Development of Atherosclerosis in Alloxan Diabetic Rats

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Rats with alloxan-induced diabetes developed severe atherosclerotic lesions when they were maintained on a 0.25% cholesterol diet for one year. The atheromatous changes developed at the aortic arch, appeared as early as 3 months after the start of the experiment, and increased thereafter. The diabetic rats also developed atherosclerosis when they were fed standard rat chow, but the area of the atheromatous lesion was about one tenth of that in rats fed the high-cholesterol diet. Normal rats did not develop atherosclerosis even when fed the high-cholesterol diet for one year. The alloxan diabetic rats showed no increase in body weight, but developed serum glucose levels as high as 600-800 mg/dl as well as high serum cholesterol levels and lower serum HDL-cholesterol levels. The development of atherosclerosis in these rats was significantly related to an increase in the serum cholesterol/phospholipid ratio, the atherogenic index (TC-HDL/HDLC), and the serum total cholesterol level, but was not related to the serum glucose, HDL-cholesterol, triglyceride, or lipid peroxide levels. These relationships were found as early as 8-16 weeks after the start of the experiment. These data suggest that the serum cholesterol/phospholipid ratio, the atherogenic index, and the total cholesterol level are important risk factors for the development of atherosclerosis in rats with alloxan diabetes.

Key words: Hypercholesterolemia, Fibrous plaque-like lesion, Lipoproteins, Apolipoproteins

The incidence of atherosclerosis is known to be high in patients with diabetes mellitus (1-9). Disorders of lipid metabolism, hyperglycemia, enhanced platelet function, abnormal blood coagulation, and hormonal imbalances have been suggested as risk factors for the development of atherosclerosis in diabetic patients.

In diabetic animals, elevated of lipid and lipoprotein levels have been reported after feeding with a high cholesterol diet (10-14), but few experiments have succeeded in producing atherosclerosis in rats (15-17), probably because of the difficulty of maintaining diabetic rats for a long period. We recently found that rats with alloxan-induced diabetes could survive for over one year and that these diabetic rats developed marked hypercholesterolemia when fed a high-cholesterol diet (18). When alloxan diabetic rats were maintained for one year on a high cholesterol diet, they developed atherosclerotic changes in the aorta. In the present study, we investigated the development of these atherosclerotic changes in relation to serum lipid, lipoprotein, and glucose levels.

Materials and Methods

Male Sprague-Dawley rats (Jcl-SD, 11 weeks old) were obtained from Japan Clea Ltd. (Tokyo, Japan) and were kept in an air-conditioned room (25±1°C, 50-60% humidity), with free access to rat chow (Japan Clea CA-1 diet) and tap water. The composition of the diet was as follows: 25.5% protein, 4.0% lipids, 53.5% carbohydrate, 4.0% fiber, 7.0% ash, and 6.0% water. The cholesterol content was 0.04-0.05%. A high-cholesterol diet was prepared by adding 0.25% cholesterol to the above standard diet.
Alloxan monohydrate (Tokyo-Kasei Kogyo Co., Tokyo, Japan) was dissolved in saline (20 mg/ml) and a dose of 40 mg/kg was injected intravenously into 12-week-old rats. The serum cholesterol level was determined 3 weeks after the injection and rats showing levels over 100 mg/dl were selected for subsequent experiments.

Normal and alloxan diabetic rats were divided into two groups at the age of 16 weeks; one group was fed the standard diet and the other was given the high-cholesterol diet. Blood (ca. 200 μl) samples were obtained from the orbital sinus at 4-week intervals. Five or six rats from each group were killed at 14, 26, and 51 weeks after the start of the experiment.

These rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and blood was withdrawn from the inferior vena cava. Two % glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) was infused via the left ventricle to fix the aorta. The aorta was dissected from the arch to the bifurcation of the common iliac arteries. Then it was opened tangentially and photographs of the lumen were taken using a Nikon binocular microscope (×6 magnification). The photographs were traced onto transparent plastic sheets, and the total surface area of the aorta and the area of the atheromatous lesions were measured using an image analyzing system (Nippon Avionics, TVIP, Tokyo, Japan). Then the ratio of the atherosclerotic lesion to the total surface area of the aorta was calculated.

Histological investigation of the atherosclerotic lesions was performed at 6 and 12 months. Small pieces of the lesion were excised from the glutaraldehyde-fixed aortas and rinsed in 0.1 M phosphate buffer (pH 7.4) at 4°C. The tissues were post-fixed in 1% osmium tetroxide in the same buffer for 1.5 hours at 4°C, stained en bloc with 3% aqueous uranyl acetate for 1 hour at 4°C, dehydrated through a graded series of ethanols, and embedded in an epoxy resin. For light microscopy, thick Epon-embedded sections were cut by a Reichert Ultracut E (Vienna, Austria) and stained with toluidine blue. For electron microscopy, ultrathin sections were double-stained with uranyl acetate and lead citrate, then examined with a JEOL 100CX electron microscope.

The lipid levels and other parameters studied were measured using the following commercially available kits: total cholesterol by the Determiner-TC 555 kit (Kyowa Medics Co., Tokyo, Japan), free cholesterol by the Determiner-FC 555 kit (Kyowa Medics Co.), triglycerides by the Triglycisme-V Eiken kit (Eiken Co., Tokyo, Japan), phospholipids by the phospholipid B-Test Wako (Wako Pure Chemical Industries, Osaka, Japan), serum HDL-cholesterol by the HDL-cholesterol test Wako (Wako Pure Chemical Industries), serum glucose by the New Blood Sugar Test (Boeringer Mannheim-Yamanouchi, Tokyo, Japan), and the serum lipid peroxide level by the Lipoperoxide-Test Wako (Wako Pure Chemical Industries). The atherogenic index was expressed by total cholesterol–HDL/HDLC.

Table 1. Development of atheromatous lesions in normal and alloxan diabetic rats.

<table>
<thead>
<tr>
<th>Rats</th>
<th>Diet</th>
<th>3 Months</th>
<th>6 Months</th>
<th>12 Months</th>
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<tr>
<td>Healthy</td>
<td>Standard</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Healthy</td>
<td>Cholesterol</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Standard</td>
<td>–</td>
<td>0.03±0.028 (1/5)</td>
<td>3.6±1.42 (5.5)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Cholesterol</td>
<td>3.9±1.12 (6/6)</td>
<td>19.4±6.32 (5/5)</td>
<td>32.2±8.14 (6/6)</td>
</tr>
</tbody>
</table>

nd: Not detected. ( ): No. of rats developing atheromatous lesions. [ ]: Range of the values.

*: Mean ± S.E. (Percentage of atheromatous lesion area to the total aortic area).

Results

The incidence of atheromatous lesions in diabetic rats and the area of the lesions are given in Table 1. Healthy rats did not develop lesions even when fed a high-cholesterol diet for 12 months, but diabetic rats developed lesions within 3 months and the area of the lesions increased as the duration of the high-cholesterol diet increased. Diabetic rats also developed atherosclerotic lesions when fed the standard diet, but the changes were only slight (Fig. 1).

The atherosclerotic lesions that developed in diabetic rats fed a high-cholesterol diet for 6 months were typical fatty streaks. The subendothelial space was predomi-
nantly filled with foam cells derived from macrophages. Well-developed fatty streaks were seen in the aortic arch and the abdominal aorta, consisting of several layers of foam cells in the thickened intima. These foam cells were large cells containing numerous lipid inclusions that filled most of their cytoplasm (Fig. 2). A moderate number of residual bodies containing membrane fragments and myelin forms were seen in most of the foam cells. Smooth muscle cells were rarely observed in the intimal lesions that formed the fatty streaks. The endothelial cells covering the lesions were elongated but expanded locally around the nucleus. The endothelial cells also contained a few lipid droplets. Adherent mononuclear cells were frequently observed on the endothelial surface of the lesions (Fig. 3). Most adherent mononuclear cells were ovoid with short microvilli and an indented nucleus containing coarse, peripherally distributed chromatin. Their cytoplasm routinely contained a few large mitochondria, numerous free ribosomes, and a few lipid droplets. Some of the medial smooth muscle cells beneath the lesions also contained a few lipid droplets.

In the diabetic rats fed a high-cholesterol diet for 12 months, some of the lesions in the aortic arch and abdominal aorta were markedly elevated. These advanced lesions were of the fibroproliferative type, and contained relatively small amounts of lipids (Fig. 4). Lipid-filled foam cells were mainly clustered in the deeper portion of the thickened intima, while a moderate number

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Fig. 1. Electron micrograph of a lesional area in the thoracic aorta of the diabetic rat after 6 months on a standard diet. A lipid-laden macrophage (M) was located in the intimal space. The endothelial cell (EC) also contained a few lipid droplets. Arrowheads indicate some of the lipid droplets. IEL, internal elastic layer. SMC, medial smooth muscle cell. ×3,600.

Fig. 2. Electron micrograph of a fatty streak after 6 months on the cholesterol diet. Large lipid-filled foam cells (FC) were present under the markedly stretched and thinned endothelium (EC). IEL, internal elastic lamina. ×2,200.

Fig. 3. Electron micrograph of a monocyte (M) adherent to an endothelial cell (EC) overlying the lesion after 6 months on the high-cholesterol diet. ×5,500.
of small foam cells were also found just beneath the endothelium. The greater portion of the midsection of these lesions contained randomly distributed intimal smooth muscle cells (Fig. 5). The extracellular matrix surrounding these cells contained numerous collagen fibers and cholesterol crystals. The intimal smooth muscle cells were relatively small and varied in appearance. Some of them appeared to be contractile smooth muscle cells, because of their extensive myofilament bundles and the perinuclear distribution of organelles, while others appeared to be synthetic smooth muscle cells containing well-developed rough endoplasmic reticulum and Golgi apparatus (Fig. 6). Both types of intimal smooth muscle cells contained a few lipid droplets. Adherent mononuclear cells were occasionally observed on the endothelial surface of the fibrous plaque lesions.

Changes in the body weight of the rats which were maintained for 12 months are shown in Fig. 7. The normal rats gained weight continuously during the experiment and the increase was greater in the rats fed a high-cholesterol diet. The diabetic rats, although they ate about twice as much as the normal rats, showed no weight gain, but rather had a slight decrease in body weight.

The alloxan diabetic rats in all 4 groups showed almost constant hyperglycemia of about 700 mg/dl throughout the year, while the control rats had a normal serum glucose level.

Figure 8 shows the serum total cholesterol levels. The cholesterol level increased in the diabetic rats even when they were on the standard diet, reaching 500-1,000 mg/dl. When fed the high-cholesterol diet, cholesterol levels in the diabetic rats increased markedly to a maximum of approximately 4,000 mg/dl at 32 weeks and then declined gradually to about 2,000 mg/dl at 48 weeks due to decreased consumption of the diet. In contrast, normal rats fed a high-cholesterol diet showed almost no increase in serum cholesterol levels. Changes in the serum free and esterified cholesterol levels were almost parallel with that of total cholesterol, suggesting that the percentage of esterified cholesterol remained constant throughout the experiment.

Changes in the serum HDL-cholesterol level are shown in Fig. 9. The HDL-cholesterol level in normal rats on either the standard diet or the high-cholesterol diet increased gradually with age. The level in the diabetic rats decreased within 4 weeks and remained low with only a gradual increase thereafter.

The atherogenic indices did not change in the control rats fed either standard or high-cholesterol diet for 48 weeks, but these indices increased markedly in diabetic rats during the early period on the high-cholesterol diet and remained almost constant during the experiment. This index also increased in the diabetic rats on the standard diet, but the change was much smaller than that in rats on the high-cholesterol diet (Fig. 10).

Serum triglyceride and phospholipid levels remained approximately parallel to the serum cholesterol level, so the cholesterol/phospholipid (C/P) ratios of all the groups were almost constant except for an acute increase in diabetic rats 4 weeks after the high-cholesterol diet was begun (Fig. 11). This ratio was highest in diabetic rats on the high-cholesterol diet and lowest in normal rats on the standard diet.

Changes in the protein and lipid composition of the serum lipoproteins at 12 months is shown in Table 2. Although there were marked fluctuations in the diabetic rats, HDL decreased and other lipoproteins (especially chylomicrons) increased. Cholesterol feeding did not change either the total or relative amounts of proteins and lipids in healthy rats, but it increased the protein and cholesterol content (especially in VLDL and IDL) in diabetic rats. HDL decreased in the diabetic rats on a high-cholesterol diet, but the change was not statistically significant. There were no significant changes in the percentage of any component of lipoproteins in the control rats fed a high-cholesterol diet. In the diabetic rats, the percentage of cholesterol increased, while the percentage of triglyceride decreased. After receiving a high-cholesterol diet, these tendencies in the diabetic rats were remarkable.

The ratios of small soluble apoproteins in VLDL are shown in Fig. 12. Apoprotein E was increased in the diabetic rats and was further increased by the high-cholesterol diet. In contrast, apoprotein C, especially apoprotein C-III, was decreased in diabetic rats.

Apoproteins in HDL were also examined (Fig. 13). Apoprotein A-IV was increased and apoprotein A-IV was decreased in the diabetic rats, but their ratios were similar to those in normal rats receiving a high-cholesterol diet. In contrast, apoprotein E was high in diabetic rats fed the
The correlations between the severity of atherosclerosis and the various biochemical parameters in diabetic rats on the high-cholesterol diet are shown in Table 3. The atherogenic index, serum cholesterol level, and C/P ratio all showed a strong correlation with the severity of atherosclerosis, while the serum glucose, triglyceride, HDL-cholesterol, and lipid peroxide levels showed no correla-
Figure 14 shows the time course of changes in the correlation coefficients for the atherogenic index, serum cholesterol level, C/P ratio, and serum glucose level. Coefficients for the atherogenic index, serum cholesterol, and C/P ratio gradually increased with a longer time on the high-cholesterol diet; significant correlations (p < 0.05) were found at 16 weeks for serum cholesterol and the atherogenic index, and as early as 4 weeks for the C/P ratio. However, there was no significant correlation for the serum glucose level during the observation period.

Discussion

The serum lipid concentrations and the effects of dietary cholesterol on these concentrations differ among animal species (21, 22). Serum cholesterol levels are low in rats and are only slightly increased by dietary cholesterol because of the rapid feedback regulation of cholesterol metabolism in this species (23). Furthermore, the serum level of HDL, which is considered an antiatherogenic factor in humans, is relatively high in rats compared with serum levels of other lipoproteins (21, 22). By contrast, LDL concentration which is considered contributory factor for atherosclerosis is very low. Consequently, atherosclerosis does not develop in rats even when they are fed a high-cholesterol diet over a long period (24-26).

In an attempt to induce hyperlipidemia and vascular lesions in rats, antithyroid drugs, bile acids, or large amounts of saturated fats have been added to the diet together with a high dose of cholesterol, but these methods are not really satisfactory (11, 15-17).

In diabetic rats, however, the serum cholesterol level increases rapidly after consuming cholesterol (10-14). This may be due to increased intestinal absorption of cholesterol (27-30), increased intestinal synthesis of cholesterol (31-33), delayed catabolism of serum lipoproteins due to impaired lipoprotein lipase activity (36-37), a decrease in the receptor affinity of diabetic LDL due to increased glycation (38-40), or hyperphagia associated with the diabetic state.

Wexler reported that vascular lesions did develop in alloxan diabetic rats, although only early fatty plaques appeared to develop (41, 42). The rats used in his experiments were established breeder rats, which had hyperlipidemia, hyperglycemia, and hypertension prior to the induction of severe alloxan-induced diabetes. In the present experiment, aortic atherosclerotic lesions associated with marked hyperlipidemia were induced by feeding diabetic rats with a diet containing a relatively low cholesterol content (0.25%) without using any other...
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Figure 9. Changes in the serum HDL-cholesterol level in normal and alloxan diabetic rats fed either a standard diet (SD) or a high-cholesterol diet (HCD). Each point and bar represents the mean ± SE in 5-6 rats. * Significant difference from normal rats fed a standard diet (p < 0.05). † Significant difference from normal rats fed a high-cholesterol diet (p < 0.05).

Figure 10. Changes in the atherogenic index in normal and alloxan diabetic rats fed either a standard diet (SD) or a high-cholesterol diet (HCD). Each point and bar represents the mean ± SE in 5-6 rats. * Significant difference from normal rats fed a standard diet (p < 0.05). † Significant difference from normal rats fed a high-cholesterol diet (p < 0.05). • Significant difference from diabetic rats fed a standard diet (p < 0.05).

Figure 11. Changes in the cholesterol/phospholipid ratio in normal and alloxan diabetic rats fed either a standard diet (SD) or a high-cholesterol diet (HCD). Each point and bar represents the mean ± SE in 5-6 rats. * Significant difference from normal rats fed a standard diet (p < 0.05). † Significant difference from normal rats fed a high-cholesterol diet (p < 0.05). • Significant difference from diabetic rats fed a standard diet (p < 0.05).
Table 2. Serum lipoprotein compositions in normal and alloxan diabetic rats at 12 months.

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<th></th>
<th>Healthy rats</th>
<th>Alloxan diabetic rats</th>
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<tbody>
<tr>
<td></td>
<td>Standard diet</td>
<td>High-cholesterol diet</td>
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<tr>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Chylomicron</td>
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<tr>
<td>Protein</td>
<td>45.5 ± 4.90</td>
<td>47.4 ± 12.08</td>
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<tr>
<td>Cholesterol</td>
<td>5.4 ± 0.44</td>
<td>6.6 ± 1.22</td>
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<tr>
<td>Triglycerides</td>
<td>31.5 ± 4.67</td>
<td>31.5 ± 9.42</td>
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<tr>
<td>Phospholipids</td>
<td>8.6 ± 0.72</td>
<td>9.1 ± 1.92</td>
</tr>
<tr>
<td>VLDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>147.4 ± 18.38</td>
<td>162.4 ± 36.40</td>
</tr>
<tr>
<td>Protein</td>
<td>14.7 ± 1.28</td>
<td>17.0 ± 2.78</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5.7 ± 0.66</td>
<td>8.4 ± 1.42</td>
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<tr>
<td>Triglycerides</td>
<td>106.9 ± 14.38</td>
<td>113.5 ± 27.05</td>
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<tr>
<td>Phospholipids</td>
<td>20.2 ± 2.61</td>
<td>23.5 ± 5.57</td>
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<td>IDL</td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>26.1 ± 1.30</td>
<td>28.9 ± 4.35</td>
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<tr>
<td>Protein</td>
<td>3.7 ± 0.33</td>
<td>4.1 ± 0.33</td>
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<tr>
<td>Cholesterol</td>
<td>3.9 ± 0.72</td>
<td>5.9 ± 0.53</td>
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<td>Triglycerides</td>
<td>14.1 ± 0.89</td>
<td>13.9 ± 3.46</td>
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<td>Phospholipids</td>
<td>4.4 ± 0.53</td>
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<td>LDL</td>
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<tr>
<td>Total</td>
<td>71.2 ± 12.61</td>
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<td>18.6 ± 3.98</td>
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<td>8.8 ± 0.92</td>
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<tr>
<td>Protein</td>
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<tr>
<td>Cholesterol</td>
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<td>66.0 ± 7.92</td>
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<tr>
<td>Triglycerides</td>
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<tr>
<td>Phospholipids</td>
<td>56.4 ± 5.05</td>
<td>59.3 ± 11.93</td>
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* Mean ± S.E.

b: Significant difference from normal rats fed a high-cholesterol diet (P < 0.05).

c: Significant difference from alloxan diabetic rats fed a standard diet (P < 0.05).

Fig. 12. Composition of soluble VLDL apoproteins in normal and alloxan diabetic rats fed either a standard diet (SD) or a high-cholesterol diet (HCD).

Fig. 13. Composition of HDL apoproteins in normal and alloxan diabetic rats fed either a standard diet (SD) or a high-cholesterol diet (HCD).
lipoproteins became more distinct in rats fed the high-cholesterol diet. In particular, the level of IDL (intermediate metabolites of VLDL) was markedly elevated by the high-cholesterol diet. IDL has been proven to be atherogenic, because patients with Type III hyperlipidemia have increased IDL concentrations and develop vascular lesions as well as xanthomas. Accordingly, diabetic rats can be regarded as susceptible to developing atherosclerosis because of increased IDL and LDL and decreased HDL levels.

IDL contains a relatively large amount of apo E compared with apo C proteins. In general, serum apo E concentrations increase in patients with hyperlipidemia and are well correlated with the serum triglyceride and cholesterol levels (44). Apo E has a high affinity for B/E receptors as well as E receptors (45-47). Lipoproteins or artificial liposomes relatively rich in apo E are more readily taken up by the liver than those with a low content of apo E (48, 49). It has been reported that cholesterol feeding rats elevates the serum apo E concentrations (43, 50), suggesting that this increase is an adaptive reaction which aims to eliminate the excess serum lipids.

Bar-On et al. (51) and O’Looney et al. (52) have reported that VLDL from diabetic rats were deficient in apo E, but that apo C was decreased more strikingly than apo E (52). Other experiments on diabetic rats have indicated that apo E is significantly decreased in HDL, but increased in VLDL, LDL, and the d >1.21 fraction (13).

An increased apo E level has also been reported in spontaneously diabetic rats (53). Including both cholesterol and cholic acid in the diet given for approximately one month to rats with drug-induced diabetes elevated the apo E content of VLDL relative to that of apo C (14). In the present experiment, the proportion of apo E in the apoproteins of VLDL was increased in diabetic rats on the standard diet without cholesterol supplementation, although the increase was only slight. However, in diabetic rats on a high-cholesterol diet, the percentage of apo E rose markedly in both VLDL and HDL. A cooperative effect between cholesterol feeding the diabetic state is considered to induce these high apo E levels.

The net concentration of VLDL proteins was increased 2-fold in diabetic rats fed a standard diet and 7-fold in those fed a high-cholesterol diet. Consequently, the net apo E level in VLDL was estimated to increase about 4-fold and 27-fold, respectively. In contrast, the apo E content of HDL might not have changed, because HDL apoproteins decreased to one half and one fourth of the control level in diabetic rats on a standard diet and on a high-cholesterol diet, respectively.

Some of the discrepancies in the changes of apo E levels noted in various studies of diabetic rats are probably derived from variations in the degree of hyperlipidemia and the duration of cholesterol feeding.

As for apo C-III proteins in VLDL, Bar-On et al. (13) showed that apo C-III, increased and apo C-III, decreased in streptozotocin diabetic rats. They suggested that sialation of apo C-III is stimulated in diabetic rats and that cholesterol feeding further reduces the apo C-IIIb level. However, the true meaning of this change in apo C-III protein subclasses remains unclear.

In this study, the area of the aortic lesions did not
correlate with the serum HDL level but correlated well with the atherogenic index and the C/P ratio from the early period of cholesterol feeding. The reason for the lack of correlation between the area of lesions and the HDL level was not clear. Some differences have been suggested in the metabolism of serum lipoproteins between human and rats. Since LDL uptake by the liver is very active, delivery of cholesteryl ester from LDL to extrahepatic tissues is low (54-57) and cholesteryl ester transfer from LDL to apo B rich-lipoproteins is almost absent in rats (58), the reverse transport role of HDL may not be as important in rats as it is in humans. Furthermore, atherogenic lipoproteins such as IDL and LDL in the diabetic rats fed cholesterol increased about 24-fold and 5-fold compared with those of the control rats, while variations in the HDL level remained in a narrow range.

The C/P ratio also had a very intimate relationship with the area of aortic lesions. LDL uptake by smooth muscle cells in vitro was reported to decrease when the proportion of phospholipids in LDL increased (59). Therefore, an increase in the C/P ratio might stimulate LDL uptake by these cells. The activity of acid cholesterol esterase in the aortic smooth muscle cells was decreased in diabetic rats (60). In addition, it was proven that a decreased percentage of phospholipids in artificial liposomes, which were incubated with smooth muscle cells in vitro, reduced the cellular enzyme activity and might enhance the accumulation of cholesteryl ester in artery wall (61). These results suggest that an increase in the C/P ratio enhances atherosclerotic changes. Further investigation is necessary to elucidate these mechanisms and their clinical significance.

References

(28) Uchida K, Takase H, Kadowaki M, Nomura Y, Matsubara...
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(33) Young NL, Saudek CD, and Crawford SA: Total hydroxyester transfer activity in sixteen vertebrate species. Comp Biochem Physiol, 71B: 265-269, 1982


(41) Mahley RW and Innerarity TL: Lipoprotein receptors and cholesterol homeostasis. Biochim Biophys Acta, 737: 197-222, 1983


(44) Young NL, Saudek CD, and Crawford SA: Total hydroxyester transfer activity in sixteen vertebrate species. Comp Biochem Physiol, 71B: 265-269, 1982