects. Statistical analysis was performed using the Mann-Whitney "U" test.

		Diabetic patients	
	Control (n = 20)	HbA_{1c} < 7 % (n = 14)	HbA_{1c} > 7 % (n = 17)
HDL-Cholesterol	1.38 ± 0.03	1.37 ± 0.10	1.26 ± 0.07
HDL-Tryglycerides	0.13 ± 0.02	0.20 ± 0.03[a]	0.30 ± 0.03[ab]
HDL-Phospholipids	1.15 ± 0.04	1.05 ± 0.04[a]	0.94 ± 0.09[ab]
Tiglycerides/Cholesterol ratio	0.09 ± 0.01	0.14 ± 0.04[a]	0.24 ± 0.02[ab]
Apoprotein AI	140.51 ± 3.80	122.50 ± 1.92[a]	123.40 ± 6.03[a]
Apoprotein AII	37.80 ± 0.76	36.25 ± 0.70	34.54 ± 1.50
Apoprotein AI/Apoprotein AII ratio	3.72 ± 0.39	3.38 ± 0.40	3.57 ± 0.52

[a] $p < 0.010$ comparing vs control.
[b] $p < 0.010$ comparing diabetic patients with HbA_{1c} > 7 % vs diabetic patients with HbA_{1c} < 7 %.

Furthermore cholesterol/triglyceride ratio were increased in diabetic patients with higher HbA_{1c} levels compared to diabetics with lower HbA_{1c} levels. Increases in the apo E/apo C ratio were also seen in diabetics with HbA_{1c} of more than 7 % compared to diabetics with HbA_{1c} of less than 7 %. apo CII/apo CIII ratio decreased in diabetic patients with HbA_{1c} > 7 % with respect to diabetics with HbA_{1c} < 7 %.

Both groups of diabetic patients, Table IV, showed increases in HDL-triglycerides and triglycerides/cholesterol ratio with respect to control. Decreases in HDL-apo AI were also seen in both groups of diabetic patients compared to control, while HDL-apo AII and HDL-apo AI/HDL-apo AII ratio did not differ significantly from control.

Discussion

In this work, plasma triglycerides were high in both groups of diabetic patients with respect to control and in diabetics with HbA_{1c} levels of > 7 % compared to diabetics with HbA_{1c} < 7 %. These results were in agreement with those found previously (6, 7, 20).

In agreement with the findings of ISHIBASHI *et al.* (12) in the patients with HbA_{1c} > 7 % a lowered significant apo CII/apo CIII ratio was found. In addition these patients had a high apo E/apo C ratio, elevated cholesterol/triglyceride ratio and increased apo B in VLDL, which are assumed to reflect a tendency to raise the concentration of remnant particles (11). This accumulation of remnant particles implies an alteration in the catabolic pathway of triglyceride-rich-lipoproteins. One explanation may be the altered distribution of apo C in VLDL, favouring a reduced activity of lipoprotein lipase (26). Another possibility is an altered turnover rate of apo B (24). In normal subjects remnant particles are rapidly removed by the splachnic region, but in the insulin-resistant state, this uptake is significantly reduced (15). Remnant particles are capable of crossing the capillary endothelial barrier (21) and can bind to specific receptors at the smooth muscle cells (1). Their

uptake by peripheral tissues could provide an increased supply of cholesterol to those peripheral tissues (9) and may contribute to increased cardiovascular risk in diabetes.

In contrast, diabetic patients with HbA_{1c} levels of less than 7 % showed an improvement in the apo composition. An analysis of soluble VLDL-apo reported similar composition between these diabetic patients and control subjects.

The apo CII/apo CIII ratio and apo E/apo C ratio were also not significantly different from those of the controls. In addition VLDL-apo B and cholesterol/-triglyceride ratio were also not significantly different. These results may be associated with a better clearance of VLDL. The improvement in the catabolic pathway of triglyceride-rich lipoproteins could reflect a lowering of the remnant particle concentrations and decreased cardiovascular risk in diabetic patients with HbA_{1c} levels of less than 7 %.

In agreement with the findings of SCHONFELD *et al.* (24) both groups of diabetic patients showed enriched triglyceride HDL.

Our data suggesting that HDL from diabetic patients was enriched in triglycerides support the finding of NILSSON-EHLE (18) that type II diabetes is associated with increases in HDL-triglycerides although the amount of HDL particles remains unaffected.

On the other hand both groups of diabetic patients showed decreases in HDL-apo AI, while HDL-apo II and HDL-apo AI/HDL-apo AII did not differ from control. CASTELLI *et al.* (4) found decreased levels of HDL-apo AI in diabetic patients.

The presented data demonstrate that HDL_2-cholesterol and HDL_3-cholesterol plasma levels were only decreased in diabetic patients with HbA_{1c} levels of > 7 % compared to control. These data suggest the existence of impaired catabolism of triglyceride rich lipoproteins in this group of diabetic patients as described before. These results allow us to point out the usefulness of HbA_{1c} determination and it could be a reference value of the lipid metabolism status in Type II diabetic patients, treated by the oral hypoglycemic agent glibenclamide and a weight maintaining diet.

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Se estudian los niveles de lípidos y la composición de las VLDL y HDL plasmáticas en varones varones no diabéticos (control, n = 20) y en pacientes diabéticos no insulino dependientes (n = 31) de mediana edad tratados con un hipoglucemiante oral glibenclamide y dieta para mantener un peso adecuado. Los pacientes diabéticos se subdividieron en dos grupos en función de que el porcentaje de hemoglobinas glicosiladas HbA_{1c} fuese inferior o superior al 7 %. Las alteraciones de la VLDL son más frecuentes en los pacientes diabéticos con HbA_{1c} > 7 %. Las VLDL-colesterol, VLDL-triglicéridos y VLDL-fosfolípidos se incrementan en todos los pacientes diabéticos, mientras que la VLDL-Apo B se incrementa sólo en los diabéticos con HbA_{1c} > 7 %. Estos pacientes presentan también disminuidos los niveles de apo-CII, apo-CIII y la relación apo-CII/apo-CIII junto con un incremento en la apo-E y en la relación apo-E/apo-C. No obstante la concentración de apo-CII y apo-CIII y la relación apo-CII/apo-CIII permanecen inalteradas en los pacientes diabéticos

con niveles de HbA_{1c} menores al 7 %. En estos pacientes se observan incrementos de apo-E, mientras que la relación apo-E/apo-C no se altera. Todos los diabéticos presentan aumentada la concentración de HDL-triglicéridos y la proporción triglicéridos/colesterol total con respecto al control. En ambos grupos de diabéticos se observa un descenso de HDL-apo AI aunque la relación HDL-apo AI/HDL-apo AII no difiere del grupo control.

Palabras clave: HbA_{1c}, Lipoproteínas, Apoproteínas, Diabetes Tipo II.

References

1. Bierman, E. L., Eisenberg, S., Stein, Y. *et al.* (1973): *Biochim. Biophys. Acta*, 329, 163-169.
2. Biesbroeck, R. C., Albers, J. J., Wahl, P. W., Weinberg, C. R. *et al.* (1982): Diabetes, 31, 126-130.
3. Betteridge, D. (1989): *Diabetic. Med.*, 6, 195-218.
4. Castelli, W. P., Garrison, R. J. and Wilson, P. W. F. (1986): *J. Am. Med. Assoc.*, 256, 2835-2842.
5. Cuesta, C., Sánchez-Muniz, F. J., García-La Cuesta, A. *et al.* (1989): *Atherosclerosis*, 80, 33-39.
6. Fontobone, A., Eschwegw, E. and Cambien, F. (1989): *Diabetología*, 32, 300-304.
7. Gibbson, G. F. (1986): *Clin. Sci.*, 71, 477-486.
8. Goldstein, D. E. Walker, B. and Rawlings, S. S. (1980): *Diabetes Care*, 3, 503-510.
9. Goldstein, J. L., Ho, Y. K., Brown, M. S. *et al.* (1980): *J. Biol. Chem.*, 255, 1839-1848.
10. Havel, R. J., Eder, H. A. and Bragden, J. H. (1955): *J. Clin. Invest.*, 34, 1345-1353.
11. Howard, B. V., Abbot, W. G. H., Beltz, W. Z. *et al.* (1987): *Metabolism*, 36, 870-877.
12. Ishibashi, S., Yamada, N., Shimano, H. *et al.* (1989): *Clin. Chem.*, 35, 808-812.
13. Jeppson, J. O., Franzen, B. and Nilson, K. O. (1978): *Science Tools*, 25, 69-72.
14. Kane, J. P. (1973). *Anal. Biochem.*, 53, 350-364.
15. Kissebah, A. H., Alfarsi, S. and Vydelingum, N. (1979): *Clin. Res.*, 27, 79l-707.
16. Lipid Research Clinic Program. (1974). In "Publication (NIH) US Department of Health Education and Welfare, eds.): Manual of Laboratory Operation. Lipid and Lipoprotein Analysis. Vol. 1. Bethesda, pp. 75-628.
17. Lowry, O. H., Rosebrough, N. J., Far, A. L. *et al.* (1951): *J. Biol. Chem.*, 193, 265-268.
18. Nilsson-Ehle, P. (1987): In "Druggs affecting lipid metabolism" (Paoletti, R., Kritchevsky, D. and Holmes, W. L., eds.). Springer-Verlag. Berlin. pp. 83-87.
19. Owen, O. E. (1988): In "Nutrition and Metabolism in Patient Care. Obesity" (Rinney, J. M., eds.). Saunder Co. P. Philadelphia, P. A. pp. 167-192.
20. Pfeifer, M. A., Brunzell, J. D., Best, J. D. *et al.* (1983): *Diabetes*, 32, 525-531.
21. Reichl, D., Myant, N. B., Pflug, J. J. *et al.* (1977). Clin. Sci. Mol. Med., 53: 221-226.
22. Sánchez-Cabezudo, M., Martín, M.C. and Ródenas, S. (1987): *An. Real Acad. Farm.*, 53, 103-114.
23. Sánchez-Cabezudo, M. (1990): Tesis Doctoral. Facultad de Farmacia. Universidad Complutense de Madrid.
24. Schonfeld, G., Birge, L., Miller, J. P. *et al.* (1974): *Diabetes*, 23, 827-834.
25. Stamler, J. (1972): Preventive Cardiology. The National Cooperative Pooling Project in the United States, Stockholm. pp. 42-53.
26. Taskinen, M. R. and Nikkila, E. A. (1979): *Diabetología*, 17, 351-356.
27. WHO (1975). In "Energy and Protein requeriments". FAO Nutr. Meetings Rep. Ser N° 52. WHO TECH: Rep. Ser. N° 522, Geneve.
28. WHO (1980): In "Problemas relacionados con el consumo de alcohol". Informe de un Comité de Expertos de la WHO. Serie de Informes Técnicos. 650, Geneve.