Hypertension Does Not Stimulate the Development of Hypercholesterolemia or Fatty Liver Induced by a High Cholesterol/Cholate Diet in Rats

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A high cholesterol/cholate diet induced hypercholesterolemia and fatty liver in both spontaneously hypertensive rats (SHR) and normotensive control rats (WKY). However, in contrast to previous concepts, the levels of cholesterol ester, triacylglycerol and phosphatidylcholine in plasma as well as triacylglycerol in liver were higher in WKY than in SHR fed a normal diet. The high cholesterol/cholate diet elevated the levels of plasma cholesterol, plasma cholesterol ester and hepatic triacylglycerol, and the extent of elevation was significantly higher in WKY than in SHR. Increases both in monoene/saturated ratios, an indication of elevated Δ^9-desaturase activity, and in linoleate/arachidonate ratios, a possible indication of impaired desaturation-elongation activity, were observed in hepatic and plasma lipids of both strains fed the high cholesterol/cholate diet. The increases in monoene/saturated ratios were similar in both strains, but the increases in the linoleate/arachidonate ratios were higher for the plasma cholesterol esters of WKY than of SHR. The *n*-6/*n*-3 ratios of plasma and hepatic lipids were higher in WKY than in SHR throughout the experiments. These diet-induced changes observed in hepatic and plasma lipids were not reflected in the aortic lipids.

Thus, hypertension *per se* does not promote the development of hyperlipemia and fatty liver induced by a high cholesterol/cholate diet. Our results also suggest that the metabolism of polyenoic fatty acids is different between SHR and WKY.

Keywords high cholesterol diet; spontaneously hypertensive rat; Wistar Kyoto rat; hyperlipemia; hypercholesterolemia; fatty liver

Hypercholesterolemia and hypertension are considered to be important risk factors for atherosclerosis. There are species differences in the responses to dietary cholesterol: in contrast to rabbits, cholesterol feeding alone usually does not induce hypercholesterolemia[1] or atherosclerotic lesions[2] in normotensive rats. However, when atherogenic diets are given together with cholate,[3-5] arterial fat deposition is reported to develop rapidly in spontaneously hypertensive rats (SHR), but not in normotensive control rats (WKY).[6,7] The degrees of hyperlipemia and hepatic lipid accumulation are reported to be higher in SHR than in WKY.[6,8] Consequently, hypertension has been considered to be a major factor contributing to the development of the arterial fat deposition induced by atherogenic diets.[6]

Previously, we have noted elevations of plasma and hepatic cholesterol oleate levels and increases in the linoleate/arachidonate ratios in SHR fed a high cholesterol/cholate diet,[9] and we have investigated the enzymatic basis for the increased cholesterol oleate and triacylglycerol levels.[10] In the study reported here, we have compared the plasma, hepatic, and aortic lipids of SHR and WKY fed a high cholesterol/cholate diet. Contrary to current concepts, the hyperlipemia and fatty liver induced by the diet were more severe in normotensive WKY than in hypertensive SHR.

Materials and Methods

Male SHR and normotensive Wistar Kyoto rats (WKY) at five weeks of age (Charles River of Japan, Kanagawa, Japan) were fed *ad libitum* for up to 45 d either a conventional diet (MF; Oriental Yeast Co., Tokyo) or a diet supplemented with 5% cholesterol and 0.5% cholate (high cholesterol/cholate diet). The MF diet contained, by weight, 24% protein, 5.1% fat, 6.2% minerals, 3.2% fibers, and 54.5% nonnitrogenous compounds supplemented with vitamins D_3 and K_3. The major fatty acid constituents were linoleate (50%), oleate (22%), palmitate (16%), α-linolenate (4%), and stearate (2%).

Tail systolic blood pressure was measured by the plethysmographic tail method with an apparatus produced by Natsume Co. (Tokyo, Japan). From 2 to 4 rats were sacrificed at each point as indicated in the legends to the figures and tables. After fasting for 14 h, rats were sacrificed by decapitation. Then, the livers were excised and the blood samples were collected in a test tube containing ethylenediaminetetraacetic acid (EDTA). Plasmas were separated by centrifugation of the blood samples. The abdominal and thoracic aortas were freed of surrounding fat tissue under light microscopy. Samples were kept frozen at −80 °C. Lipids extracted from liver and plasma with chloroform–methanol mixture were chromatographically separated on silica gel plates (Merck 60, prewashed with developing solvents). Petroleum ether–diethyl ether–acetic acid (80 : 30 : 1, v/v/v) and chloroform–methanol–water (70 : 30 : 5, v/v/v) were used as solvents for separating neutral lipids and phospholipids, respectively. Fatty acids were analyzed as methyl esters by gas liquid chromatography (GLC) using heptadecanoic acid as an internal standard. Positional isomers of octadecenoate were determined as described elsewhere.[11] The amounts of lipids were expressed as mg of heptadecanoic acid per g of wet weight or per dl of plasma. Cholesterol was quantified by GLC as the trimethylsilyl ether using ergosterol as an internal standard.

Glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities in plasma were measured colorimetrically by using commercial assay kits (GOT/GPT kit-S; Nippon Shoji Co., Ltd., Osaka).

Results

The diets fed to SHR and WKY for 45 d did not affect the body weights or blood pressures in either strain: SHR developed a typical hypertension with systolic blood pressures of ~180 mmHg, while no changes in blood pressures were observed with WKY (~100 mmHg).

Symptoms of fatty liver were apparent by the 20th day of the high cholesterol/cholate diet as judged by a fading of liver color to that typical of fatty liver, and the liver weights were about 70% greater at the 45th day in the high cholesterol/cholate groups than in normal diet groups both in SHR and in WKY.

Plasma GOT and GPT activities were increased by feeding the high cholesterol/cholate diet for 20 d. GOT activities were higher by 21% in SHR and by 60% in WKY, while GPT activities were higher by 40% in SHR and by 63% in WKY. Since the administration of CCl_4 induces more that 5- to 10-fold increase in these transaminase

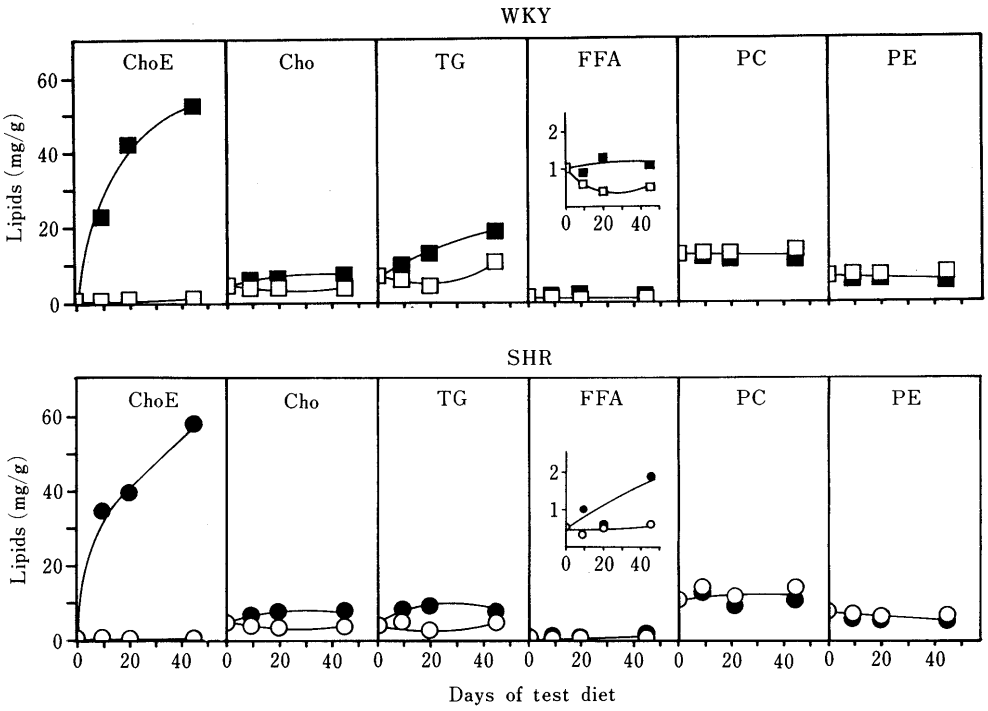

Fig. 1. Effect of a High Cholesterol/Cholate Diet on Hepatic Lipid Contents

Rats ($n=2$) were sacrificed on the indicated days after overnight fasting. Lipids were extracted from plasma with chloroform–methanol and individual lipid components were separated by Silica Gel thin-layer chromatography and quantified as described in the text. Cholesterol ester (ChoE), cholesterol (Cho), triacylglycerol (TG), free fatty acid (FFA), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were determined. The maximal deviations from the means of ChoE, Cho, TG, FFA, PC and PE were 65, 17, 23, 57, 28 and 21% in WKY, and 34, 21, 64, 70, 15 and 18% in SHR, respectively. Normal diet group (open symbols) and high cholesterol/cholate diet group (closed symbols) in WKY (—□—, —■—) and SHR (—○—, —●—) were determined.

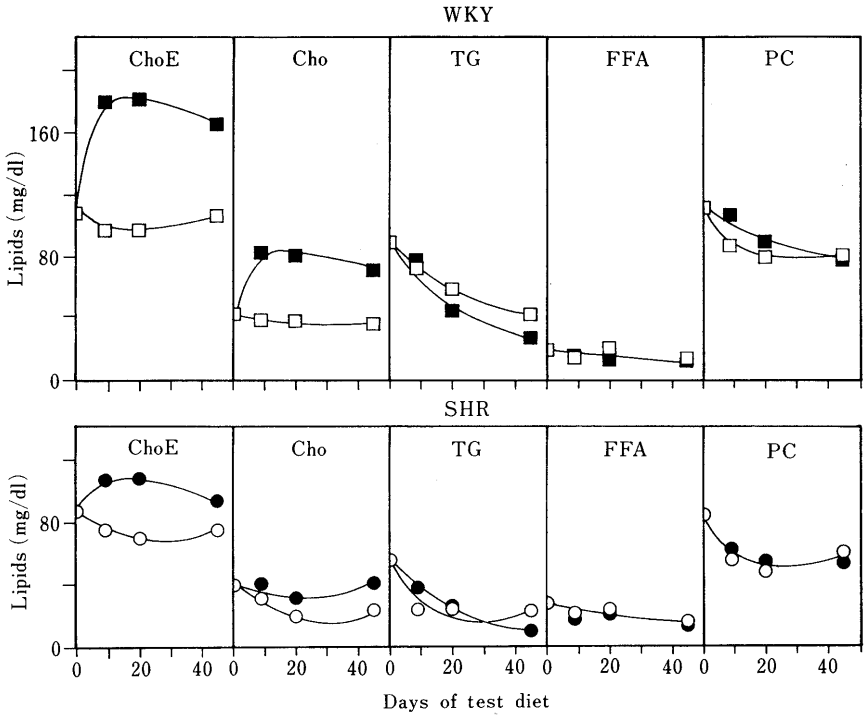

Fig. 2. Effect of a High Cholesterol/Cholate Diet on Plasma Lipid Contents

Lipids were quantified as described in the legend to Fig. 1. The maximal deviations from the means of ChoE, Cho, TG, FFA and PC were 34, 21, 24, 38 and 22% in WKY and 16, 17, 24, 12 and 25% in SHR, respectively. The values for normal diet group (open symbols) and high cholesterol/cholate diet group (closed symbols) in WKY (—□—, —■—) and SHR (—○—, —●—) were determined ($n=2$—4).

activities,[12] the hepatic tissue damage observed in our study was judged to be relatively minor at this stage. The levels of hepatic lipids were followed for up to 45 d under the test diets (Fig. 1).

Hepatic cholesterol ester levels increased approximately 80-fold in rats fed the high cholesterol/cholate diet: free

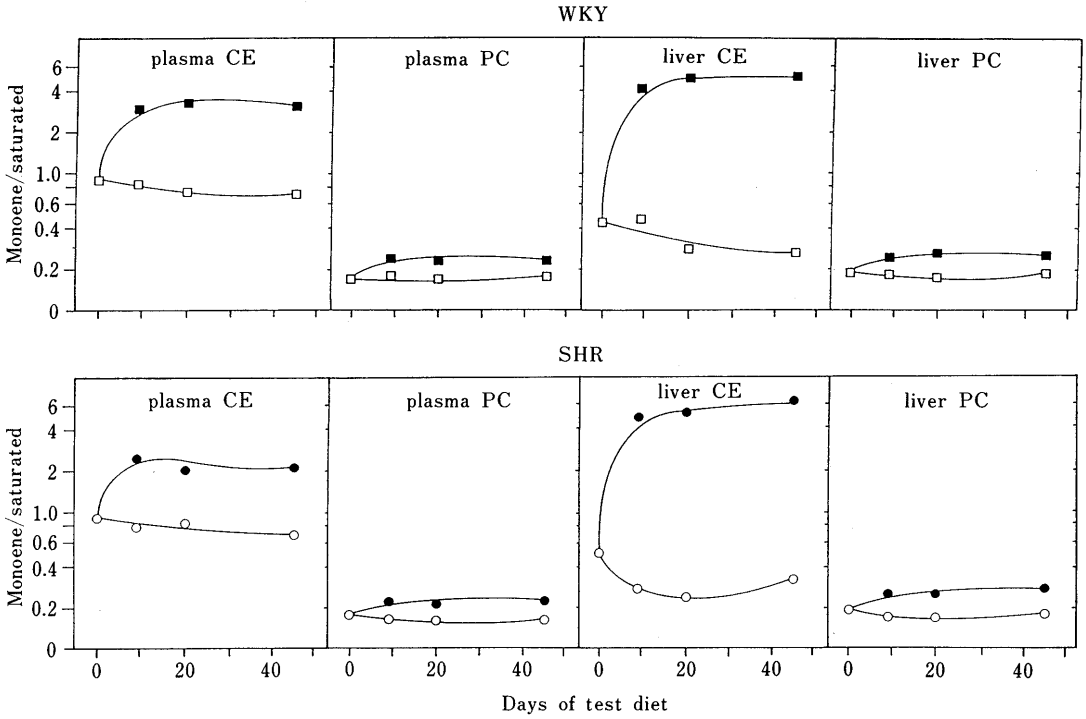

Fig. 3. Effect of a High Cholesterol/Cholate Diet on Monoene/Saturated Fatty Acid Ratios for Cholesterol Ester and Phosphatidylcholine in Plasma and Liver

Lipids were separated and fatty acids were determined as methyl esters by GLC as described in the text, and the ratios of monoene fatty acid (monoene) to saturated fatty acid (saturated) in cholesterol ester and phosphatidylcholine were determined. Averages of two to four separate determinations are presented. Normal diet group (open symbols) and high cholesterol/cholate diet group (closed symbols) in WKY (—□—, —■—) and SHR (—○—, —●—) were determined.

cholesterol, triacylglycerol and free fatty acid levels increased about 2-fold in both strains. However, the levels of phosphatidylcholine and phosphatidylethanolamine were relatively unchanged. The responses of the two strains to the high cholesterol/cholate diet were similar with respect to hepatic lipids, except that the triacylglycerol level in WKY was 2.5-fold higher than that in SHR at the 45th day of the high cholesterol/cholate diet.

Changes in the plasma lipid levels were followed during the 45 d of the test diets (Fig. 2). In contrast to liver where lipid levels more or less increased, the levels of plasma lipids, particularly those of triacylglycerol and phosphatidylcholine, tended to decrease during the 45 d of the test diets. The basal levels of plasma triacylglycerol, phosphatidylcholine and cholesterol ester observed in the normal diet were higher by ~2.3, ~1.5, and ~1.3-fold, respectively, in WKY than in SHR. The high cholesterol/cholate diet elevated plasma cholesterol and cholesterol ester levels, and the increases of plasma cholesterol and cholesterol ester of WKY were 1.8—2.6-fold and 1.7—1.8-fold higher than those of SHR, respectively. In both strains, a more than 100-fold elevation of the hepatic cholesterol ester level was induced by the high cholesterol/cholate diet, but this was not reflected in plasma cholesterol ester levels.

Feeding the high cholesterol/cholate diet increased the monoene/saturated fatty acid ratios, particularly in hepatic and plasma cholesterol esters, to a lesser extent in phosphatidylcholine, and to a slight degree in the other lipid fractions (Fig. 3). The increased octadecenoate was found to be the Δ^9-isomer (oleate): the proportions of oleate in hepatic cholesterol ester were 84.1, 91.0, 91.5 and 92.2% of total octadecenoate isomers at 0, 9, 20 and 45 d of the test diets, respectively. The other monoene was mainly the Δ^{11}-isomer (*cis*-vaccenate). There were no significant differences in the extents of elevation of monoene/saturated ratios between SHR and WKY.

Another feature of changes in the acyl compositions of lipids induced by feeding the high cholesterol/cholate diet was an increase in linoleate/arachidonate ratios. Presumably, this is a reflection of impaired desaturation-elongation activities. The increase in the linoleate/arachidonate ratios was particularly prominent in plasma and hepatic cholesterol ester (Fig. 4). Curiously, the linoleate/arachidonate ratios of plasma cholesterol ester were higher in WKY than in SHR fed the high cholesterol/cholate diet, while no significant differences were observed in the other lipid fractions between the two strains.

As to the *n*-6/*n*-3 ratio of polyunsaturated fatty acids, which are found mainly in phospholipids, the high cholesterol/cholate diet did not induce significant changes but the ratio did show a tendency to increase with age (Fig. 5). The *n*-6/*n*-3 ratio was higher in WKY than in SHR in hepatic and plasma phospholipids throughout the experimental period.

Lipids of abdominal and thoracic aortas from WKY at the 45th day of the test diets were analyzed (Table I). Despite 2-fold increases in plasma cholesterol and 1.6 to 1.9-fold increases in plasma cholesterol ester induced by feeding the high cholesterol/cholate diet, no significant differences in aortic phospholipid or cholesterol contents were observed between the two dietary groups. The hepatic cholesterol ester content increased from 0.7 mg/g wet weight in the normal diet group to 52.6 mg/g wet weight in the high cholesterol/cholate diet group, but aortic cho-

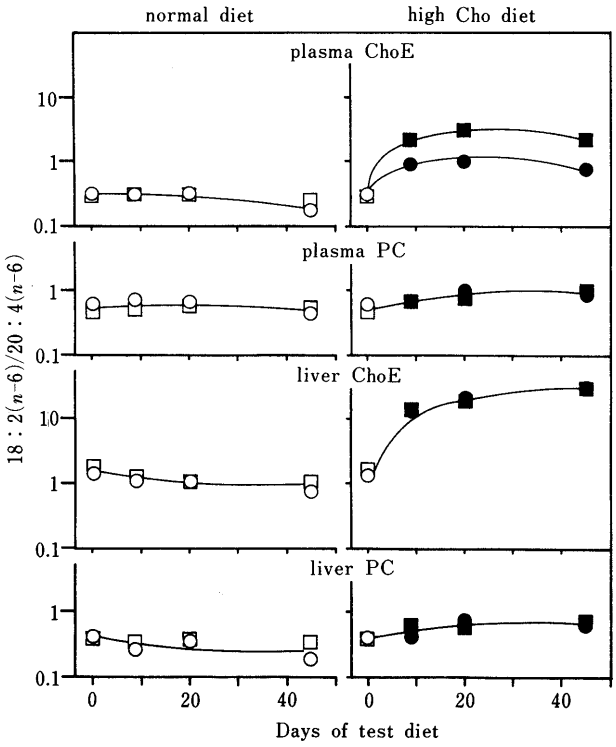

Fig. 4. Effect of a High Cholesterol/Cholate Diet on Linoleate (18:2)/Arachidonate (20:4) Ratios for Cholesterol Ester and Phosphatidylcholine in Plasma and Liver

Experimental conditions were the same as in Fig. 3. Normal diet group (open symbols) and high cholesterol/cholate group (closed symbols) values were determined in WKY (—□—, —■—) and SHR (—○—, —●—).

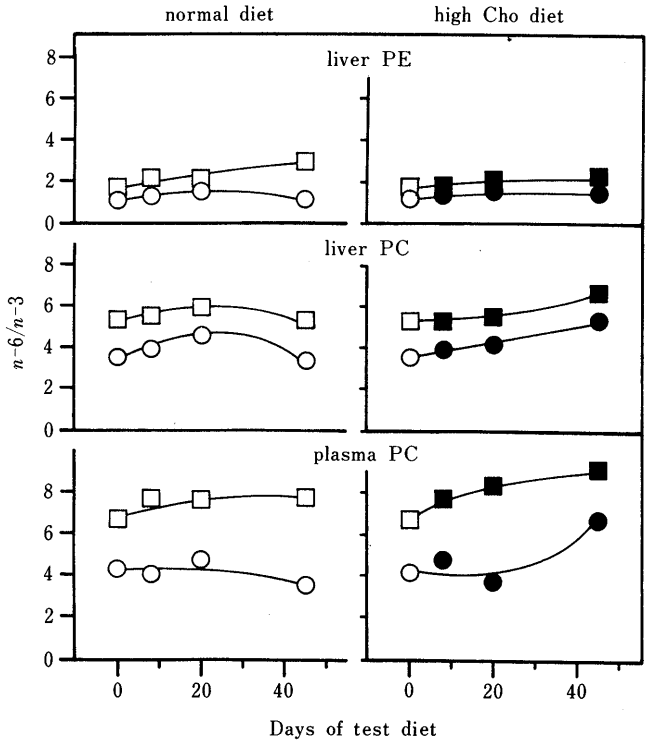

Fig. 5. Effect of a High Cholesterol/Cholate Diet on *n*-6/*n*-3 Ratios of Hepatic and Plasma Phospholipids

Experimental conditions were the same as in Fig. 3. WKY fed normal diet (—□—) or high cholesterol/cholate diet (—■—) and SHR fed normal diet (—○—) or high cholesterol/cholate diet (—●—) were examined.

TABLE I. Lipid Compositions of Abdominal and Thoracic Aortas

Diet[a]	Lipid contents[b] (mg/g wet weight)				
	PC	PE	TG	Cho	ChoE
Normal diet	2.3±0.1[c]	1.4±0.1	1.9±1.3	3.0±0.0	0.2±0.1
High cholesterol/cholate diet	2.5±0.1	1.3±0.2	0.9±0.2	3.8±0.4	0.2±0.1

a) Rats (WKY) were fed the test diets for 45 d. *b*) Lipids were extracted from aortas with chloroform–methanol and individual lipid components were separated by silica gel thin-layer chromatography and quantified as described in the text. *c*) All values represent the averages of two separate determinations.

lesterol esters remained below 0.1 mg/g wet weight.

Fatty acid compositions of aortic phospholipids from WKY fed either the normal diet or the high cholesterol/cholate diet, are compared with those of plasma and hepatic phospholipids in Table II. Increases in the proportions of oleate and linoleate and decreases in the proportions of arachidonate noted in plasma and hepatic phospholipids were barely noticeable in the fatty acids of aortic phospholipids. Thus, the very large changes in plasma and hepatic lipids induced by the high cholesterol/cholate diet were not reflected in aortic lipids under the conditions examined.

Discussion

Basal levels of plasma cholesterol ester, triacylglycerol and phosphatidylcholine were higher in WKY than in SHR (Fig. 1) as reported by Iritani *et al.*[13] On feeding a high cholesterol/cholate diet, plasma cholesterol and cholesterol ester reached higher levels in WKY than in SHR (Fig. 1). These results contrast with the previous concepts that hypertension augments hypercholesterolemia[8] and that hypertension and hemodynamic derangements induced by hypertension promote fat deposition in arteries in rats.[6,7] Differences in the contents of the diets might account for the observed discrepancies. A greater hypercholesterolemia was reported in SHR as compared with WKY using a diet supplemented with 2% cholesterol.[8] Furthermore, the report on arterial fat deposition was from experiments in which the diet included 20% suet, 5% cholesterol, and 2% cholic acids.[6,7] In our experiments, a diet supplemented with 5% cholesterol and 0.5% cholate was used. Our results on hyperlipemia and fatty liver are consistent with those of Cohen and Krause,[14] who used a diet supplemented with 20% olive oil, 2% cholesterol, and 0.3% taurocholate; these authors reported higher cholesterol levels in WKY than in SHR. The reasons for the discrepancies among the various studies remain unclear. However, our results and those of Cohen and Krause[14] clearly show that hypertension does not stimulate hyperlipemia in rats fed high cholesterol/cholate diets.

In human atherosclerotic lesions, the major lipid component has been shown to be cholesterol ester, particularly cholesterol oleate.[15-20] On feeding the high cholesterol/cholate diet to WKY, tremendous amounts of cholesterol ester accumulated in the liver (Fig. 2), and plasma cholesterol oleate increased more than 10-fold. However, no accumulation of either cholesterol or cholesterol ester was observed in abdominal and thoracic aortas from SHR[9] or WKY (Table I) fed a high cholesterol/cholate diet. Therefore, feeding a high cholesterol/cholate diet to SHR or WKY may provide a good experimental model for

TABLE II. Fatty Acid Compositions of Aortic Phospholipids in Comparison with Those of Plasma and Hepatic Phospholipids

Fatty acid	Plasma		Liver				Aorta			
	PC[b]		PC		PE		PC		PE	
	ND	HCD	ND[a]	HCD	ND	HCD	ND	HCD	ND	HCD
14:0[d]	0.1[c]	0.2	0.2	0.2	0.0	0.0	0.5	0.5	0.1	0.1
14:1 *n*-5	0.3	0.4	0.2	0.3	0.2	0.6	0.4	0.6	0.0	1.7
16 DMA	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	13.7	14.2
16:0	23.8	25.2	24.3	21.4	19.9	17.4	32.5	33.4	5.5	6.5
16:1 *n*-7	0.5	0.8	0.9	1.1	0.2	0.1	1.3	1.5	0.7	0.5
18 DMA	0.0	0.0	0.0	0.0	0.0	0.0	0.1	tr.	9.6	8.8
18:0	21.2	18.4	19.3	20.4	27.1	25.7	24.2	21.6	22.1	24.5
18:1	5.6	8.1	5.9	8.6	4.6	7.2	9.6	10.5	5.5	6.6
18:2 *n*-6[e]	14.5	20.1	11.2	18.1	8.0	11.7	4.3	5.1	1.3	1.5
18:3 *n*-6	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.0
18:3 *n*-3	0.1	0.2	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.1
20:1	0.4	0.6	0.3	0.4	0.0	0.4	0.3	0.3	0.1	0.1
20:3 *n*-9	0.4	0.4	0.3	0.6	0.0	0.2	0.0	0.0	0.5	tr.
20:3 *n*-6	0.6	1.2	0.3	2.1	0.0	0.7	1.3	2.0	0.5	0.8
20:4 *n*-6	27.3	19.6	29.6	21.2	26.6	21.7	20.1	17.2	23.4	22.3
20:4 *n*-3	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.4	0.0	0.3
20:5 *n*-3	0.2	0.6	0.3	0.8	0.2	1.1	0.1	0.0	0.2	0.1
22:4 *n*-6	0.1	0.4	0.2	0.1	0.0	0.0	1.8	1.7	6.4	5.9
22:5 *n*-6	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.0	1.9	1.5
22:5 *n*-3	1.1	0.9	1.1	1.0	0.9	1.8	0.3	0.0	1.4	0.0
22:6 *n*-3	4.1	3.0	6.2	4.2	10.9	12.3	1.4	2.1	3.8	1.3

a) Rats (WKY) were fed the test diets for 45 d. *b*) Lipids were extracted, individual phospholipids were separated by silica gel thin-layer chromatography, and fatty acids were determined as methylesters by GLC as described in the text. *c*) All values represent the averages of two separate determinations. The maximal deviation from the mean was below 29% of the values given when the proportions were about 5%. *d*) Fatty acids are expressed as the number of carbons: the number of double bonds. *e*) The abbreviations *n*-9, *n*-6, and *n*-3 denote the positions of the first double bond numbered from the methyl terminus.

hyperlipemia and fatty liver, but not for atherosclerosis. Thus, our results lead us to conclude that hypertension is not a stimulatory factor in the development of hyperlipemia and fatty liver induced by a high cholesterol/cholate diet, but we cannot exclude the possibility that hypertension is a risk factor for the development of atherosclerotic lesions.

Independently of the diets, the metabolism of *n*-3 and *n*-6 fatty acids appears to be different between SHR and WKY strains. The *n*-6/*n*-3 ratios of phospholipid acyl chains were higher in WKY than in SHR throughout the experimental period. In this context, it is interesting to note that dietary supplementation of *n*-3 fatty acids (α-linolenate or eicosapentaenoate) but not *n*-6 fatty acid (linoleate) lowers the systolic blood pressure of SHR.[21,22] The molecular mechanism for the hypotensive actions of dietary *n*-3 fatty acids remains to be elucidated. It may be that the consumption of *n*-6 fatty acids is higher in SHR than in WKY, since the production of arachidonate metabolites is reported to be higher in SHR than in WKY.[23-27] Prostaglandins E_2 and I_2 are known to have hypotensive activities, and the elevated production of these eicosanoids is interpreted to be secondary to the development of hypertension in SHR.[25,27]

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