Hemodynamic Sequelae of Regression of Experimental Atherosclerosis

MARK L. ARMSTRONG, DONALD D. HEISTAD, MELVIN L. MARCUS, DONALD J. PIEGORS, and FRANCOIS M. ABBOUD, *Cardiovascular Division, Department of Internal Medicine and the Cardiovascular Center, University of Iowa College of Medicine and Veterans Administration Hospital, Iowa City, Iowa 52242*

ABSTRACT Regression of experimental atherosclerosis is characterized by decreased intimal thickness and luminal enlargement, but intimal fibrosis becomes more dense. We tested the hypothesis that fibrosis of arteries during regression might limit vasodilator capacity and restrict hemodynamic improvement despite luminal improvement. We studied limb, coronary, and cerebral hemodynamics in 11 normal cynomolgus monkeys, 10 monkeys given an atherogenic diet for 20 mo and 8 monkeys given a regression diet for an additional 18 mo. The atherogenic diet induced lesions of moderate severity (50–60% stenosis); owing to characteristic vessel growth during the atherogenic period, luminal size did not decrease correspondingly. Regression monkeys showed typical changes of regression with luminal enlargement but increased fibrosis. The iliac artery was perfused at constant blood flow and maximal vasodilatation was produced with papaverine. Blood flow was measured with microspheres during maximal vasodilatation in the coronary bed (adenosine) and cerebral bed (hypercapnia). In normal monkeys, minimal vascular resistances were 1.95±0.19 mm Hg/ml/min × 100 g (mean±SE) (limb), 0.13±0.01 (coronary), and 0.44±0.02 (cerebral). In atherosclerotic monkeys minimal resistance increased ($P < 0.05$) 108, 62, and 166% in the limb, coronary, and cerebral beds, respectively. In regression monkeys, minimal resistance increased from values found in atherosclerotic animals in the limb (+22%), decreased inconsistently in the coronary bed (−19%), and decreased significantly in the cerebral bed (−44%, $P < 0.05$). Thus morphologic regression was accompanied by significant hemodynamic improvement during maximal dilatation only in cerebral vessels. We conclude that increases in luminal size during regression of atherosclerotic lesions may not be associated with increases in vasodilator capacity, as intimal fibrosis may limit physiologically important hemodynamic improvement.

INTRODUCTION

Experimental atherosclerosis has been shown to regress in primates when the atherogenic stimulus is removed or attenuated (1–4). The principal morphologic changes that characterize regression are a reduction in intimal mass and an increase in lumen diameter. Reduction in intimal size is largely caused by decreases in intimal lipid and cellularity (5–7). In most regression studies in which the preceding atherosclerosis is more advanced than simple fatty streaks (8), there is also a tendency for intimal fibrosis to become more marked. Intimal fibrosis is particularly marked in cynomolgus monkeys during regression of atherosclerosis (9–11).

Luminal enlargement that occurs during regression would be expected to produce functional improvement, but one might speculate that persistence of connective tissue with more advanced intimal fibrosis may limit the capacity for vasodilatation and may restrict hemodynamic responses to increased demand for blood flow. There have been no studies of the effect of regression of experimental atherosclerosis on tissue or organ blood flow, a point that is critical in interpretation of the significance of morphologic changes. In this study, we have compared effects of moderately severe atherosclerosis, and of subsequent regression, on blood flow to the hindlimb, heart, and brain in cynomolgus monkeys.

A preliminary report of this study was presented at the Annual Meeting of the Association of American Physicians, 25 April 1981, San Francisco, CA, and published as an abstract (1981. *Clin. Res.* **29**: 561a). Another preliminary report, containing details of the methods used, has been submitted for publication.

Received for publication 18 January 1982 and in revised form 23 August 1982.

METHODS

Dietary induction of atherosclerosis and regression

Three groups of adult male Malaysian cynomolgus monkeys, 5.1±0.9 kg were studied. One group of 11 animals (normal group) was given commercial laboratory chow (Purina Monkey Chow, Ralston Purina Co., St. Louis, MO). By analysis this diet was essentially cholesterol-free and contained 5% fat (unsaturation index 0.89, polyenoic fatty acid content 28%). 18 monkeys were fed an atherogenic diet that contained 0.8% cholesterol and 41% fat (unsaturation index 0.69, polyenoic fatty acid content 12%) for 20 mo. The animals were then divided into two groups matched for hypercholesterolemia and body weight. 10 monkeys (atherosclerotic group) were studied and 8 monkeys (regression group) were fed commercial chow during a regression period of 18 mo.

At intervals of 1–2 mo during the dietary regimens, plasma was obtained from venous blood samples drawn in EDTA (disodium ethylenediamine tetraacetic acid), 1 mg/ml, after injection of ketamine hydrochloride (12 mg/kg, i. m.). Total cholesterol was determined by the method of Abell et al. (12) as modified by the Lipid Research Clinics Protocol for the Technicon Auto-Analyzer II (Technicon Instruments Corp., Tarrytown, NY) (13). Triglycerides were measured by the corresponding protocol for glyceride analysis.

Hemodynamic studies

Measurement of hindlimb hemodynamics. The animals were sedated with ketamine hydrochloride (12 mg/kg, i. m.) and anesthetized with chloralose (75 mg/kg, i. v.). They were intubated and ventilated with room air and supplemental oxygen via a Harvard model 661 small animal respirator (Harvard Apparatus Co., S. Natick, MA). Rectal temperature was maintained at 37.5°C with a heating pad.

Polyethylene catheters (PE-90) were inserted into each brachial artery, the splenic artery and the right femoral vein. Decamethonium bromide (0.5 mg/kg, i. v.) was used for skeletal muscle paralysis and heparin (500 U/kg, i. v.) was used for anticoagulation. Blood gases were monitored at frequent intervals throughout the study using an IL 113 Blood Gas Analyzer (Instrumentation Laboratories, Lexington, MA).

The bifurcation of the abdominal aorta and the proximal left iliac artery were exposed. The left iliac artery and hindlimb were perfused at constant flow with a Harvard model 1210 peristalic pump while perfusion pressure was recorded. The limb was perfused with pulsatile pressure (~30 mm Hg pulse pressure). The left dorsal pedal artery was cannulated to measure the iliac-dorsal pedal pressure gradient and to estimate resistance of large arteries of the limb.

Base-line perfusion pressure of the hindlimb was established by adjusting blood flow so that the perfusion pressure was similar to the animal's mean pressure measured in the right brachial artery. Pressure was measured at three levels of perfusion: base-line flow, 40% above base-line flow, and 40% below base-line flow. Thus a three-point pressure-flow curve was obtained for each animal.

To study maximal vasodilator responses of the hindlimb, papaverine hydrochloride was infused at 2.5 mg/min into the perfusion tubing. Perfusion pressure was measured during base-line flow and flows 40% above and below base-line flow. The dose of papaverine was then increased to 5.0 mg/min. The higher dose did not usually produce a further decrease in perfusion pressure, which indicates that the vessels were dilated maximally. In those experiments in which further vasodilatation was observed, pressure-flow determinations were repeated. We also studied vasodilator responses to adenosine in the limb. Adenosine (5 μM i. v./kg per min) was infused for 20 min. Limb perfusion pressure was monitored, and blood flow to the limb was increased to maintain perfusion pressure at control levels. Thus, limb vasodilator responses to adenosine were expressed as the increment in blood flow that was required to maintain perfusion pressure constant.

To study vasoconstrictor responses of the hindlimb, bolus injections of l-norepinephrine (0.2, 0.6, and 1.2 μg) were given into the hindlimb perfusion tubing and changes in perfusion pressure were recorded.

Coronary and cerebral blood flow. Through a thoracotomy, a catheter was placed in the left atrium for injection of microspheres. For determination of organ blood flow, microspheres, 15 μM mean Diam, were injected into the left atrium (14). Reference arterial samples were withdrawn (1.03 ml/min) from the splenic and left brachial artery starting 30 s before injection and continuing for 2 min thereafter. We injected spheres labeled with ^{46}Sc, ^{85}Sr, ^{141}Ce, and ^{125}I.

Measurement of coronary blood flow was made during a control period and during maximal vasodilatation. Maximal vasodilatation was obtained by infusion of adenosine (5 μM i. v./kg/min). A pulsed Doppler flow probe (15) was placed over the left anterior coronary artery. Vasodilatation was shown to be maximal when a dose of 5 μM/kg/min was used, because no further increase in blood velocity occurred after infusion of adenosine at 10 μM i. v./kg per min, or during reactive hyperemia after occlusion of the coronary artery for 30 s.

Measurement of cerebral blood flow was made during a control period of normocapnia, and after maximal vasodilatation during 15–20 min of hypercapnia. We have shown previously that cerebral vascular resistance is similar during hypercapnia and seizures, which suggests that hypercapnia produces maximal cerebral vasodilatation (15). Hypercapnia was induced by ventilating the animals with a gas mixture of 10% CO_2-90% N_2 and O_2 to maintain normal PO_2. The ventilatory rate was reduced by two to three breaths per minute.

The monkeys were then killed by exsanguination, and the heart and brain were removed. Measurement of radioactivity in tissue and in arterial blood samples was performed as described previously (14). Coronary and cerebral blood flows were calculated from the formula, $BF = C_{ti} \times 100\ RBF/C_r$, where BF is organ blood flow in milliliters per minute × 100 g tissue, C_{ti} is counts per gram of tissue, RBF is reference blood flow (rate of withdrawal of reference arterial blood samples, in milliliters per minute) and C_r is total counts per milliliter of reference arterial blood. The counts in the two reference blood samples were averaged. Vascular resistance was calculated by dividing mean systemic arterial pressure by total organ blood flow.

Morphological studies

The overall extent of morphologic change was determined by gross examination. Notations were made of wall stiffness, calcification, approximate lumen size, and macroscopic lesion appearance. Maximal percent stenosis was estimated grossly in the carotid and hindlimb arteries; maximal percent stenosis occurred in the proximal sites of arterial segments in this series, and the histologic sample obtained at these

sites was considered to provide a quantitative estimate of maximal stenosis. Intimal surface involvement was estimated visually in 5% units by two observers, with average difference of 3%, and expressed as mean and range of involvement. In the coronary arteries estimates of intimal changes were unreliable because of prior postmortem coronary arteriography, so we used angiographic evaluation of these vessels. The angiographic procedure has been described elsewhere (16). Briefly, the coronary ostia were cannulated, a barium-gelatin-formalin mix was infused at 100 mm Hg, and angiograms obtained in the frontal and oblique views of the heart. Angiograms were carefully evaluated for focal stenotic changes. Histologic study was carried out on carbowax sections of formalin-fixed tissue that were taken at standardized sites. These included the following arterial locations: iliac and femoral vessels (proximal and midsegment sites); coronary epicardial arteries at five sites (left main coronary, proximal left anterior descending, circumflex near origin, right coronary near ostium, and right coronary at the acute margin), and common carotid (proximal and midsegment sites), internal carotid, vertebral, and basilar, and branches of the circle of Willis arteries.

Arterial cross-sections, prepared for microscopy, were evaluated quantitatively. Morphometric determination was performed with an image analyzer to quantify the degree of lumen stenosis, size of lesions, areas of calcification, and mass of media. The images were projected at 60 or 150×, areas defined with a cursor and digitized to square millimeters transverse arterial area. The cross-sectional area enclosed by the internal elastic (IE)[1] lamina was corrected to a circle by applying the form factor $\frac{l^2}{4\pi}$ to the measurement of the IE lamina, where l = length of lamina in millimeters. Correction of IE area in this way minimizes the variable effect of elastic recoil on lumen size. Percent stenosis was calculated from the ratio of intimal area to corrected IE area. Lumen was calculated as corrected IE area–intimal area.

Statistical analysis

The *t* test was used to compare two groups, and analysis of variance to compare more than two groups. A significance level $\alpha = 0.05$ was used. In the *t* test comparisons, a reduced *P* value based on the Bonferroni inequality was used to indicate significance, to allow for the effects of multiple testing (17). Pressure-flow curves were initially analyzed as regression slopes. Because significant intergroup differences in slope were found, the pressure-flow data were analyzed with a nested analysis of variance as to the effects of diet and flow rate on vascular resistance (18). Ratios (e.g., percent stenosis) were compared parametrically after determining that the two components had significant linear correlations with the intercept set at zero, and nonparametrically with the Kruskal-Wallis test (19). The two types of analysis were in agreement, and only the results of the parametric tests are presented. Values are expressed as means±SE unless otherwise stated.

RESULTS

Plasma lipids

Plasma total cholesterol in normal monkeys was 98±2.4 mg/dl after 2–3 mo of control diet with little fluctuation thereafter. Plasma cholesterol was 534±30 mg/dl in atherosclerotic monkeys and 538±33 in regression monkeys during months 2–20 of atherogenic diet, and was 102±7 mg/dl in regression monkeys during the final 14 mo of the regression diet. Plasma triglycerides were similar during the control and atherogenic diets, remaining in the range of 25 to 40 mg/dl.

Morphologic changes

Gross findings. In atherosclerotic monkeys there was widespread involvement of the aorta and its primary and secondary branches with fatty streaks and plaques. Total intimal involvement of the aorta was 81% (mean of visual estimates, range 70–90%), with plaque involvement 44% (25–60%). The limb arteries had plaque areas of 20% (10–40%), and the carotid arteries averaged 27% (20–35%). The vertebral arteries had principally fatty streak lesions. The basilar artery had minimal fatty streak changes and branches of the circle of Willis had no gross changes. In the regression monkeys the fatty streaks had disappeared; the residual plaque areas averaged 42% (30–60%) in aorta, 24% (5–35%) in limb arteries, and 25% (10–45%) in carotid arteries. Thus, plaque areas did not differ significantly between atherosclerosis and regression, which indicates that regression caused little change in the amount of connective tissue grossly visible at the intimal surface.

Maximal focal stenosis estimated grossly did not exceed 75%, and no lesion in a regression animal exceeded 60% stenosis in any bed. No focally stenotic lesions were seen in postmortem coronary arteriograms in atherosclerotic or regression monkeys. Qualitatively, the arteriograms in atherosclerotic monkeys tended to show narrower lumens than did those of normals, but there was a considerable range in the size of the coronary arteries in the normal group. The lumens in regression monkeys were larger than those in either normals or atherosclerotic monkeys.

The vessel walls in all three beds were diffusely thicker in regression monkeys than in normals, and the vessels were stiffer than those of atherosclerotic monkeys. The average length *in situ* of the arterial segments in which diffuse changes occurred differed among the three beds: for limb arteries the length was 18 cm, for carotids 6 cm, and for the coronary arteries (orifice to apex) 5–6 cm. Thus, the lengths of the segments altered by connective tissue changes was greater in the limb bed than in the coronary or carotid bed by a factor of three or more. In regression foci of calcification were larger than in atherosclerotic vessels and some calcification was noted in all beds studied except in intracranial vessels.

[1] *Abbreviation used in this paper*: IE, internal elastic.

TABLE I
Arterial Morphometric Data

	Luminal stenosis		Luminal area			Intimal area		Medial area		
	AS	Reg	AS	Reg	Normal	AS	Reg	AS	Reg	Normal
	%		mm^2			mm^2		mm^2		
Limb arteries										
Proximal iliac										
Right	47±4	26±5*	1.96±0.38	3.69±0.45*	2.57±0.45	1.69±0.22	1.49±0.26	0.95±0.12	1.32±0.14	1.41±0.14
Left	45±3	39±4	2.26±0.31	3.52±0.33*	2.41±0.33	1.85±0.20	2.12±0.21	1.29±0.26	1.48±0.28	1.10±0.28
Mid-iliac										
Right	54±4	28±4*	1.41±0.15	1.98±0.16	1.48±0.21	1.64±0.21	0.99±0.23	0.90±0.07	1.12±0.74	0.78±0.09
Left	46±5	30±4	1.77±0.35	2.58±0.30	1.64±0.32	1.39±0.19	1.19±0.17	1.13±0.11	0.93±0.10	0.77±0.10
Proximal femoral										
Right	59±6	26±5*	0.88±0.21	1.53±0.17	1.04±0.21	1.37±0.21	0.69±0.17	0.94±0.11	0.89±0.09	0.70±0.11
Left	44±4	19±5*	1.41±0.18	1.71±0.19	1.06±0.24	1.11±0.14	0.51±0.15*	1.15±0.25	1.26±0.28	0.88±0.34
Mid-femoral										
Right	38±7	23±5	0.74±0.14	0.68±0.11	0.40±0.13	0.46±0.10	0.25±0.08	0.68±0.11	0.95±0.08	0.60±0.10
Left	43±4	3±2*	0.91±0.21	1.01±0.22	0.46±0.25	0.63±0.08	0.08±0.08*	1.00±0.06	0.94±0.07	0.68±0.08*
Coronary arteries										
Right proximal	63±3	23±2*	0.96±0.40	2.24±0.35	1.24±0.31	1.69±0.11	0.58±0.10*	0.81±0.23	1.07±0.20	0.41±0.18
Right "distal"	59±4	30±4*	0.81±0.16	1.42±0.16*	0.54±0.13	1.04±0.06	0.56±0.06*	0.98±0.15	0.55±0.15	0.26±0.11*
Left main	53±4	20±4*	1.53±0.46	3.58±0.46*	1.40±0.32	1.65±0.14	0.75±0.14*	0.91±0.13	0.85±0.13	0.50±0.10
Circumflex	61±3	27±2*	0.96±0.39	2.24±0.36	0.87±0.34	1.38±0.12	0.77±0.11*	0.47±0.26	1.20±0.25	0.38±0.23
Left ant. desc.	63±4	23±4*	0.85±0.12	1.25±0.11	0.58±0.10	1.42±0.11	0.42±0.11*	0.71±0.09	0.40±0.08	0.27±0.07*
Carotid arteries										
Proximal common										
Right	59±4	35±4*	1.61±0.22	3.41±0.25*	2.51±0.21	2.34±0.23	1.84±0.26	1.12±0.18	1.29±0.20	1.20±0.18
Left	62±5	29±5*	1.70±0.36	4.43±0.36*	2.48±0.41	2.77±0.32	1.79±0.32	1.42±0.16	1.21±0.16	1.14±0.18
Mid common										
Right	60±5	31±4*	1.45±0.34	3.03±0.28*	1.52±0.32	2.11±0.25	1.41±0.20	1.30±0.14	1.04±0.12	0.89±0.13
Left	57±4	30±4*	1.47±0.38	3.39±0.34*	1.58±0.42	1.80±0.25	1.40±0.22	1.01±0.24	1.40±0.21	0.85±0.26
Internal										
Right	68±5	33±7*	0.65±0.18	1.85±0.24*	0.82±0.24	1.26±0.14	0.90±0.19	0.73±0.07	0.55±0.09	0.41±0.09*
Left	53±5	36±6	1.19±0.31	2.45±0.38	0.81±0.36	1.32±0.12	1.15±0.15	1.44±0.30	1.05±0.37	0.62±0.09
External										
Right	48±7	2±1*	0.40±0.11	1.23±0.47	0.69±0.54	0.71±0.10	0.03±0.11*	0.56±0.12	0.75±0.14	0.46±0.16
Left	74±5	9±5*	0.68±0.32	1.37±0.10*	0.92±0.14	1.08±0.11	1.11±0.10	0.56±0.07	0.54±0.06	0.41±0.18

Abbreviations: AS, atherosclerosis; Reg, regression. *Abbreviations for coronary arteries*: Left ant. desc., left anterior descending artery. Sites of sampling are described in Methods. Data are means±SE. Values given for luminal stenosis and lumen size are corrected measurements as described in Methods. Not shown are values in Normal for luminal stenosis, which was <1% and for intimal area, which was minimal (<0.1 mm^2).

* Significant at $\alpha = 0.05$ after Bonferroni's adjustment for multiple simultaneous testing: asterisk at Reg indicates AS vs. Reg and at Normal indicates AS vs. Normal.

Histologic findings. Since our gross pathologic studies and angiographic data indicate that atherosclerosis and regression were diffuse, microscopic evaluation of standardized, unbiased sites is considered to provide data that reliably reflect the type and magnitude of morphologic change. Changes typical of relatively long-term atherogenic diet were seen in the aorta and branch arteries in atherosclerotic monkeys. These have been described in detail previously (9, 20, 21). Both intra- and extracellular lipid were present in marked degree. There were moderate periluminal fibromuscular thickenings ("caps") and relatively minor medial atrophy.

In regression monkeys, there was marked loss of visible intimal lipid and the intima was fibrotic. This change was seen in the aorta and in all three arterial beds in which we performed hemodynamic studies. In normal animals the intimal space was narrow and relatively acellular, but small intimal cushions or thickening and occasional fatty streaks were seen in the aorta.

Morphometric findings. The key morphometric findings are listed in Table I. Because only trivial lesions were seen in the intracranial arteries of atherosclerotic or regression groups, we have not listed these sites. Intimal area and percent stenosis are not shown for normal monkeys because intimal area was minimal and percent stenosis was < 1%. In limb arteries, extramural coronary bed, and carotid arteries, the average stenosis in atherosclerotic monkeys was 47, 60, and 68%, respectively. Because of vessel growth during atherosclerosis, lumen area in the atherosclerotic beds did not decrease correspondingly. The overall lumen/lumen ratios of atherosclerotic/normal monkeys averaged 1.02, 1.10, and 0.80 in the hindlimb, coronary, and carotid vessels, respectively. The lumen/lumen ratio of atherosclerotic/normal monkeys at the most proximal site in each bed was 0.84, 0.94, and 0.66 in the hindlimb, coronary, and carotid vessels, respectively.

In regression monkeys there was an average 35–60% reduction in degree of stenosis to 25% stenosis in each bed (Table I). The lumen/lumen ratios of regression/atherosclerotic monkeys averaged 1.47, 2.11, and 2.32 in the hindlimb, coronary, and carotid vessels, respectively. The lumen/lumen ratio of regression/atherosclerotic monkeys at the most proximal site in each bed was 1.71, 2.34, and 2.37 in the hindlimb, coronary, and carotid vessels, respectively.

In statistical comparisons between atherosclerotic and normal monkeys there were no significant differences in corrected lumen size (Table I; see Discussion). The increase in intima in atherosclerosis was massive and the differences from normals highly significant. Medial mass tended to increase in atherosclerosis, but the changes at individual sites were usually not significant. In statistical comparisons between atherosclerotic and regression monkeys, there was strong evidence of luminal enlargement, in spite of the conservative effect of multiple simultaneous testing (17). In regression monkeys, intimal mass decreased 62% and the IE area increased 17%; the combined effects increased lumen area and decreased percent stenosis significantly. Medial mass did not change significantly (+4%) in regression. Calcification (not shown in Table I) was not significantly different in the two groups.

Hemodynamic changes

Limb arteries. Pressure-flow curves during the control period and maximal vasodilatation are shown in Fig. 1. During the control period atherosclerotic animals had higher perfusion pressures than normals at base-line flow and when flow was reduced or increased by 40% ($P < 0.05$). Values in regression animals were significantly lower than those in atherosclerotic monkeys, and were significantly higher than values found in normals at all three levels of flow. During maximal vasodilatation, both atherosclerotic and regression animals had significantly higher perfusion pressures and resistances than normal animals. Thus, the vascular resistance of regression animals was less than that of atherosclerotic animals during the control period, but during maximal dilatation vascular resistance was much higher than that of normal animals and similar to or exceeding that of atherosclerotic animals (Fig. 1, Table II).

During maximal vasodilatation with systemic adenosine, the increase in flow needed to maintain perfu-

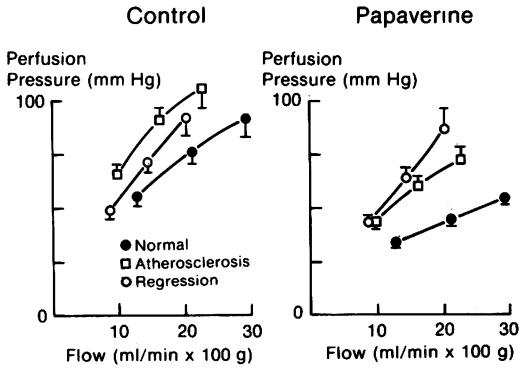

FIGURE 1 Pressure-flow curves in the perfused limb bed in the resting state (control) and after maximal vasodilatation (papaverine). Data points are group means±SE. During control, resistance was different ($P < 0.05$) in atherosclerosis vs. regression and normal, and in regression vs. normal. During papaverine, resistance was different in atherosclerosis and regression vs. normal, and at high flow rates in regression vs. atherosclerosis.

TABLE II
Vascular Resistance in the Limb Bed (mm Hg/ml/min × 100 g)

	Control			Papaverine		
	Flow rate			Flow rate		
	Low	Base line	High	Low	Base line	High
Normal	4.44±0.33	3.67±0.24	3.32±0.31	2.76±0.25	2.18±0.19	1.95±0.19
Atherosclerotic	8.18±1.21*	6.91±1.07*	5.76±0.94*	5.74±1.03*	4.75±0.85*	4.06±0.69*
Regression	6.32±1.20*‡	5.42±0.88*‡	5.06±0.93*‡	5.66±1.26*‡	4.94±0.87*	4.94±1.14*‡

* $P < 0.05$ compared with normal.
‡ $P < 0.05$ compared with atherosclerosis.

sion pressure constant is shown in Fig. 2. Perfusion pressure of the control state was restored in both atherosclerotic and regression monkeys at only one-fourth the incremental flow needed in normal animals ($P < 0.05$), which indicates marked reduction in vasodilator capacity in atherosclerosis of the limb bed, and failure of recovery of vasodilator capacity after regression. Thus, regardless of whether pressure or flow was used as the dependent variable, evidence of marked loss of vasodilator capacity was found in the limb bed of regression monkeys.

Vasoconstrictor responsiveness of limb vessels to norepinephrine was not significantly different in normal, atherosclerotic, and regression animals (Fig. 3). In regression animals there was a tendency for reduced responses.

The contribution of large arteries to alterations of pressure-flow curves was evaluated by measurement of the perfusion pressure gradient between the iliac and dorsal pedal arteries (Fig. 4). The difference in perfusion pressure across large limb arteries was greater in atherosclerotic and regression monkeys than in normal monkeys, particularly at high levels of blood flow. Thus, resistance of large arteries in the limb is increased in both atherosclerotic and regression monkeys.

Coronary vascular resistance

During the control period there were no significant differences in coronary vascular resistance among normal, atherosclerotic and regression monkeys (Table III). During maximal vasodilatation with adenosine, however, vascular resistance was higher in atherosclerotic monkeys than in normals ($P < 0.05$). Minimal vascular resistance in regression animals was intermediate, not differing significantly from either the atherosclerotic or normal groups.

Cerebral vascular resistance

During the control period there were no significant differences among the normal, atherosclerotic and the regression groups (Table IV). During maximal vasodilatation with hypercapnia, minimal vascular resistance in atherosclerotic animals was significantly higher than in normal or regression animals ($P < 0.05$). Min-

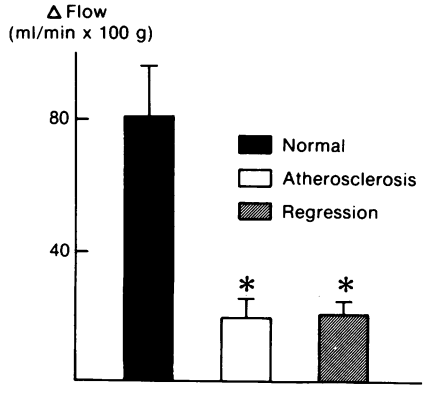

FIGURE 2 Increase in flow during systemic adenosine that was required to attain base-line perfusion pressure of the resting state (control). *$P < 0.05$ compared with normal.

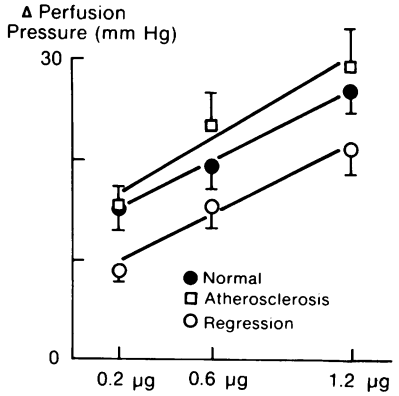

FIGURE 3 Increase in perfusion pressure in the limb bed after bolus injections of norepinephrine. Responses are not significantly different.

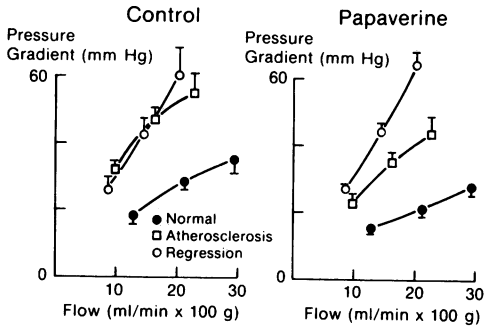

FIGURE 4 Large artery pressure-flow curves in the perfused limb bed in the resting state (control) and after maximal vasodilatation (papaverine). Data points are group means±SE. During control, large artery resistance was different ($P < 0.05$) in regression vs. normal.

imal resistances in regression animals and normals were not significantly different.

DISCUSSION

In this study of hemodynamic effects of atherosclerosis and regression, atherosclerotic lesions of moderate severity (50–60% stenosis) increased minimal vascular resistance in the limb, coronary, and cerebral vascular beds. Regression of atherosclerosis to 25% stenosis had differing hemodynamic effects on the three beds (Fig. 5). Minimal resistance did not decrease in the limb bed. Regression of atherosclerosis tended to reduce minimal resistance in the coronary bed (19%), but improvement was not consistent ($P > 0.05$). In cerebral vessels, minimal resistance was decreased 46% ($P < 0.05$) in regression of atherosclerosis. We will discuss these findings and propose a hypothesis regarding effects of fibrotic changes in arteries on hemodynamic performance in regression.

Atherosclerosis

Whether atherosclerosis causes changes in resting resistance is usually considered to be related to both the degree of stenosis and to the length of the lesion (22). This statement must be qualified by the recent recognition that the atherosclerotic process causes growth of arteries in experimental (23, 24) and human (25) atherosclerosis. The present study illustrates the problem in interpretation raised by this finding: percent stenosis, although not profound, showed more evidence of obstructive change than was found in the comparison of lumen size of atherosclerotic monkeys with that of normals, which showed no clear difference. In some experimental studies the lumens of atherosclerotic arteries are actually larger than those of age-matched controls (23). We anticipate that a full reinterpretation of the atherosclerotic process, observed under controlled conditions, will require integration of the fact of vessel growth with the observations that the altered vessels may increase vascular resistance (as in the present study), and more rarely be a cause of tissue necrosis at relatively low levels of obstructive arterial change (26).

In atherosclerosis induced by hypercholesterolemia in nonhuman primates, diffuse distribution of lesions is observed in primary and secondary branches of the aorta and isolated marked focal narrowing is uncommon (24, 27). Hemodynamic effects reported in this study are therefore those associated with diffuse change in arteries. The lengths of the diffusely altered segments in limb arteries exceeded those in the carotid and coronary arteries by a factor of three or more. This would be expected to increase resistance of the limb bed in atherosclerosis preferentially, if the changes caused by diffuse lesions in arteries are associated with increases in vascular resistance, and might explain in part the finding that of the three arterial beds only the limb bed showed increased resting resistance.

Two other factors may have contributed to increased resting resistance in limb arteries. One is that autoregulatory effects in limb arteries are less than those in coronary and cerebral circulations (28). The second is that atherosclerotic lesions may have been more advanced in the limb, or that lesions in limb arteries are

TABLE III
Coronary Hemodynamic Changes in Atherosclerosis and Regression

	Control			Adenosine		
	Normal	Atherosclerosis	Regression	Normal	Atherosclerosis	Regression
Blood flow, *mm/min × 100 g*	296±36	228±22	199±31	316±32	285±35	269±37
MAP, *mm Hg*	77±3	63±2	38±3	38±3	53±6	35±3
Heart rate	208±8	165±7	203±9	172±26	149±17	139±10
Resistance, *MAP/blood flow*	0.36±0.05	0.42±0.05	0.39±0.06	0.13±0.01	0.21±0.04*	0.17±0.04

Values are means±SE. MAP, mean arterial pressure.
* $P < 0.05$ compared with normal.

TABLE IV
Cerebral Hemodynamic Changes in Atherosclerosis and Regression

	Control			Hypercapnia		
	Normal	Atherosclerosis	Regression	Normal	Atherosclerosis	Regression
CBF, ml/min × 100 g	42±6	36±2	55±9	197±17	105±21	116±15
MAP, mm Hg	80±5	83±4	65±1	79±5	87±9	68±3
CVR, mm Hg/ml/min × 100 g	2.12±0.27	2.47±0.18	1.61±0.33	0.44±0.02	1.17±0.32*	0.66±0.08‡
PO_2, mm Hg	110±7	120±7	114±7	111±10	102±9	115±6
$PaCO_2$, mm Hg	35.9±0.4	36.1±0.6	35.6±0.5	64.1±1.9	64.6±0.9	61.6±0.6
PH_a	7.35±0.01	7.36±0.01	7.37±0.01	7.12±0.02	7.12±0.12	7.14±0.01

Values are means±SE. CBF, cerebral blood flow; MAP, mean arterial pressure; CVR, cerebral vascular resistance; PO_2, arterial PO_2; $PaCO_2$, arterial PCO_2; pH_a, arterial pH.
* $P < 0.05$, compared with normal.
‡ $P < 0.05$, compared with atherosclerosis.

more fibrogenic. Connective tissue stains of the atherosclerotic arteries did not suggest selectively increased fibrosis in the limb arteries. Biochemical estimates of collagen have not been helpful in examining this possibility; measurements of arterial fibrous proteins do not agree well with morphologic evidence of fibrosis (29, 30) or with measurements of arterial elasticity (31, 32).

During maximal vasodilatation, minimal vascular resistance was greater in atherosclerotic than in normal monkeys in all three beds. We have reported previously that vasodilator responses are impaired in the cerebral circulation of atherosclerotic monkeys (33). Maximal vasodilatation eliminates neurohumoral and autoregulatory effects, and allows examination of structural contributions to resistance. In coronary and cerebral beds, significant hemodynamic differences between atherosclerotic and normal monkeys were unmasked only during maximal vasodilatation. This finding is compatible with lesions that produce only moderate atherosclerotic changes in large arteries.

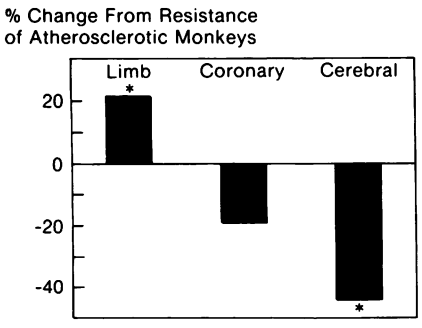

FIGURE 5 Minimal vascular resistance in regression monkeys, expressed as percent change from minimal resistance in atherosclerotic monkeys. Minimal resistance in the limb is that obtained at high flow. *$P < 0.05$ compared to atherosclerosis.

Regression

Regression of atherosclerosis increased the luminal area. In the limb bed, resting resistance was lower in regression than in atherosclerotic monkeys. Of greater physiologic importance, maximal vasodilatation produced no reduction in resistance in the limb bed in regression monkeys. Minimal resistance tended to be even higher in regression monkeys than atherosclerotic monkeys despite luminal enlargement during regression. This finding suggests marked impairment of vasodilator capacity in the limb bed of regression monkeys. We propose that loss of vasodilator capacity in limb arteries of regression monkeys was caused by extensive involvement of large arteries by dense intimal fibrosis.

We should consider several aspects of the conclusion that regression of atherosclerosis does not produce hemodynamic improvement in the limb. Adenosine as well as papaverine disclosed a marked abnormality in limb resistance in regression and atherosclerosis, and the abnormality was noted whether pressure or flow was used as the dependent variable. The finding that the large artery pressure gradient did not decrease in regression monkeys suggests that large arteries of the limb contributed importantly to absence of hemodynamic improvement. It is possible that loss of media might also contribute to impaired dilator responses, but medial mass was not decreased in regression monkeys (Table I). Average medial thickness increased slightly (+5%, not significant) in limb arteries in regression as artery size (IE area) increased 12% (not significant). Occasionally, focal lesions may encroach deeply into the media in cynomolgus monkeys (9). The physiologic significance of this finding is unknown, and it occurred to an equal extent in atherosclerosis and regression. Vasoconstrictor responses of limb arteries to norepinephrine were not significantly altered in atherosclerosis or regression. These responses suggest

that small vessels that account for most of the increase in resistance during infusion of norepinephrine (34) were functionally intact.

Strikingly different hemodynamic results were obtained by Zelis et al. (35) in studies of limb blood flow in men after correction of type III hyperlipoproteinemia. After only 3–6 mo of normalization of plasma lipids, there were increases in peak flow after reactive hyperemia, xanthomas were resorbed, and intermittent claudication improved. It is not clear why functional improvement occurred in man and no improvement was found in the present study of limb arteries in monkeys during maximal vasodilatation. Zelis et al. (35) refer to the prominent foam-cell component that may be present in the lesions of such subjects, with presumed rapid resolution during lipid-lowering programs. The lesions in nonhuman primates are similarly rich in foam cells after induction of experimental atherosclerosis. One possible explanation is that intimal fibrosis in cynomolgus monkeys is much greater in regression than it is in man, but morphologic data on regression in man are too limited to provide any comparison. A second consideration, which Zelis et al. (35) suggested was unlikely in their study, is that development of collateral flow may have produced the hemodynamic improvement that they observed. Preferential formation of collateral channels during regression seems a remote possibility that has not been excluded. Pending new information, the difference in the two studies is unresolved.

In the coronary bed there was no consistent improvement during maximal vasodilatation in regression monkeys. The most dramatic improvement occurred in the cerebral bed during maximal vasodilatation. It should be noted that only the proximal, extracranial portions of the cerebral bed had significant atherosclerosis and subsequent fibrotic regressive changes. Thus, although the degree of intimal fibrosis was histologically comparable in the three beds during regression, the length of the involved vascular segments was greater in limb vessels than in the cerebral bed.

The major implication of this study is that intimal fibrosis of atherosclerotic lesions in large arteries may restrict physiologically important increases in blood flow. Luminal enlargement in regression, following moderate atherosclerotic wall changes, may not be a significant physiologic advantage if the regression arteries have long segments of intimal fibrosis that seriously limit hemodynamic improvement.

Atherosclerosis increased resting resistance significantly in limb arteries, although the relative intimal mass was slightly less than in coronary or cerebral arteries. A major reason may well be the greater relative resistance of large artery lesions in limb vessels. After regression of the intimal lesions, the capacity for dilatation remained severely limited and even tended to worsen in limb vessels. In contrast, dilator capacity was largely restored in cerebral vessels and partially restored in coronary vessels.

ACKNOWLEDGMENTS

We thank Peter Preston, Charles Eastham, Marjorie Megan and Pamela Tompkins for expert technical assistance, and Dr. Allyn Mark for critical review of the manuscript.

Research was supported by the National Institutes of Health, program project grant HL 14388, Arteriosclerosis Specialized Center of Research grant HL 14230, research grants HL 16066 and 20827, Research Career Development Award H1 00328, and by a Medical Investigatorship and Research Grant from the Veterans Administration.

REFERENCES

1. Armstrong, M. L., E. D. Warner, and W. E. Connor. 1970. Regression of coronary atheromatosis in rhesus monkeys. *Circ. Res.* **27:** 59–67.
2. Vesselinovitch, D., and R. W. Wissler. 1978. Prevention and regression in animal models by diet and cholestryamine. *In* International Symposium: State of Prevention and Therapy in Human Atherosclerosis and Animal Models. W. H. Hauss, R. W. Wissler, and R. Lehmann, editors. Westdeutscher Verlag Opladen. **63:** 127–137.
3. Malinow, M. R., P. McLaughlin, W. P. McNulty, H. K. Naito, and L. A. Lewis. 1978. Treatment of established atherosclerosis during cholesterol feeding in monkeys. *Atherosclerosis.* **31:** 185–193.
4. Clarkson, T. B., M. G. Bond, B. C. Bullock, and C. A. Marzetta. 1981. A study of atherosclerosis regression in *Macaca mulatta*. IV. Changes in coronary arteries from animals with atherosclerosis induced for 19 months then regressed for 24 or 48 months at plasma cholesterol concentration of 300 or 200 mg/dl. *Exp. Mol. Pathol.* **34:** 345–368.
5. Armstrong, M. L., and M. B. Megan. 1972. Lipid depletion in atheromatous coronary arteries in rhesus monkeys after regression diets. *Circ. Res.* **30:** 675–680.
6. Wagner, W. D., R. W. St. Clair, T. B. Clarkson, and J. R. Connor. 1980. A study of atherosclerosis regression in Macaca mulatta: III. Chemical changes in arteries from animals with atherosclerosis induced for 19 months then regressed for 48 months at plasma cholesterol concentrations of 300 or 200 mg/dl. *Am. J. Pathol.* **100:** 633–650.
7. Armstrong, M. L., M. B. Megan, and E. D. Warner. 1980. Regression sequences after experimental atherosclerosis. *In* Atherosclerosis V. A. M. Gotto, Jr., L. C. Smith, and B. Allen, editors. Springer-Verlag, New York. pp. 731–734.
8. Tucker, C. F., C. Catsulis, J. P. Strong, and D. A. Eggen. 1971. Regression of early cholesterol-induced aortic lesions in rhesus monkeys. *Am. J. Pathol.* **65:** 493–514.
9. Armstrong, M. L., and M. B. Megan. 1973. Responses of two macaque species to atherogenic diet and its withdrawal. *In* Atherosclerosis III. G. Schettler and A. Weizel, editors. Springer-Verlag, New York. pp. 336–338.
10. Malinow, M. R., P. McLaughlin, L. Papworth, H. K. Naito, L. Lewis, and W. P. McNulty. 1976. A model for therapeutic intervention on established coronary atherosclerosis in a nonhuman primate. *Adv. Exp. Med. Biol.* **67:** 3–31.

11. Vesselinovitch, D., and R. W. Wissler. 1980. Reversal of atherosclerosis: comparison of nonhuman primate models. *In* Atherosclerosis V. A. M. Gotto, Jr., L. C. Smith and B. Allen, editors. Springer-Verlag, New York. pp. 369–374.
12. Abell, L. L., B. B. Levy, B. B. Brodie, and F. B. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195:** 357–366.
13. Manual of Laboratory Operations, Lipid Research Clinics Program, Vol. 1, Lipid and Lipoprotein Analysis. National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD. DHEW Publication No. (NIH) 76-628. May 1974.
14. Marcus, M. L., D. D. Heistad, J. C. Ehrhardt, and F. M. Abboud. 1976. Total and regional cerebral blood flow measurement with 7–10, 15-, 25-, and 50-μM microspheres. *J. Appl. Physiol.* **40:** 501–507.
15. Marcus, M. L., C. J. Bischof, and D. D. Heistad. 1981. Comparison of microsphere and xenon-133 clearance method in measuring skeletal muscle and cerebral blood flow. *Circ. Res.* **48:** 748–761.
16. Marcus, M. L., M. L. Armstrong, D. D. Heistad, C. L. Eastham, and A. L. Mark. 1982. A comparison of three methods of evaluating coronary obstructive lesions; postmortem arteriography, pathological examination and measurement of regional myocardial perfusion during maximal vasodilatation. *Am. J. Cardiol.* **49:** 1699–1706.
17. Miller, R. G., Jr. 1981. Simultaneous Statistical Inference, 2nd edition, Springer-Verlag, New York. pp. 67–81.
18. Greenhouse, W. W., and S. Geisser. 1959. Methods of the analysis of profile data. *Psychometrika.* **24:** 95–112.
19. Hollander, M., and D. A. Wolfe. 1973. Nonparametric Statistical Methods. John Wiley and Sons, New York. pp. 233–261.
20. Kramsch, D. M., and W. Hollander. 1968. Occlusive atherosclerotic disease of the coronary arterys in monkey (Macaca irus) induced by diet. *Exp. Mol. Pathol.* **9:** 1–22.
21. Wagner, W. D., R. W. St. Clair, and T. B. Clarkson. 1978. Angiochemical and tissue cholesterol changes in *Macaca fascicularis* fed an atherogenic diet for three years. *Exp. Mol. Pathol.* **28:** 140–153.
22. Hillis, W. S., and G. C. Friesinger. 1976. Reactive hyperemia: An index of the significance of coronary stenosis. *Am. Heart J.* **92:** 737–740.
23. Bond, M. G., M. R. Adams, and B. C. Bullock. 1981. Complicating factors in evaluating coronary artery atherosclerosis. *Artery.* **9:** 21–29.
24. Hollander, W., S. Prusty, S. Nagraj, J. Paddock, and M. Colombo. 1981. Morphometric changes in the coronary arteries during the induction and "regression" of atherosclerosis. *Circulation.* **64**(IV): 45.
25. Young, W., J. W. Gofman, and R. Tandy. 1960. The quantitation of atherosclerosis. 1. Relationship to artery size. *Am. J. Cardiol.* **6:** 288–293.
26. Bond, M. G., B. C. Bullock, D. A. Bellinger, and T. E. Hamm. 1980. Myocardial infarct in a large colony of nonhuman primates with coronary artery atherosclerosis. *Am. J. Pathol.* **101:** 675–691.
27. Armstrong, M. L., and E. D. Warner. 1971. Morphology and distribution of diet-induced atherosclerosis in the rhesus monkey. *Arch. Pathol.* **92:** 395–401.
28. Johnson, P. C. 1980. The Myogenic Response. *In* Handbook of Physiology, Section 2: The Cardiovascular system. D. F. Bohr, A. P. Somlyo, and H. V. Sparks, Jr., editors. American Physiological Society, Bethesda, MD. pp. 409–442.
29. Armstrong, M. L., and M. B. Megan. 1975. Arterial fibrous proteins in cynomolgus monkeys after atherogenic and regression diets. *Circ. Res.* **36:** 256–261.
30. Wagner, W. D., R. W. St. Clair, and T. B. Clarkson. 1980. A study of atherosclerosis regression in Macaca mulatta. II. Chemical changes in arteries from animals with atherosclerosis induced for 19 months then regressed for 24 months at plasma cholesterol concentrations of 300 or 200 mg/dl. *Exp. Mol. Pathol.* **32:** 162–174.
31. Farrar, D. J., H. D. Green, M. G. Bond, W. D. Wagner, and R. A. Gobbee. 1978. Aortic pulse wave velocity elasticity, and composition in a nonhuman primate model of atherosclerosis. *Circ. Res.* **43:** 52–62.
32. Farrar, D. J., H. D. Green, W. D. Wagner, and M. G. Bond. 1980. Reduction in pulse wave velocity and improvement of aortic distensibility accompanying regression of atherosclerosis in the rhesus monkey. *Circ. Res.* **47:** 425–432.
33. Heistad, D. D., M. L. Marcus, D. J. Peigors, and M. L. Armstrong. 1980. Regulation of cerebral blood flow in atherosclerotic monkeys. *Am. J. Physiol.* **239:** H539–H544.
34. Abboud, F. M., and J. W. Eckstein. 1966. Comparative changes in segmental vascular resistance in response to nerve stimulation and to norepinephrine. *Circ. Res.* **18:** 263–277.
35. Zelis, R., D. T. Mason, E. Braunwald, and R. I. Levy. 1970. Effects of hyperlipoproteinemia and their treatment on the peripheral circulation. *J. Clin. Invest.* **49:** 1007–1015.