Elevation of Plasma High Density Lipoprotein-Cholesterol in Spontaneously Diabetic Chinese Hamsters

MASAAKI ETO, KIYOSHI WATANABE, YASUNORI IWASHIMA,
AKIZUKI MORIKAWA, TAKAAKI TAKEBE and KANEO ISHII

*The Second Department of Internal Medicine, Asahikawa
Medical College, Asahikawa 078-11*

ETO, M., WATANABE, K., IWASHIMA, Y., MORIKAWA, A., TAKEBE, T. and ISHII, K. *Elevation of Plasma High Density Lipoprotein-Cholesterol in Spontaneously Diabetic Chinese Hamsters.* Tohoku J. exp. Med., 1984, 144 (3), 281-290 — The aim of this study is to define the change of plasma high density lipoprotein-cholesterol (HDL-C) in the spontaneously diabetic Chinese hamsters in the Asahikawa colony (CHA). These animals were divided into two groups according to fasting plasma glucose level; non-diabetic group and diabetic group. Plasma HDL-C was measured by a μ l-scale ultracentrifugal method using an RPL-42T rotor (Hitachi Koki Co.). The diabetic hamsters had hypoinsulinemia and hyperlipidemia. Plasma HDL-C in the diabetic group was significantly elevated as compared with the non-diabetic group. A significant positive correlation was observed in both groups between plasma HDL-C and total cholesterol. The male hamsters tended to have higher plasma HDL-C and total cholesterol levels than the female hamsters in either group. Moreover, an electrophoretic analysis showed that there was some relative increase in plasma very low density lipoprotein, low density lipoprotein and high density lipoprotein (HDL) in the diabetic hamsters. An ultracentrifugal analysis showed that plasma chylomicron appeared only in the diabetic hamsters. The heterogeneity of particle size of HDL was found by gradient gel electrophoresis. The apparent average molecular weight of HDL was approximately 265,000 in either group. It is concluded that plasma HDL-C increased with the advance of hyperglycemia and hyperlipidemia in insulin-deficient diabetic hamsters and that there was sex difference in the hamsters for plasma HDL-C and total cholesterol. ——— high density lipoprotein-cholesterol; Chinese hamster; diabetes mellitus; hyperlipoproteinemia; sex difference

In recent years attention has been focused on high density lipoprotein-cholesterol (HDL-C) as a possible anti-atherogenic factor. Many accumulated data have shown that plasma HDL-C level in diabetic patients is highly variable

Received for publication, February 21, 1984.

A preliminary report of this study was presented at the 11th Congress of the International Diabetes Federation (IDF) in Nairobi, November, 1982.

(Nikkilä 1981). On the other hand, with respect to experimental diabetic animals it is well known that streptozotocin-induced diabetic rats have hyperlipidemia and hyperlipoproteinemia, i.e. elevated levels of very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) (Schein et al. 1971; Bar-On et al. 1976a, b). Furthermore, Chobanian et al. (1974, 1982) showed that there was also hyperlipidemia in spontaneously diabetic Chinese hamsters which were insulin-deficient, non-obese diabetic animals (Gerristen and Dulin 1967). However, there is little knowledge about the change of plasma lipoproteins in the diabetic hamsters and sex difference in Chinese hamsters for plasma lipids and lipoproteins.

The aim of this study is, therefore, to define the change of plasma lipoproteins, especially HDL-C, in spontaneously diabetic Chinese hamsters and to determine whether there is sex difference in Chinese hamsters for plasma lipids and HDL-C.

ANIMALS AND METHODS

Animals

115 mature Chinese hamsters in the Asahikawa colony (CHA) (Funaki and Mikamo 1983; Watanabe et al. 1983) were studied. As shown in Table 1, these animals were divided into two groups according to fasting plasma glucose (FPG) level; non-diabetic group and diabetic group. No significant difference in age was observed between the two groups. The body weight of the diabetic group was significantly ($p < 0.001$) decreased as compared with the non-diabetic group and there was significant ($p < 0.005$) sex difference in either group for the body weight. The diabetic hamsters were confirmed by KETO-DIASTIX (Miles Laboratories Inc., Elkhart, Indiana) not to have ketouria. These hamsters were fed normal laboratory chow (MF, Oriental Kobo Co., Tokyo) and given water ad libitum. They were kept under constant laboratory conditions: 14 hr illumination, from 5 a.m. to 7 p.m.; temperature, $23 \pm 2^\circ\text{C}$; and humidity, 50–60%.

TABLE 1. *Age, body weight, fasting plasma glucose level and non-fasted plasma insulin level in Chinese hamsters*

Group	Sex	Number	Age (months)	Body weight (g)	Fasting plasma glucose (mg/100 ml)	Insulin ($\mu\text{U/ml}$)
Non-diabetic	Male	41	8.0 ± 0.2	41.6 ± 0.6	61 ± 2	127.0 ± 14.7
	Female	35	7.9 ± 0.2	38.0 ± 1.0	63 ± 2	132.6 ± 22.8
	Total	76	7.9 ± 0.2	40.3 ± 0.6	62 ± 2	128.6 ± 12.2
Diabetic	Male	18	8.1 ± 0.4	$36.4 \pm 0.9^*$	$413 \pm 11^*$	$5.9 \pm 0.4^*$
	Female	21	7.9 ± 0.3	$32.0 \pm 1.0^*$	$419 \pm 10^*$	$6.8 \pm 1.3^*$
	Total	39	8.0 ± 0.3	$33.8 \pm 0.7^*$	$416 \pm 9^*$	$6.3 \pm 0.6^*$

Values are mean $\pm$ S.E.

* $p < 0.001$ vs. non-diabetic; † $p < 0.005$ between male and female.

Fig. 1. Polyacrylamide disc gel electrophoresis of the lipoprotein fractions separated by a μ l-scale ultracentrifugation at $d=1.063$. No cross-contamination was observed between the top (left) and bottom (right) fractions.

Measurement of plasma glucose, lipids and insulin

Blood samples were collected in mg/ml EDTA from the orbital venous plexus after an approximately 16-hr overnight fast and plasma were separated by centrifugation. Fasting plasma triglyceride (TG) (Bucolo and David 1973), phospholipids (PL) (Takayama et al. 1977), free fatty acids (FFA) (Takahashi et al. 1975) and total cholesterol (TC) (Allain et al. 1974) were measured enzymatically. Fasting plasma glucose was measured by the glucose oxidase method (Teller 1956). Non-fasted plasma insulin was measured by the plain charcoal method of radioimmunoassay (Asano et al. 1975).

Measurement of plasma HDL-C

Fasting plasma HDL-C was measured by a μ l-scale ultracentrifugal method. 90–100 μ l of plasma samples were adjusted to a density of 1.063 by adding the same volume of KBr solution ($d=1.120$) to 230 μ l polycarbonate tube (0.23 PC tube, Hitachi Koki Co., Tokyo). The adjusted samples were centrifuged in a Hitachi Model 65P-7 ultracentrifuge using an RPL-42T rotor (radius 10.85 cm, tube $\times$ 72, Hitachi Koki Co.) at $160,000 \times g$ and 16°C for 3 hr. The bottom fraction was collected for determination of cholesterol. Cholesterol (HDL-C) was measured enzymatically. This procedure made it possible to analyze very small quantities of many samples by means of the ultracentrifuge. As shown in Fig. 1, the absence of other classes of lipoproteins was verified by polyacrylamide disc gel electrophoresis (Narayan 1966) using samples pre-stained with 1% Sudan Black B in ethylene glycol. The intraassay and interassay coefficients of variation ($n=10$) for HDL-C were 2.5% and 3.7%, respectively. There was a highly significant correlation ($r=0.937$, $p<0.01$) between the human plasma HDL-C values measured by this ultracentrifugal method and by the phosphotungstate- MgCl_2 precipitating method.

Separation and average molecular weight determination of HDL

HDL ($d=1.063$ – 1.210) was separated from the pooled plasma in each group by the method of Havel et al. (1955). It was carried out in a Hitachi Model 65P-7 ultracentrifuge using an RP-55 rotor (Hitachi Koki Co.). The separated HDL was dialyzed against 0.15 M NaCl solution with 0.001 M EDTA, pH 8.0. The dialyzed HDL was applied on gradient gel

Fig. 2. Gradient gel (PAA 2/16) electrophoresis of whole plasma pre-stained with 1% Sudan Black B from non-diabetic (left) and diabetic (right) Chinese hamsters.

electrophoresis to determine apparent average molecular weight. Electrophoresis was carried out on a Pharmacia Electrophoresis Apparatus GE-2/4 loaded with gradient gel PAA 4/30 (Pharmacia Fine Chemicals, Uppsala, Sweden) at 120 V and 10°C for 15 hr in a Tris/borate buffer (90 mM Tris HCl, 80 mM borate, 2.5 mM EDTA, PH 8.4). The gel was stained for protein with 1% Coomassie Blue R-250 in 25% methanol and 10% acetic acid for 10 hr and destained with 10% acetic acid and 30% methanol for 24 hr. The migration distances were measured from the top of the gel to the middle of the stained bands. The apparent average molecular weight was measured from the migration distances of the HDL and the protein markers which had been plotted on a semi-logarithmic chart. Calibration kits for high molecular weight determination (Pharmacia Fine Chemicals) were used as the protein markers.

Analysis of whole plasma lipoprotein patterns

100 μ l of freshly prepared plasma was mixed with 50 μ l of 1% Sudan Black B in ethylene glycol and 25 μ l of 40% sucrose solution. After overnight incubation at 4°C in a dark room, dyes in excess were removed by centrifugation at 2,000 rpm for 5 min. 20 μ l of the supernatant was applied on the gradient gel PAA 2/16 (Pharmacia Fine Chemicals) electrophoresis. It was carried out in the same condition as described above. Moreover, chylomicron, VLDL (d 1.006), LDL (d 1.006-1.063) and HDL (d 1.063-1.210) were separated as standard lipoproteins from the pooled plasma by the method of Havel et al. (1955). Chylomicron, which appeared only in the diabetic hamsters, was separated by ultracentrifugation at 10,000 $\times$ g for 30 min. These lipoproteins were pre-stained and applied on the gradient gel PAA 2/16 electrophoresis in the same condition as described above. Thus, VLDL (part of chylomicron), LDL and HDL bands were confirmed on the gel (Fig. 2). They were clearly separated according to their particle size, i.e. molecular weight. This procedure using gradient gel PAA 2/16 electrophoresis is thought to be useful for analysis of whole plasma lipoprotein patterns.

Statistical analysis

Statistical analysis was performed with Student's *t* test. All values were expressed as mean $\pm$ s.e..

RESULTS

Non-fasted plasma insulin level was significantly ($p < 0.001$) lower in the diabetic group than in the non-diabetic group (Table 1). Plasma TG, PL and FFA levels were significantly ($p < 0.001$) higher in the diabetic group than in the non-diabetic group (Table 2). There was no sex difference in the diabetic or non-diabetic group for insulin, TG, PL and FFA. Plasma TC level was significantly ($p < 0.001$) higher in the diabetic group than in the non-diabetic group (Table 2). In the non-diabetic group, the male hamsters had significantly ($p < 0.001$) higher TC level than the female hamsters. In the diabetic group, there was also a tendency of the same sex difference, but it was not significant.

Plasma HDL-C and HDL-C/TC ratio are shown in Table 2. Plasma HDL-C level was significantly ($p < 0.001$) higher in the diabetic group (89 ± 3 mg/100 ml) than in the non-diabetic group (58 ± 1 mg/100ml). In the diabetic group, the male hamsters had significantly ($p < 0.001$) higher HDL-C level than the female hamsters. In the non-diabetic group, there was also a tendency of the same sex difference, but it was not significant. HDL-C/TC ratio was significantly ($p < 0.001$) lower in the diabetic group than in the non-diabetic group. In the non-diabetic group, the female hamsters had significantly ($p < 0.001$) higher HDL-C/TC ratio than the male hamsters. However, there was no sex difference

Fig. 3. Correlation between plasma HDL-C and total cholesterol in both non-diabetic and diabetic groups.

TABLE 2. Plasma lipid and high density lipoprotein-cholesterol (HDL-C)

Group	Sex	Triglyceride (mg/100 ml)	Phospholipids (mg/100 ml)
Non-diabetic	Male ($n=41$)	66 ± 6	204 ± 6
	Female ($n=35$)	68 ± 10	201 ± 8
	Total ($n=76$)	67 ± 5	203 ± 6
Diabetic	Male ($n=18$)	$465 \pm 64^*$	$388 \pm 20^*$
	Female ($n=21$)	$449 \pm 51^*$	$372 \pm 21^*$
	Total ($n=39$)	$456 \pm 39^*$	$379 \pm 14^*$

Values are mean $\pm$ s.e.

* $p < 0.001$ vs. non-diabetic; † $p < 0.001$ between male and female.

Fig. 4. Gradient gel (PAA 4/30) electrophoresis of HDL from non-diabetic (right) and diabetic (middle) Chinese hamsters. Calibration kit for high molecular weight determination is shown on the left; a: thyroglobulin (molecular weight 669,000), b: ferritin (440,000), c: catalase (232,000), d: LDH (140,000), e: albumin (67,000).

in the diabetic group for HDL-C/TC ratio.

A significant ($p < 0.01$) positive correlation was observed between plasma HDL-C and TC in both diabetic and non-diabetic groups (Fig. 3).

In the gradient gel electrophoresis the migration distances were determined according to the particle size, i.e. molecular weight. The heterogeneity of the particle size of HDL was found (Fig. 4). The apparent average molecular weight was approximately 265,000 in either group.

Whole plasma lipoprotein patterns are shown in Fig. 2. A gradient gel electrophoretic analysis showed that plasma LDL scarcely existed in the Chinese hamster and that there was some relative increase in plasma VLDL, LDL and HDL in the diabetic hamsters. In addition, an ultracentrifugal analysis showed that plasma chylomicron appeared only in the diabetic hamsters.

levels, and HDL-C/total cholesterol ratio in Chinese hamsters

Free fatty acids (mEq/liter)	Total cholesterol (mg/100 ml)	HDL-C (mg/100 ml)	HDL-C/total cholesterol ratio (%)
0.79±0.05	118± 4	60±2	50±1
0.74±0.06	90± 2	55±2	62±2
0.76±0.04	105± 3	58±1	56±1
1.26±0.16*	250±16*	101±3*	42±2*
1.57±0.20*	211±13*	79±3*	40±2*
1.40±0.13*	229±12*	89±3*	41±2*

DISCUSSION

Our observation of elevated levels of plasma lipids in the diabetic hamsters as compared with the non-diabetic controls agree with the previous reports by Gerristen and Dulin (1967), and Chobanian et al. (1974, 1982), who found hyperlipidemia in the diabetic hamsters in the Upjohn colony.

On the other hand, there are few studies on plasma lipoprotein levels in the Chinese hamsters. Chobanian et al. (1982) reported that the major increases occurred in the $d < 1.006$ fraction in the diabetic hamsters. However, other characterization of the lipoproteins had not been performed in the hamsters in the Upjohn colony partly because plasma volume obtained was too small. Now, our μ -scale ultracentrifugal method made it possible to analyze very small quantities of plasma samples. By this method, we have shown that plasma HDL-C in the diabetic hamsters was significantly elevated as compared with the non-diabetic hamsters. In addition, we have shown some relative increase in plasma VLDL, LDL and HDL, and the appearance of plasma chylomicron in the diabetic hamsters.

Bar-On et al. (1976a) reported that sucrose-fed, streptozotocin-induced diabetic rats had elevated levels of plasma lipids and all lipoproteins as compared with the controls. This finding has some resemblance to ours. Plasma lipoprotein pattern of the hamster is thought to be similar to that of the rat because plasma LDL scarcely exists in both animals. Accordingly, in these animals the HDL might carry the major part of the plasma cholesterol-ester (Bar-On and Eisenberg 1978) and all plasma lipoproteins including HDL might be elevated in the diabetic state of these animals.

Since the change of plasma lipoproteins has not yet been clear in diabetes mellitus of humans, this finding of elevation of plasma HDL-C in the diabetic Chinese hamsters is of considerable interest. However, the mechanism by which plasma HDL-C could increase is unknown. The factors which influence plasma HDL-C level are the activities of lipoprotein lipase and hepatic TG lipase, the synthetic and secretory rate in liver and intestine, insulin level, sex hormone level,

diet and exercise (Nikkilä 1981). It is likely that hypoinsulinemia observed in the diabetic hamster may lead to low activity of lipoprotein lipase (Nikkilä et al. 1978), resulting in the increase in chylomicron and VLDL (Schnatz and Williams 1963). In humans, VLDL and HDL levels seem to be reciprocally related, whereas in the hamster or rat both VLDL and HDL increased. It is unknown how both increase simultaneously in these diabetic animals. On the other hand, it was previously reported that inhibition of hepatic TG lipase by an antiserum was followed by elevation of HDL-C (Kuusi et al. 1979) and that the activity of hepatic TG lipase fell in conditions of insulin deficiency or ineffective insulin action (Elkeles and Hambley 1977). In addition, it was reported that a decrease in catabolic rate and an increase in synthetic rate of HDL could contribute to the elevation of plasma HDL-C in the sucrose fed, streptozotocin-induced diabetic rats (Bar-On and Eisenberg 1978) and that there was an increase in HDL in liver perfusion of the diabetic rats (Berry et al. 1981). Bar-On et al. (1976a) also found alterations in VLDL and HDL apoprotein patterns in the diabetic rats. The further studies will be required to clarify the relationship between plasma HDL-C and these factors.

In the present study, it has proved that the male hamsters tend to have higher levels of plasma TC and HDL-C than the female hamsters in either group. The same sex difference in TC was also observed in the rat (Dupont et al. 1978) and in humans (Kritchevsky 1980). In contrast to the result from this study, it is well known in humans that HDL is higher in women than in men (Anderson et al. 1978). Moreover, the sex difference in HDL-C/TC ratio was observed in the diabetic group. The effect of sex hormone on plasma lipids and lipoproteins remains to be elucidated in the Chinese hamsters.

Finally, the heterogeneity of the particle size of HDL was found by gradient gel electrophoresis. The apparent average molecular weight was defined to be approximately 265,000 in either group. The particle size of hamsters' plasma HDL differed from that of human plasma HDL which has 2-3 subfractions (Anderson et al. 1977).

In this study, it is concluded that plasma HDL-C increased with the advance of hyperglycemia and hyperlipidemia in the insulin-deficient diabetic Chinese hamsters and that there was sex difference in the Chinese hamsters for plasma TC and HDL-C.

Acknowledgments

We are grateful to Prot. Kazuya Mikamo and Dr. Kenji Funaki, Department of Biological Sciences, Asahikawa Medical College, for supplying Chinese hamsters, and to Mrs. Keiko Hidano for typing the manuscript.

References

- 1) Allain, C.G., Poon, L.S., Chan, C.S.G., Richmond, W. & Fu, P.C. (1974) Enzymatic

- determination of total serum cholesterol. *Clin. Chem.*, **20**, 470-475.
- 2) Anderson, D.W., Nichols, A.V., Forte, T.M. & Lindgren, F.T. (1977) Particle distribution of human serum high density lipoproteins. *Biochim. biophys. Acta*, **493**, 55-68.
 - 3) Anderson, D.W., Nichols, A.V., Pan, S.S. & Lindgren, F.T. (1978) High density lipoprotein distribution. Resolution and determination of three major components in a normal population sample. *Atherosclerosis*, **29**, 161-179.
 - 4) Asano, T., Sasaki, H., Nishida, K., Okumura, M. & Yuki, M. (1975) The use of plain charcoal to separate free insulin from antibody-bound insulin. A comparison between dextran coated and uncoated charcoal for the adsorption of free insulin. *J. Jap. diab. Soc.*, **18**, 468-476. (Japanese)
 - 5) Bar-On, H., Roheim, P.S. & Eder, H.A. (1976a) Hyperlipoproteinemia in streptozotocin-treated rats. *Diabetes*, **25**, 509-515.
 - 6) Bar-On, H., Roheim, P.S. & Eder, H.A. (1976b) Serum lipoproteins and apoproteins in rats with streptozotocin-induced diabetes. *J. clin. Invest.*, **57**, 714-721.
 - 7) Bar-On, H. & Eisenberg, S. (1978) The metabolic fate of high density lipoprotein (HDL) in the diabetic rat. *Diabetologia*, **14**, 65-69.
 - 8) Berry, E.M., Ziv, E. & Bar-On, H. (1981) Lipoprotein secretion by isolated perfused livers from streptozotocin-diabetic rats. *Diabetologia*, **21**, 402-408.
 - 9) Bucolo, G. & David, H. (1973) Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.*, **19**, 476-482.
 - 10) Chobanian, A.V., Gerristen, G.C., Brecher, P.I. & Kessler, M. (1974) Cholesterol metabolism in the diabetic Chinese hamster. *Diabetologia*, **10**, 595-600.
 - 11) Chobanian, A.V., Arquilla, E.R., Clarkson, T.B., Eder, H.A., Howard, C.F., Regan, T. J., Jr. & Williamson, J.R. (1982) Cardiovascular complication. *Diabetes*, **31**, Suppl. 1, 54-64.
 - 12) Dupont, J., Spindler, A.A. & Mathias, M.M. (1978) Dietary fat saturation and cholesterol and fatty acid synthesis in aging rats. *Age*, **1**, 93-99.
 - 13) Elkeles, R.S. & Hambley, J. (1977) The effect of fasting and streptozotocin diabetes on hepatic triglyceride lipase activity in the rat. *Diabetes*, **26**, 58-60.
 - 14) Funaki, K. & Mikamo, K. (1983) Developmental stage-dependent teratogenic effects of maternal spontaneous diabetes in the Chinese hamster. *Diabetes*, **32**, 637-643.
 - 15) Gerristen, G.C. & Dulin, W.E. (1967) Characterization of diabetes in the Chinese hamster. *Diabetologia*, **3**, 74-84.
 - 16) Havel, R.J., Eder, H.A. & Bragdon, J.H. (1955) The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. clin. Invest.*, **34**, 1345-1353.
 - 17) Kritchevsky, D. (1980) Age-related changes in lipid metabolism. *Proc. exp. Biol. Med.*, **165**, 193-199.
 - 18) Kuusi, T., Tinnunen, P.J.K. & Nikkilä, E.A. (1979) Hepatic endothelial lipase antiserum influences rat plasma low and high density lipoproteins in vivo. *FEBS Letters*, **104**, 384-388.
 - 19) Narayan, K.A., Creinin, H.L. & Kummerow, F.A. (1966) Disc electrophoresis of rat plasma lipoproteins. *J. Lipid Res.*, **7**, 150-157.
 - 20) Nikkilä, E.A. (1981) High density lipoproteins in diabetes. *Diabetes*, **30**, Suppl 2, 82-87.
 - 21) Nikkilä, E.A., Taskinen, M.R. & Kekki, M. (1978) Relation of plasma high-density lipoprotein cholesterol to lipoprotein-lipase activity in adipose tissue and skeletal muscle of man. *Atherosclerosis*, **29**, 497-501.
 - 22) Schnatz, D.J. & Williams, R.H. (1963) The effect of acute insulin deficiency in the rat on adipose tissue lipolytic activity and plasma lipids. *Diabetes*, **12**, 174-178.
 - 23) Schein, P.S., Alberti, K.G.M.M. & Williamson, D.H. (1971) Effect of streptozotocin on carbohydrate and lipid metabolism in the rat. *Endocrinology*, **89**, 827-834.

- 24) Takahashi, J., Fujii, K. & Hayashi, C. (1975) An approach to develop a new enzymatic method for determination of free fatty acid. Preliminary report. *Jap. J. clin. Chem.*, **4**, 179-185. (Japanese)
 - 25) Takayama, M., Itoh, S., Nagasaki, T. & Tanimizu, I. (1977) A new enzymatic method for determination of serum choline-containing phospholipids. *Clin chim. Acta*, **79**, 93-98.
 - 26) Teller, J.D. (1956) Direct, quantitative, colorimetric determination of serum or plasma glucose. In: *Abstracts of Papers, 130th Meeting of Amer. Chem. Soc.*, P 69c.
 - 27) Watanabe, K., Mikamo, K. & K. Ishii (1983) Characterization of diabetic Chinese hamsters in the Asahikawa colony. In: *Diabetic Microangiopathy*, edited by H. Abe & M. Hoshi, University of Tokyo Press, Tokyo, pp. 465-476.
-