Abnormal Arachidonate Distribution in Low-Density Lipoprotein and Thoracic Aorta in Hyperinsulinemia

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The mechanism by which hyperinsulinemia promotes atherogenesis is unknown. The effects of hyperinsulinemia on risk factors for atherosclerosis were investigated by subcutaneously injecting rats daily with an insulin-zinc suspension (20 U/kg) for 12 weeks. After this period, body mass and food consumption did not differ significantly between control and insulin-treated animals. Daily insulin injection significantly increased urinary excretion of epinephrine and decreased urinary excretion of norepinephrine and dopamine, but had no significant effect on blood pressure or heart rate. Although insulin decreased plasma triglyceride concentration by 44% ($P < .01$), the triglyceride to protein ratio in plasma low-density lipoprotein (LDL) was increased by 34% ($P < .05$) in insulin-treated rats; the cholesterol to protein and triglyceride to protein ratios remained unaffected, indicating a change in the quality of the LDL particle. Insulin also increased the percentage of arachidonic acid (20:4) in LDL triglycerides by 37% ($P < .05$). In contrast, cholesteryl esters and triglycerides in the thoracic aorta were significantly increased (49% and 91%, respectively) by insulin treatment. Insulin increased the percentage of monounsaturated fatty acids and decreased the percentage of n-6 fatty acids, including arachidonate, in aortic triglycerides. Insulin also increased the percentage of palmitoleic acid (16:1) and decreased the percentages of saturated fatty acids and n-6 fatty acids in aortic cholesteryl esters. These results indicate that insulin induced deposition of cholesteryl esters and triglycerides, especially those containing monounsaturated fatty acids, and abnormal arachidonate distribution in LDL and tissues. The data further suggest that the development of atherosclerosis in response to hyperinsulinemia may be associated with arachidonate-rich triglycerides in LDL.

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HYPERINSULINEMIA and insulin resistance are closely associated with the development of atherosclerosis.[1,2] They are highly prevalent among individuals with such cardiovascular risk factors as non–insulin-dependent diabetes mellitus, hypertension, and obesity, and are related to the frequent occurrence of coronary artery disease.[3,4] Hyperinsulinemia is induced by a state of cellular resistance to insulin action. Thus, endogenous hyperinsulinemia linked with insulin resistance is more closely associated with atherosclerosis than hyperinsulinemia without insulin resistance. However, in experimental animals, insulin deficiency is not related to the development of atherosclerosis, but instead, appears to protect against atherosclerosis.[4,5] Insulin, independent of its effects on blood pressure and plasma lipids, enhances the transport of cholesterol into cells by activating the low-density lipoprotein (LDL) receptor[6] and increases the endogenous synthesis of lipid.[7] Insulin also promotes proliferation of arteriolar smooth muscle cells in the vascular wall[8] and synthesis of collagen in connective tissue.[9] Accordingly, Stout[10] suggested that insulin is an atherogenic hormone.

Rodents such as rats and mice are generally considered resistant to experimentally induced atherosclerosis because induced hyperlipidemia does not produce atherosclerotic lesions in appropriate arteries.[11] However, the rat may be a suitable model for identifying factors that promote atherosclerosis.[12] Sato et al[13] showed that atherosclerosis-like lesions can be induced in rat aortas by long-term injection of insulin. We have now used this model, injecting insulin-zinc suspension over a period of 12 weeks, rather than 12 months, to investigate the effects of hyperinsulinemia on the characteristics of plasma LDL and the lipid composition of plasma and the thoracic aorta. Cholesteryl esters have previously been shown to be the main contributors to excess cellular lipid accumulation in atherosclerotic lesions.[14] Because increased renal tubular sodium reabsorption[15,16] and sympathetic nervous activity in response to hyperinsulinemia are implicated in the genesis of hypertension,[17] we also determined blood pressure, heart rate, and urinary catecholamine excretion.

MATERIALS AND METHODS

Treatment of Animals

Male Wistar rats weighing 180 to 200 g were divided into two groups. One group was subcutaneously injected daily with insulin-zinc suspension (20 U/kg body mass, Monotard Human; Novo-Nordisk, Bagsvaerd, Denmark) for 12 weeks, and the other group was similarly treated with saline. Standard rat chow and water were available ad libitum throughout the study. Ten weeks after the first injection, animals were housed individually in metabolic cages so that urine could be collected for determination of catecholamine excretion. Twelve hours after the last injection of insulin or saline, systolic blood pressure and heart rate were measured with a photoelectric, tail-cuff, pulse-detection system in conscious, prewarmed, restrained animals. Rats were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg), the abdominal cavity was opened, and the animals were exsanguinated from the abdominal aorta. NaN_3 (0.01%), merthiolate (0.005%), and Na_2 EDTA (1 mg/mL) were added to the blood, and plasma was prepared. The entire thoracic aorta from the ascending aorta was removed, and the adventitia and periadventitial fat were dissected away by use of a dissecting microscope in ice-cold saline. The aortic tissue was immediately frozen in liquid nitrogen.

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Lipoprotein Isolation

Lipoproteins were prepared from unfrozen plasma by KBr density gradient centrifugation as previously described.[18] Gradients consisted of 1 mL distilled water, 3 mL KBr and NaCl solution (d, 1.019 g/mL), 3 mL of another KBr and NaCl solution (d, 1.063 g/mL), and 2 mL plasma, which was adjusted to a density of 1.21 g/mL with solid KBr; all salt solutions contained EDTA (0.1 mg/mL). After centrifugation at 38,000 rpm for 15 hours in an SW 41-Ti swinging-bucket rotor (Beckman, Fullerton, CA), fractions in the density range 1.019 to 1.063 g/mL were collected. The protein content of this pooled LDL fraction was determined by the method of Lowry et al.[19]

Lipid Analysis of Plasma

Total cholesterol, free cholesterol, triglyceride, and phospholipid concentrations of plasma and LDL fractions were determined by enzymatic methods. The concentration of cholesteryl esters was calculated as the difference between total and unesterified cholesterol. Plasma high-density lipoprotein cholesterol level was measured with enzymatic reagents and after precipitation with heparin, Ca^{2+}, and Ni^{2+}. Total very–low-density lipoprotein mass and LDL mass were calculated from the difference in precipitation with three different heparin-$CaCl_2$ solutions.[20] For fatty acid analysis of LDL cholesteryl esters, triglycerides, and phospholipids, total lipids were extracted from lyophilized LDL with 5 mL of an ice-cold chloroform:methanol mixture (2:1 vol/vol) containing 0.01% butylated hydroxytoluene. Dried lipids were separated by thin-layer chromatography in hexane:diethyl ether:glacial acetic acid (80:20:1 vol/vol). Individual lipids were transmethylated,[21] and their fatty acid composition was assessed by splitless capillary gas–liquid chromatography (GC 14-A, Shimadzu, Kyoto, Japan) with a flame ionization detector and an HR-SS-10 capillary column (30 m × 0.25-mm ID, Shinwakakoh, Kyoto, Japan). Helium was used as the carrier gas. Peaks were identified by comparison with standards (Nu-Chek-Prep, Elysian, MN), and the relative percentages of peak areas were calculated.

Lipid Analysis of Tissue

The frozen tissue was freeze-dried and homogenized in 5 mL ice-cold chloroform:methanol (2:1 vol/vol) containing 0.01% butylated hydroxytoluene and cholesteryl acetate as an internal standard. After quantification of individual lipids with an Iatrosca (Tokyo, Japan) thin-layer chromatography–flame ionization detection method as previously described, [22] the remaining lipids were separated by thin-layer chromatography on silica gel plates for analysis of fatty acid composition as described earlier.

Other Assays

Twenty-four-hour urine samples were collected in a solution containing 6 mol/L HCl for determination of catecholamines by high-performance liquid chromatography and electrochemical detection. Plasma insulin concentration was measured by radioimmunoassay, and plasma glucose level was measured by the glucose oxidase method.

Statistical Analysis

Results are expressed as the mean ± SEM. The two groups were compared with Student's *t* test. Comparisons of percentages of fatty acids were performed using the Mann-Whitney *U* test. A *P* value less than .05 was considered statistically significant.

RESULTS

Body mass and food intake were similar in rats treated with insulin for 12 weeks and control animals (Table 1).

Table 1. Characteristics of Rats Treated With Insulin

Characteristic	Control (n = 11)	Insulin (n = 15)
Body mass (g)	499.7 ± 6.7	511.2 ± 11.4
Food intake (g/d)	25.5 ± 0.6	25.8 ± 1.1
Systolic blood pressure (mm Hg)	120.3 ± 3.0	119.6 ± 2.3
Heart rate (beats/min)	413.7 ± 11.8	389.6 ± 9.9
Plasma glucose (mg/dL)	197 ± 5	219 ± 22
Plasma insulin (μU/mL)	10.8 ± 1.3	12.2 ± 1.9
Urinary excretion (pg/d)		
Epinephrine	57.3 ± 11.4	116.9 ± 15.3†
Norepinephrine	809.4 ± 107.7	535.5 ± 61.6*
Dopamine	3,756 ± 473	2,259 ± 220†

NOTE. Values are the mean ± SEM.
*$P < .05$.
†$P < .01$.

Twelve hours after the last injection of insulin, there was no significant difference in systolic blood pressure and heart rate between insulin-treated and control rats. Plasma glucose and insulin concentrations were also similar in the two groups. Urinary epinephrine excretion after 10 weeks was significantly higher (+104%) in the insulin-treated group than in the controls. In contrast, urinary norepinephrine (−34%) and dopamine (−40%) excretion were lower in insulin-treated animals than in controls.

The plasma triglyceride concentration in insulin-treated rats was 56% of that in control rats ($P < .01$; Table 2). However, concentrations of other plasma lipids did not differ significantly between control and insulin-treated groups. The ratio of triglyceride to protein in plasma LDL was 34% greater in insulin-treated than in control rats (Fig 1). The ratios of total cholesterol, unesterified cholesterol, cholesteryl esters, or phospholipids to protein in LDL did not differ significantly between the two groups. The major fatty acids in LDL triglycerides of control rats were linoleic (18:2, n-6) (26%), palmitic (16:0) (23%), and oleic (18:1) (22%) acids (Table 3). Arachidonic acid (20:4, n-6) content in LDL triglycerides from insulin-treated rats was only 4.8%, but this value was 37% higher ($P < .05$) than in control rats. In LDL phospholipids, the percentage of

Table 2. Effects of 12 Weeks of Hyperinsulinemia on Plasma Lipid Concentrations

	Lipid Concentration (mg/dL)	
	Control (n = 13)	Insulin (n = 14)
Triglycerides	97.7 ± 10.0	54.6 ± 3.3†
Total cholesterol	56.3 ± 2.4	52.8 ± 1.6
Unesterified cholesterol	8.9 ± 0.8	7.3 ± 0.6
Cholesteryl esters	47.4 ± 2.3	45.5 ± 1.5
HDL cholesterol	39.6 ± 10.0	38.3 ± 1.5
Phospholipids	98.0 ± 14.0	109.1 ± 2.3
LDL mass	42.8 ± 4.4	29.5 ± 5.5
VLDL mass	21.9 ± 2.6	17.6 ± 2.8

NOTE. Values are the mean ± SEM.
Abbreviations: HDL, high-density lipoprotein; VLDL, very–high-density lipoprotein.
†$P < .01$ *v* control.

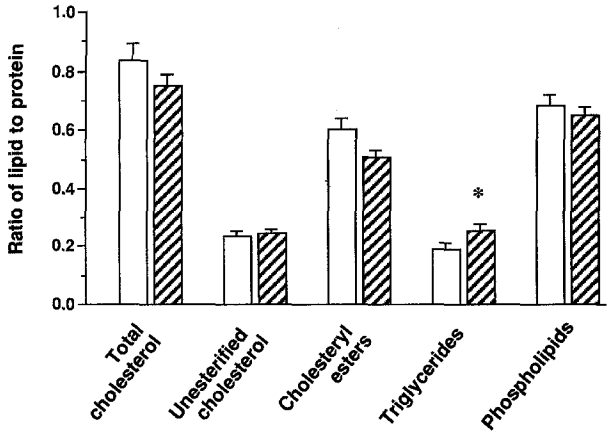

Fig 1. Ratios of lipid to protein in plasma LDL from control (□, n = 16) and insulin-treated (▨, n = 13) rats. Mean ± SEM. *$P < .05$ *v* control.

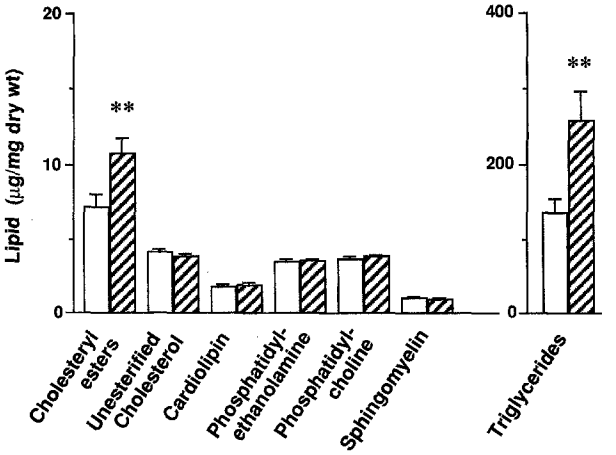

Fig 2. Lipid abundance in the thoracic aorta from control (□, n = 16) and insulin-treated (▨, n = 13) rats. Mean ± SEM. **$P < .01$ *v* control.

arachidonic acid was lower ($P < .05$) and the percentage of saturated fatty acids was higher ($P < .01$) in insulin-treated rats than in controls. LDL cholesteryl esters were enriched in linoleic and arachidonic acids, but there was no significant difference in fatty acid composition between the two groups.

The thoracic aorta was enriched in triglycerides (Fig 2). Insulin treatment significantly increased triglyceride and cholesteryl ester abundance in the aorta by 91% and 49%, respectively, whereas the amounts of cholesterol and individual phospholipids remained unchanged. The fatty acid composition of triglycerides and cholesteryl esters in the thoracic aorta was markedly altered by insulin treatment. Oleic (18:1, n-9) and palmitoleic (16:1, n-7) acids in triglycerides were increased by 51% and 58%, respectively ($P < .01$), whereas linoleic (18:2, n-6) and arachidonic (20:4, n-6) acids were decreased by 53% and 67%, respectively ($P < .01$), in the insulin-treated group (Table 4). Total monounsaturated and polyunsaturated fatty acids of aortic triglycerides increased by 47% ($P < .01$) and decreased by 53% ($P < .01$), respectively, in response to insulin treatment. In particular, the percentage of total n-6 polyunsaturated fatty acids decreased by 53% ($P < .01$) in insulin-treated rats. Cholesteryl esters of the aorta con-

Table 3. Effect of Hyperinsulinemia on Fatty Acid Composition of Plasma LDL

	Phospholipids		Cholesteryl Esters		Triglycerides	
Fatty Acid	Control (n = 15)	Insulin (n = 14)	Control (n = 15)	Insulin (n = 14)	Control (n = 15)	Insulin (n = 14)
14:0	1.9 ± 0.2	1.9 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	2.9 ± 0.3	2.1 ± 0.1*
16:0	30.6 ± 0.3	32.3 ± 0.4†	13.3 ± 0.4	14.1 ± 0.3	23.4 ± 1.1	23.5 ± 0.8
16:1(n-7)	1.3 ± 0.1	1.2 ± 0.1	3.9 ± 0.3	4.2 ± 0.1	3.4 ± 0.3	3.4 ± 0.3
18:0	18.1 ± 0.5	18.2 ± 0.4	1.2 ± 0.1	1.3 ± 0.1	3.5 ± 0.2	2.9 ± 0.1
18:1(n-7,9)	8.0 ± 0.3	7.1 ± 0.2†	13.7 ± 0.9	12.6 ± 1.0	21.8 ± 0.6	23.3 ± 0.3
18:2(n-6)	23.9 ± 0.5	24.2 ± 0.7	31.7 ± 0.5	33.1 ± 0.9	26.3 ± 1.0	24.7 ± 1.2
18:3(n-3)	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
20:3(n-6)	0.5 ± 0.0	0.6 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:4(n-6)	10.9 ± 0.5	9.5 ± 0.4*	28.9 ± 1.5	26.7 ± 1.0	3.5 ± 0.3	4.8 ± 0.4*
22:0	0.5 ± 0.0	0.6 ± 0.0	2.6 ± 0.1	3.4 ± 0.3	3.4 ± 0.4	4.9 ± 0.4*
22:1(n-9)	1.6 ± 0.2	1.6 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	2.5 ± 0.3	1.5 ± 0.1†
22:5(n-3)	0.3 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.7 ± 0.2	1.3 ± 0.1
22:6(n-3)	1.6 ± 0.1	1.6 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	4.7 ± 0.6	5.3 ± 0.5
24:0	ND	ND	ND	ND	1.4 ± 0.1	0.9 ± 0.1
24:1(n-9)	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.2	0.5 ± 0.0
Saturates	51.1 ± 0.4	53.1 ± 0.4†	18.7 ± 0.5	20.5 ± 0.4	34.5 ± 0.9	34.3 ± 0.8
Monoenes	11.6 ± 0.6	10.7 ± 0.3	19.2 ± 1.0	18.4 ± 1.1	28.3 ± 0.8	28.6 ± 0.5
Polyenes	37.3 ± 0.6	36.2 ± 0.4	62.1 ± 1.3	61.2 ± 1.0	37.2 ± 1.3	37.1 ± 1.2
n-3	2.0 ± 0.1	2.0 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	7.2 ± 0.7	7.4 ± 0.6
n-6	35.4 ± 0.5	34.3 ± 0.5	60.8 ± 1.3	59.9 ± 1.0	30.0 ± 1.0	29.7 ± 1.0

NOTE. Values are the mean ± SEM and represent the amount of each fatty acid expressed as a percentage of total fatty acids identifiable by gas-liquid chromatography.

Abbreviation: ND, not detectable.

*$P < .05$, †$P < .01$: *v* corresponding control as determined by the Mann-Whitney *U* test.

Table 4. Effect of Hyperinsulinemia on Fatty Acid Composition of Thoracic Aorta

	Phospholipids		Cholesteryl Esters		Triglycerides	
Fatty Acid	Control (n = 16)	Insulin (n = 13)	Control (n = 16)	Insulin (n = 13)	Control (n = 16)	Insulin (n = 13)
14:0	2.1 ± 0.1	2.0 ± 0.1	7.0 ± 0.6	5.4 ± 0.5	3.0 ± 0.1	2.7 ± 0.1
16:0	25.7 ± 0.4	25.2 ± 0.6	23.4 ± 0.6	17.2 ± 1.0†	29.1 ± 0.6	31.1 ± 0.4*
16:1(n-7)	1.5 ± 0.1	1.9 ± 0.1†	24.7 ± 1.8	43.9 ± 3.3†	6.0 ± 0.6	9.5 ± 0.4†
18:0	20.6 ± 0.2	20.0 ± 0.2*	7.5 ± 0.4	5.4 ± 0.6†	4.0 ± 0.2	3.9 ± 0.2
18:1(n-9)	8.1 ± 0.2	9.3 ± 0.2†	13.1 ± 0.6	8.8 ± 0.7†	23.0 ± 0.4	34.8 ± 0.7†
18:1(n-7)	3.8 ± 0.1	3.9 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	2.4 ± 0.1	2.1 ± 0.0†
18:2(n-6)	9.7 ± 0.3	10.0 ± 0.2	3.7 ± 0.2	2.3 ± 0.1†	29.0 ± 1.3	13.7 ± 0.7†
18:3(n-3)	0.4 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.3 ± 0.0	1.4 ± 0.1	0.5 ± 0.0†
20:3(n-6)	1.0 ± 0.0	0.9 ± 0.1	ND	ND	ND	ND
20:4(n-6)	19.1 ± 0.3	18.5 ± 0.6	2.0 ± 0.2	1.6 ± 0.3	0.6 ± 0.0	0.2 ± 0.0†
22:0	0.5 ± 0.0	0.7 ± 0.0†	ND	ND	ND	ND
22:1(n-9)	1.3 ± 0.1	1.4 ± 0.1	13.2 ± 0.9	10.8 ± 0.8	0.3 ± 0.0	0.5 ± 0.1
22:5(n-3)	1.0 ± 0.1	0.9 ± 0.0	3.4 ± 0.1	3.0 ± 0.4	0.2 ± 0.0	0.1 ± 0.0†
22:6(n-3)	2.4 ± 0.1	2.3 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.0	0.3 ± 0.1
24:0	0.6 ± 0.1	0.6 ± 0.1	ND	ND	ND	ND
24:1(n-9)	2.1 ± 0.1	2.0 ± 0.1	ND	ND	0.6 ± 0.1	0.5 ± 0.1
Saturates	49.6 ± 0.5	48.4 ± 0.6	37.9 ± 1.2	28.1 ± 1.8†	36.0 ± 0.7	37.6 ± 0.5
Monoenes	16.9 ± 0.4	18.5 ± 0.3	51.9 ± 1.2	64.3 ± 2.1†	32.3 ± 0.9	47.4 ± 0.7†
Polyenes	33.6 ± 0.6	33.1 ± 0.7	10.2 ± 0.3	7.6 ± 0.8†	31.7 ± 1.3	15.0 ± 0.7†
n-3	3.7 ± 0.2	3.6 ± 0.2	4.5 ± 0.1	3.8 ± 0.5	2.1 ± 0.1	1.0 ± 0.1†
n-6	29.9 ± 0.5	29.4 ± 0.6	5.7 ± 0.2	3.8 ± 0.4†	29.6 ± 1.3	14.0 ± 0.7†

NOTE. Values are the mean ± SEM and represent the amount of each fatty acid expressed as a percentage of total fatty acids identifiable by gas-liquid chromatography.

*$P < .05$, †$P < .01$: *v* corresponding control as determined by the Mann-Whitney *U* test.

tained predominately palmitoleic acid (16:1, n-7), which was 78% higher ($P < .01$) in insulin-treated rats than in controls. The percentages of n-6 polyunsaturated fatty acids and saturated fatty acids in cholesteryl esters significantly decreased, whereas that of monounsaturated fatty acids significantly increased, in response to insulin treatment. Although phospholipids in the aorta showed statistically significant changes in several fatty acids by percentage, these changes were small as compared with those for triglycerides and cholesteryl esters. There were no differences in the percentages of saturated, monounsaturated, or polyunsatured fatty acids of tissue phospholipids between insulin-treated and control groups.

DISCUSSION

Insulin resistance, which is frequently associated with obesity, hypertension, and hyperlipidemia,[23] is suggested to play a role in the pathogenesis of atherosclerosis—in particular, coronary artery disease. Insulin resistance, which is the impaired sensitivity of tissue to the action of insulin, results in hyperinsulinemia. Although hyperinsulinemia usually does not occur in isolation and almost always provides a clue to the presence of underlying insulin resistance, it is impossible to distinguish between insulin resistance and hyperinsulinemia. Experiments have suggested that insulin is atherogenic.[4,5] The atherosclerotic plaque is composed of excessive amounts of lipids and collagen, foam macrophages, and proliferating arterial smooth muscle cells. All these constituents are affected by insulin. Lipid synthesis in vascular smooth muscle cells is stimulated by insulin via activation of the lipogenic enzymes glucose-6-phosphate dehydrogenase, malic enzyme, and 3-hydroxyacyl coenzyme A dehydrogenase.[24] Proliferation of cultured smooth muscle cells is markedly stimulated[8] and collagen synthesis is augmented by exposure to insulin.[25] Insulin is not only a growth-promoting hormone, it also stimulates the synthesis of other growth factors, including insulin-like growth factor-I,[26] which contributes to the atherosclerotic process by inducing cell proliferation.

Sato et al[13] showed that exogenous insulin administered over a 12-month period induced atherosclerosis-like lesions in the aorta of rats. Light microscopy revealed that the thickened aorta and subendothelial tissues consisted of eosinophilic fiber bundles, amorphous ground substances, and irregularly arranged cells. We studied factors that contribute to atherosclerosis in rats injected daily with insulin for 12 weeks. We observed no significant difference in plasma insulin concentrations 12 hours after insulin injection between insulin-treated rats and controls, consistent with the diurnal variations described by Sato et al.[13] Epinephrine is an insulin antagonist, and the marked increase in urinary epinephrine excretion that we observed in insulin-treated rats is attributable to adrenal medullary epinephrine released to counteract insulin-induced hypoglycemia.[27] In contrast, urinary excretion of norepinephrine and dopamine, both of which originate predominantly from sympathetic neurons in a variety of tissues,[28,29] was reduced in insulin-treated rats. Infusion of insulin while maintaining normal blood glucose concentrations results in dose-dependent increases in plasma norepinephrine in humans.[27,30] However, the dose of injected insulin in our study was so large that the increase in plasma epinephrine

appeared to inhibit norepinephrine release from sympathetic neurons. Long-term insulin administration did not affect blood pressure or heart rate 12 hours after the last injection. Similarly, chronic exogenous hyperinsulinemia for 8 weeks did not result in an increase in blood pressure.[31]

Atherosclerosis is characterized by massive accumulation of lipids in the arterial wall.[14] Cholesteryl ester droplets are deposited in the intima of the aorta.[32] We observed a 49% increase in cholesteryl esters and a 91% increase in triglycerides in the aorta of insulin-treated rats, suggesting a tendency to the development of atherosclerosis despite the absence of atherosclerotic plaques. Sato et al[13] also observed a 45% increase in aortic triglyceride content after 12 months of hyperinsulinemia. Accumulation of cellular triglyceride is associated with changes in cellular cholesteryl ester content and in LDL receptor binding.[33] Moreover, the coexistence of triglycerides and cholesteryl esters within cells impairs the mobilization of cholesteryl esters.[33] Deposits of triglycerides and cholesteryl esters are thought to target the degenerated vessels for atherosclerosis.

Alavi et al[34] observed changes in phospholipid fatty acid composition, as well as in cholesteryl ester fatty acid composition, in areas of endothelial regeneration in the rabbit aortic wall after removal of endothelium with a balloon catheter. These researchers showed that palmitic (16:0) and palmitoleic (16:1, n-7) acids increased and arachidonic acid (20:4, n-6) decreased markedly in phospholipids. Insulin-induced changes in phospholipid fatty acids were not as marked in our study, but the insulin-induced alterations in triglyceride fatty acids are in part consistent with the changes in phospholipid fatty acids observed by Alavi et al[34] The small changes in phospholipid fatty acid composition induced by insulin may explain in part why rats are resistant to atherosclerotic change as compared with rabbits. The reduced arachidonic acid content in atherosclerotic vessels suggests a diminished formation of prostacyclin. Similarly, our observation that the abundance of polyunsaturated n-6 fatty acids, especially linoleate (18:2, n-6), in aortic triglycerides and cholesteryl esters was reduced in insulin-treated rats implies an alteration in the formation of prostanoids, because arachidonate is formed from linoleate as a result of elongation-desaturation catalyzed by Δ5- and Δ6-desaturases. The significant relation between blood pressure and platelet phospholipid n-3 and n-6 fatty acid incorporation suggests that the changes in lipid composition of the aorta may also affect the function of the endothelium and smooth muscle cells.[35]

The quality of LDL particles has been implicated in the development of atherosclerosis. Small LDL particles have been associated with coronary artery disease, independent of age, sex, and relative body mass.[36,37] Usually, LDL particle size correlates inversely with plasma triglyceride concentration. Thus, the mass composition of LDL, which is related to particle size, is an atherogenic factor.[38] Despite a decrease in plasma triglyceride, we detected an increase in the triglyceride to protein ratio in LDL in insulin-treated rats, suggesting that the quality of LDL particles was altered. Fatty acid compositions of LDL lipids did not differ as markedly as those of aortic lipids between insulin-treated and control rats. The arachidonic acid in LDL triglycerides increased by 37% in the insulin-treated group, even though this fatty acid is not a major component of LDL triglycerides. Recently, Phinney et al[39] described an abnormal arachidonate distribution in serum and liver in the genetically obese Zucker rat. Insulin may have the ability to induce abnormal arachidonate distribution in plasma and tissues, although the mechanism of such an effect is not clear. Our results imply that Δ5- and Δ6-desaturase activities are not affected by insulin because of the decrease in both arachidonic acid and linoleic acid (18:2, n-6) in the aorta of insulin-treated rats.

In conclusion, we demonstrated that exogenous hyperinsulinemia induced deposition of cholesteryl esters and triglycerides, especially those containing monounsaturated fatty acids, and abnormal arachidonate distribution in LDL and thoracic aorta. These results suggest that atherosclerosis promoted by hyperinsulinemia and insulin resistance may be associated with arachidonate distribution in LDL and tissues.

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