# Effects of Hyperlipoproteinemias and their Treatment on the Peripheral Circulation

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ABSTRACT The purpose of this study was to determine the effect of familial hyperlipoproteinemia (HLP) on peripheral vascular disease (PVD) and the extent to which the vascular disease is modified by treatment of the lipoprotein disorder. PVD was detected plethysmographically by observing a diminished peak reactive hyperemia blood (PRHBF) following ischemia. The value for PRHBF in the extremity demonstrating the lowest response in 32 normal subjects (age 19–50 yr) was 39.6 ±1.5 SEM, ml/min per 100 g. Patients with untreated HLP, who had PRHBF below the lower limit of normal, were 2 of 11 type II, 9 of 12 type III, 1 of 10 type IV. As a group, patients with type III HLP showed diminished PRHBF (26.6 ±3.0 ml/min per 100 g, $P < 0.01$). In view of the high incidence of PVD and the striking reduction in serum lipids and complete resorption of xanthomas observed in type III HLP with therapy, six patients were studied before and after 3–6 months of treatment with a therapeutic diet and clofibrate. PRHBF in the most severely affected extremity increased markedly, from 20.4 ±1.6 to 31.9 ±1.8 ml/min per 100 g ($P < 0.01$), indicating a dramatic increase in maximum blood flow to this extremity. In two type III patients with PVD not treated, no change in PRHBF occurred over 5 months. In two other type III patients the PRHBF increased 17% during the first 25 days of therapy concomitant with a 30% reduction in whole blood viscosity. Over the next 120 days, blood viscosity decreased only an additional 4.6% whereas the PRHBF increased 57%, indicating that the observed changes seen in the PRHBF with therapy of type III patients can be only minimally accounted for by changes in the viscosity of the blood. Thus, patients with type III HLP are particularly susceptible to the development of PVD and objective improvement of PVD can occur with medical treatment of this lipid transport disorder.

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## INTRODUCTION

Whereas it is generally accepted that subjects with hyperlipidemia have an increased risk of premature coronary artery disease (1–6), it is unclear whether efforts designed to lower serum lipids will arrest or reverse the course of the atherosclerotic process. Since population studies have thus far yielded equivocal results (7–9), it was considered desirable to examine the response of a specific vascular bed to treatment designed to lower blood lipids. In view of the difficulties inherent in measuring serial changes in the coronary circulation, it was our plan to study the vasculature of the extremities, in which the functional evaluation is simple, atraumatic, and accurate (10–14). Patients with well defined lipid abnormalities from the National Institutes of Health registry of familial hyperlipoproteinemias were selected for this study (15, 16).

Type II or hyperbetalipoproteinemia characterized by hypercholesterolemia and xanthomatosis and type IV or hyperprebetalipoproteinemia characterized by endogenous hypertriglyceridemia with or without hypercholesterolemia have both been associated with premature coronary vessel disease (15, 16, 18). Type III hyperlipoproteinemia has been characterized by the presence of abnormal beta lipoproteins, hypercholesterolemia, hypertriglyceridemia, xanthomatosis, and premature coronary and peripheral vessel disease. Combined drug and diet therapy has been shown to normalize the plasma lipids in these patients completely and to result in early resolution of external xanthomatosis (17). This dramatic responsiveness to treatment makes the type III subject an ideal one to answer the question of whether lowering

TABLE I
*Patients with Hyperlipoproteinemia*

| Type | II | III | IV |
|---|---|---|---|
| Total number | 11 | 12 | 12 |
| Average age, *yr* | 36.5 ±2.5 | 45.5 ±3.1 | 45.9 ±2.9 |
| Myocardial infarction or angina pectoris, *No. of patients* | 6 | 5 | 8 |
| Intermittent claudication, *No. of patients* | 0 | 4 | 0 |
| Abnormal glucose tolerance, *No. of patients* | 0 | 0 | 1 |
| Average serum cholesterol, *mg/100 ml* | 338 ±31 | 287 ±27 | 236 ±15 |
| Average serum triglyceride, *mg/100 ml* | 112 ±13 | 383 ±76 | 295 ±37 |

blood lipids can reverse or alter the atherosclerotic process.

However, the association between peripheral vascular disease and these specific lipoprotein abnormalities has not been established. Accordingly, there were two purposes of this investigation. The first was to evaluate the peripheral circulation of patients with type III hyperlipoproteinemia before and after their serum lipids had been lowered and maintained in the normal range from 3 to 6 months, and the second was to examine the peripheral circulation of patients with types II and IV hyperlipoproteinemia for evidence of impaired peripheral vessels. A preliminary report of this study has been presented elsewhere (19).

## METHODS

Studies were carried out on a total of 32 normal subjects between 19 and 50 yr of age (average 29.3 yr) and 35 patients with familial hyperlipoproteinemia from the NIH registry. The characteristics of the patients with familial hyperlipoproteinemia are shown in Table I. A separate group of 11 normal control subjects above age 30 (average 41.9 yr) was selected for comparison with the patients with lipoprotein abnormalities. Classification was established by paper electrophoresis and ultracentrifugation techniques coupled with lipid determinations and family screening as described elsewhere (15, 16). None of the patients had congestive heart failure or hypertension. Forearm and calf blood flow were measured bilaterally, with the limbs at heart level and the subject recumbent. The venous occlusion technique was employed (20), which utilized a single-strand mercury-in-rubber strain gauge plethysmograph (14). Circulation to the hand or foot was arrested by inflating a cuff around the wrist or ankle for at least 1 min before each determination of blood flow (21). All measurements were performed with a venous collecting pressure of 30 mm Hg (22) with the subjects in the basal, postabsorptive state with at least a 15 min period of equilibration being allowed to elapse after placement of all instruments and before blood flow recordings.

Control blood flow was taken as the average of 6–10 flow measurements made at 15-sec intervals before each intervention. Ischemia of the forearm or calf was produced by inflating the proximal cuff to suprasystolic pressure for 1, 3, 5, and 10 min. After release of arterial occlusion, blood flow was measured initially at 5 and 15 sec after release and every 15 sec thereafter until it returned to control levels. The first determination of PRHBF was always discarded (22). Since the peak reactive hyperemia blood flow (PRHBF) was not appreciably increased when the duration of ischemia was prolonged beyond 5 min, this level of PRHBF was considered to represent the maximal ability of the blood vessels to dilate to an ischemic stimulus (10–13, 22, 23). The PRHBF of any extremity was taken as the average of two determinations of peak flow after release of 5 or 10 min of arterial occlusion.

PRHBF was determined after release of 5 and 10 min of arterial occlusion in 23 normal subjects and was repeated after an interval of 32 ±5.1 days in 16 subjects. PRHBF was determined in duplicate for each extremity of all patients with hyperlipoproteinemia. Six patients with type III hyperlipoproteinemia were studied an average of 131.5 ±10.2 days after treatment with an isocaloric diet low in cholesterol (< 300 mg/day) and balanced in fat and carbohydrate (40% of calories of each) and clofibrate (2 g/day) (17). In three patients ideal body weight was achieved by caloric restriction over 6–8 wk before the isocaloric diet was instituted.

The whole blood viscosity measured with a capillary viscometer was examined in two patients with type III hyperlipoproteinemia before and after lowering of the serum lipid levels by marked caloric restriction to 600 calories a day for 25 days, and again after 120 days of treatment with diet and clofibrate as described above. The PRHBF was determined on each of the three occasions. All comparisons between control subjects and patients with hyperlipoproteinemia and before and after an intervention in individuals were based on the values for the lowest RHBF observed in any of the four extremities.

## RESULTS

*Normal subjects.* The characteristics of the blood flow response after restoration of the circulation to a temporarily ischemic limb are illustrated in Fig. 1. As the duration of arterial occlusion was increased to 5 min, the subsequent peak blood flow rose progressively. However, when arterial occlusion was prolonged beyond 5 min, there was little or no further increase in the peak blood flow (Fig. 1 C); rather, the duration of the hyperemic response became more prolonged (12, 23) (Fig. 1 A). Therefore, as the duration of arterial occlusion was prolonged, the total blood flow in excess of control values (total reactive hyperemia blood flow) continued to increase (Fig. 1 B).

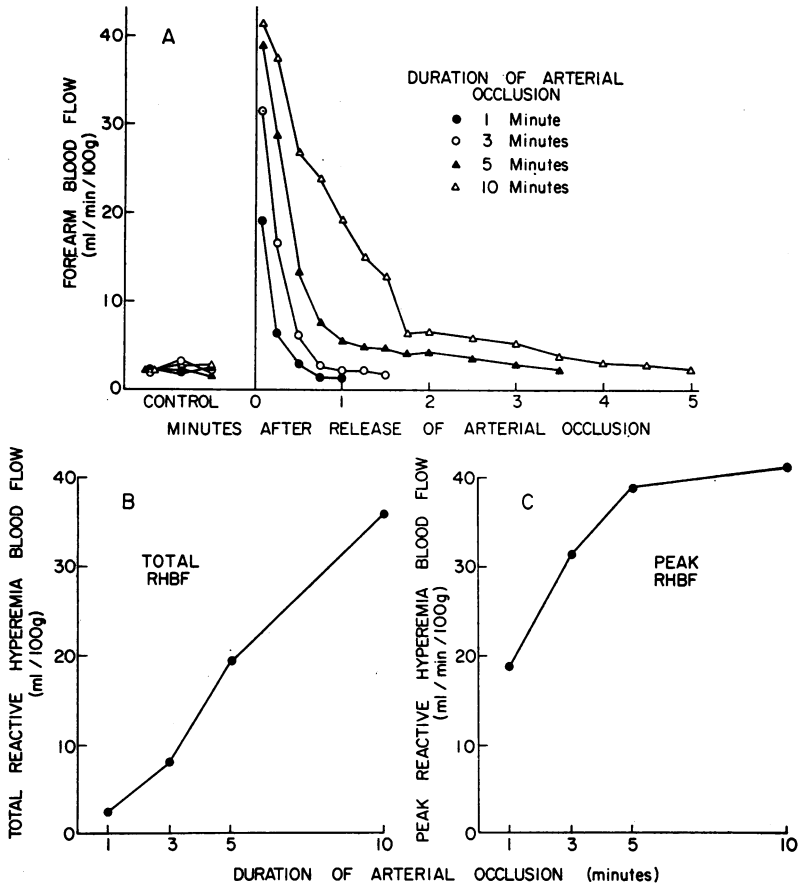


FIGURE 1 Reactive hyperemia blood flow response of a normal subject. (A) Forearm blood flow response before and after release of 1, 3, 5, and 10 min of arterial occlusion. The zero point on the abscissa represents the time of release of the arterial occlusion. (B) Total reactive hyperemia blood flow (RHBF) determined as the total blood flow in excess of the control blood flow, as a function of the duration of arterial occlusion. (C) Peak reactive hyperemia blood flow after release of arterial occlusions of various durations.

In 23 subjects the PRHBF following 5 min of arterial occlusion was 38.3 ±2.52 ml/min per 100 g (SEM) and that following 10 min of arterial occlusion was 41.8 ±2.38 ml/min per 100 g, a difference which was not significant ($P > 0.5$) (Fig. 2 A). Likewise in nine subjects, the PRHBF measured in the forearm and the calf was not appreciably different and averaged respectively 50.4 ±4.90 ml/min per 100 g (forearm) and 46.6 ±3.82 ml/100 g ($p > 0.5$) (calf). The similarity of the response of the forearm and the calf is also evident in Fig. 3, in which it is apparent that the plateaus of PRHBF occurred at similar levels; the calf PRHBF, however, reached this plateau after a shorter duration of arterial occlusion (3 min) than that in the forearm (5 min). The PRHBF of the left and right extremities measured sequentially on the same day was similar ($P > 0.5$) (Fig. 2 C). In 16 control subjects studied an average of 32 ±5.1 days apart, without any intervention between the two studies, PRHBF was unchanged, averaging 36.8 ±1.68 ml/min per 100 g at the time of the first study and 39.2 ±1.79 ml/min per 100 g at the time of the second study ($P > 0.3$) (Fig. 2 D).

The relationship between the lowest PRHBF found in any extremity of a subject and the age of the normal control subject is seen in Fig. 4. This value has no relation to the age of the subject. The lowest value for PRHBF observed in any extremity of any of the normal subjects tested was 27 ml/min per 100 g and was taken as the lower limit of normal. PRHBF in the extremity demonstrating the lowest blood flow, averaged for all normal subjects, was 39.6 ±1.50 ml/min per 100 g and for the older normal subjects was 37.8 ±2.52 ml/min per 100 g (Fig. 5 A and B).

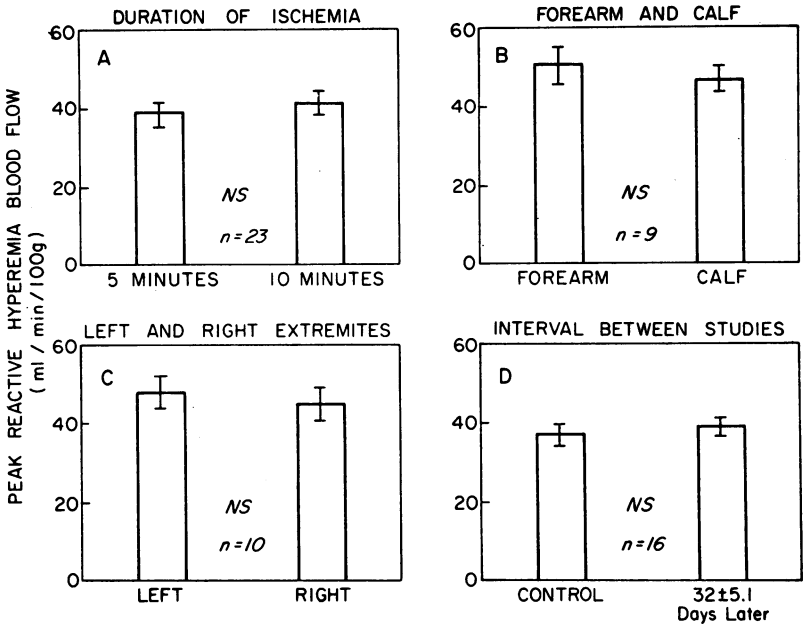


FIGURE 2 Factors found not to influence peak reactive hyperemia blood flow. (A) Peak reactive hyperemia blood flow following release of 5 and 10 min of arterial occlusion, (B) measurements in the forearm and calf of the same individual, (C) measurements in the left and right extremities of the same individual, and (D) peak reactive hyperemia blood flow measured in the same individual on two different occasions. NS, not significant.

*Patients with hyperlipoproteinemia.* In the 11 patients with type II hyperlipoproteinemia the mean value for PRHBF in the extremity with the lowest blood flow was 34.3 ±1.9 ml/min per 100 g, a value which was not significantly different from that observed in either all of the normal subjects ($P > 0.05$) or the subgroup of normal subjects over 30 yr of age ($P > 0.05$) (Fig. 5 C). Only two patients with type II hyperlipoproteinemia had values just below the lower limit of normal. The average PRHBF for the extremity with the lowest blood flow of the patients with type IV disease was 34.6 ±2.9 ml/min per 100 g, a value which did not

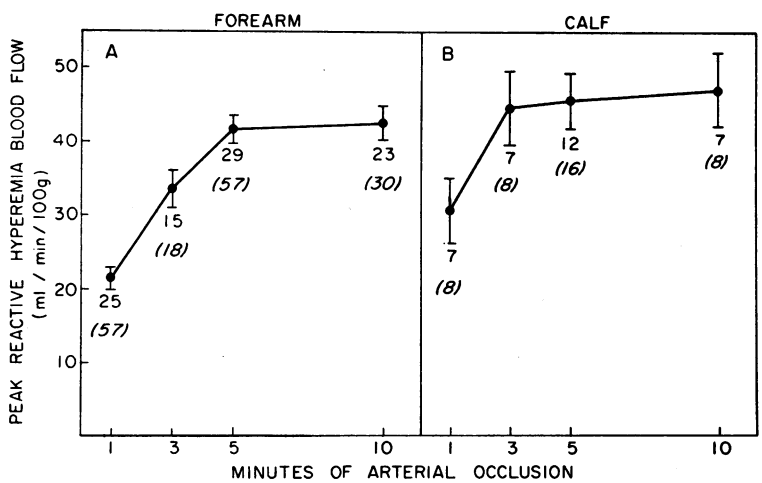


FIGURE 3 Average values (±SEM) of peak reactive hyperemia blood flow measured in the forearm (A) and calf (B) following release of occlusion of varying durations. The number of subjects is shown beneath each point and the total number of observations in parentheses.

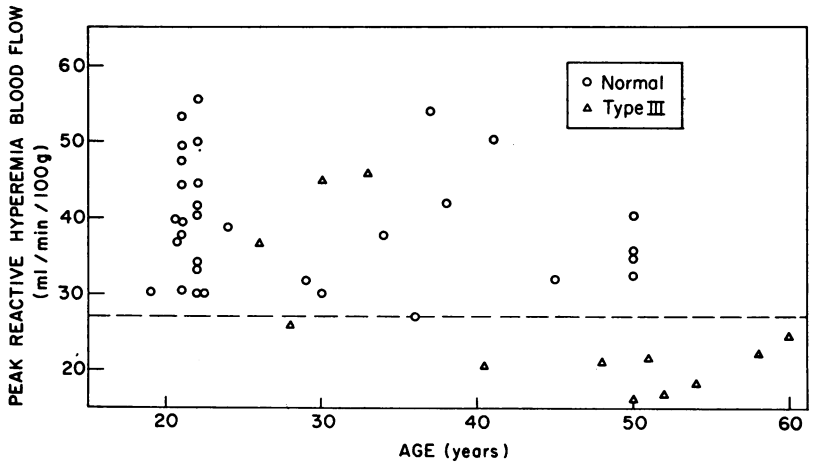


FIGURE 4 The lowest peak reactive hyperemia blood flow in any extremity as a function of age. Normal subjects are shown as open circles. Patients with type III hyperlipoproteinemia as open triangles. RHBF, reactive hyperemia blood flow.

differ from that observed in the normal subjects ($P > 0.05$) or the older normal subgroup ($P > 0.05$) (Fig. 5 E). Only one patient with type IV disease had a value for PRHBF of any extremity less than the lower limit of normal. The PRHBF for the extremity with the lowest blood flow in patients with type III disease averaged 26.6 ±3.0 ml/min per 100 g, a value which was significantly lower than that observed in any normal subjects ($P < 0.01$) (Fig. 5 D). 9 of the 12 patients with type III disease had values for PRHBF less than 27 ml/min per 100 g, i.e., below the lower limit of normal. The PRHBF of the patients with type III disease significantly decreased as the age of the subject increased (Fig. 4). A regression equation fitted to these data had a correlation coefficient of 0.6322 ($P < 0.05$).

*Response to treatment in patients with type III hyperlipoproteinemia.* Six patients with type III hyperlipoproteinemia were studied before and after 131.5 ±10.2 days of treatment. Their serum cholesterol decreased from 379 ±12.1 to 181 ±14.7 mg/100 ml ($P < 0.01$) and their serum triglyceride from 542 ±130.3 to 111 ±22.9 mg/100 ml ($P < 0.02$). After treatment the PRHBF value for the lowest extremity rose from 20.4

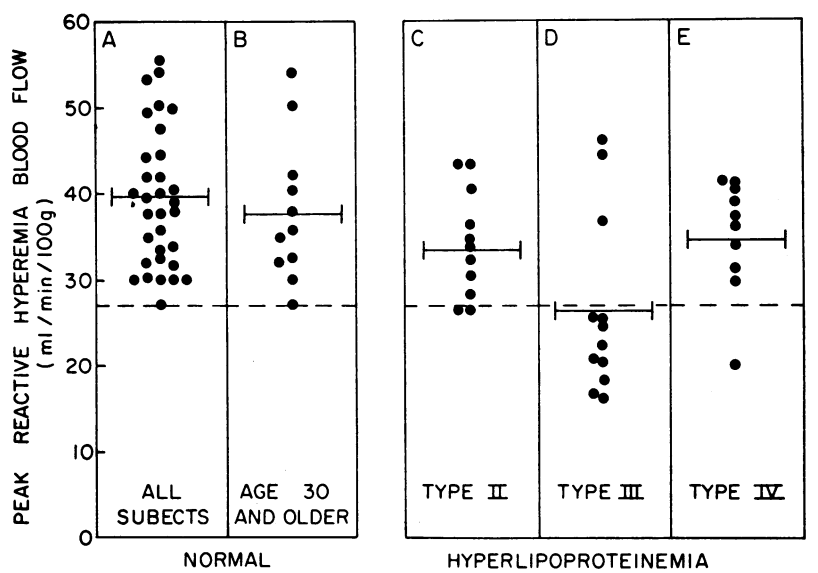


FIGURE 5 Effects of age and various forms of hyperlipoproteinemia on peak reactive hyperemia blood flow (RHBF).

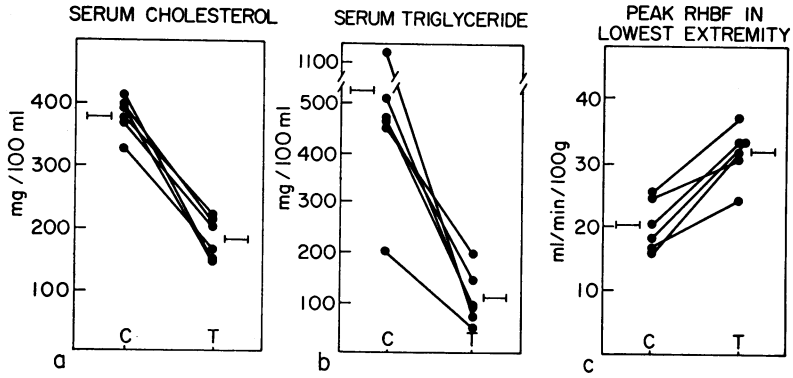


FIGURE 6 Response of six patients with type III hyperlipoproteinemia to treatment. Panel *a* serum cholesterol, panel *b* serum triglyceride, panel *c* the lowest peak reactive hyperemia blood flow in any extremity. C, control observation; T, treatment.

±1.6 to 31.9 ±1.8 ml/min per 100 g, a significant improvement ($P < 0.01$) (Figs. 6 and 7). The resting blood flow in this extremity did not change significantly with treatment (3.5 ±0.42 to 4.9 ±0.94 ml/min per 100 g ($P > 0.1$), nor was there a significant change in cuff blood pressure.

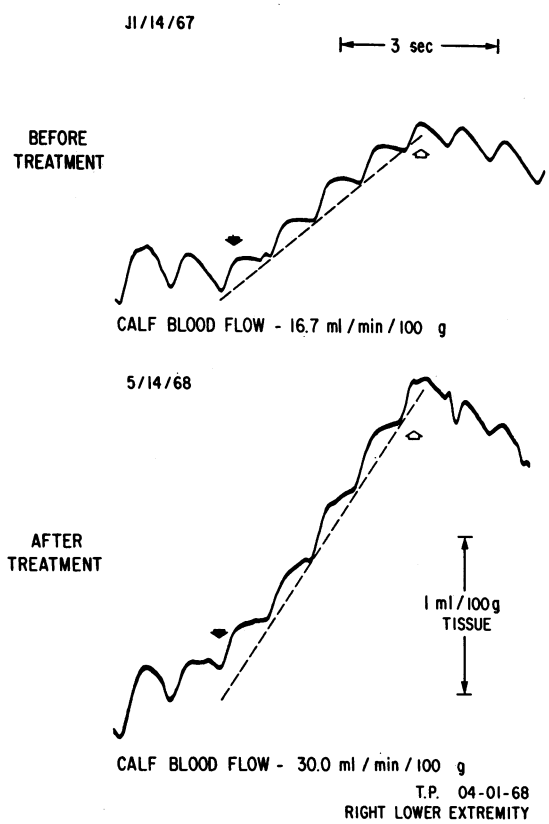


FIGURE 7 Plethysmographic tracings of the peak reactive hyperemia blood flow before and after treatment in a patient with type III hyperlipoproteinemia. The closed arrows indicate the onset of venous occlusion at 30 mm Hg. The open arrows indicate deflation of the venous collecting cuff.

In two patients whose lipids were lowered over a period of 21 days, the whole blood viscosity measured at 37°C at a shear rate of 1600 $sec^{-1}$ fell from 6.97 and 6.11 to 4.28 and 4.90 centipoise respectively, whereas the PRHBF rose from 19.5 and 16.1 ml/min per 100 g to 22.6 and 19.1 ml/100 g respectively. After prolonged therapy, the whole blood viscosity was 4.09 and 4.66 centipoise at the same shear rate and the PRHBF of the same extremities which were previously measured rose to 33.7 and 31.7 ml/min per 100 g respectively (Fig. 8).

Two patients who were not treated were studied on two occasions, 154 and 193 days apart. Their lipid values were essentially unchanged and the value for the PRHBF in the most severely affected extremity rose only minimally, from 22.7 and 22.9 ml/min per 100 g to 24.9 and 26.8 ml/min per 100 g respectively.

## DISCUSSION

The plateau in PRHBF which is achieved following arterial occlusion is considered to approach the maximal ability of the resistance blood vessels to dilate to an ischemic stimulus (22, 23). The PRHBF has been repeatedly demonstrated to be lowered in patients with peripheral vascular disease, even though the basal blood flow usually is normal (10–13). An ischemic stimulus presumably dilates the arterioles sufficiently so that the caliber of the larger arteries, i.e. the inflow vessels, becomes the critical site of resistance. Therefore, a reduction in the peak blood flow to an extremity indicates a reduced caliber in these large blood vessels if there is no evidence of arteriolar disease (14, 24, 25).

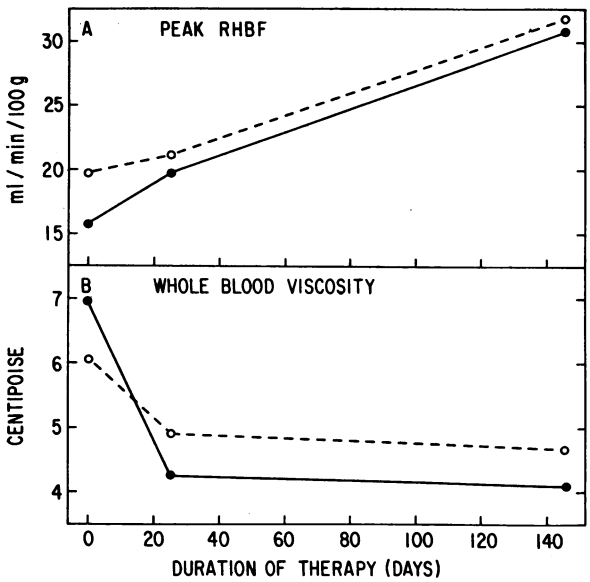


FIGURE 8 The response of the peak reactive hyperemia blood flow (RHBF) (A), and the whole blood viscosity (B), to treatment in two patients with type III hyperlipoproteinemia. During the first 25 days, therapy consisted of marked caloric restriction. During the last 120 days the patients received an isocaloric, low cholesterol diet and clofibrate as described in the text.

The reproducibility of this measurement in normal subjects was demonstrated by the finding that the PRHBF is similar in the forearm and calf as well as in both upper and lower extremities. Also, in 16 subjects studied on two different occasions the PRHBF showed little variation. In trying to determine which individuals with familial hyperlipoproteinemia had abnormal values of PRHBF, it was important to determine the lowest value which might be expected in a normal subject. This value was found to be 27 ml/min per 100 g in the 32 subjects studied and any value below this was considered to be abnormal.

Since the ages of the patients with familial hyperlipoproteinemia were greater than those of the normal subjects, comparisons were made not only between the patients with the entire group of normal subjects but also with a separate subgroup of normal subjects of comparable age. It was demonstrated that the PRHBF in patients with types II and IV hyperlipoproteinemia was not significantly different from that seen in the entire group of normal subjects or in the older subgroup. Furthermore, only 2 of the 11 patients with type II disease and 1 of the 12 patients with type IV disease had values of PRHBF which were abnormally low and this one type IV patient was the only patient studied who also had diabetes mellitus and required tolbutamide. It was of interest that 6 of the 11 patients with type II disease and 8 of the 12 patients with type IV disease had a significant degree of coronary artery disease. Thus, the coronary circulation appears to be more severely affected in these individuals than the peripheral circulation.

The patients with type III disease had an incidence of myocardial infarction or angina pectoris similar to that exhibited by patients with type II and type IV disease (Table I), but four of them also suffered from intermittent claudication. The mean value for PRHBF was significantly below the average observed in the normal subjects as well as in the subgroup of older normal subjects. None of the patients with type III disease had congestive heart failure (19) or hypertension (24), conditions which have been shown to reduce PRHBF. Therefore, it would appear that the metabolic abnormality in patients with type III hyperlipoproteinemia not only is responsible for premature coronary artery disease but is also important in the etiology of premature peripheral vascular disease. Moreover, the severity of the vascular disease appears to be greater as the age of the patient increases (Fig. 4). The only type III patients whose PRHBF fell into the normal range were less than 34 yr.

Patients with type III hyperlipoproteinemia are ideal for studying the response of peripheral blood vessels to treatment of the hyperlipoproteinemic state. Not only can their serum cholesterol and triglyceride levels be lowered to the normal range with weight reduction, a balanced low cholesterol diet, and clofibrate, but they also demonstrate a remarkable ability for the xanthoma to resorb (17). This can occur in 3 months when the lipid levels are maintained in a normal range (17). Both the serum cholesterol and triglycerides were reduced to the normal range in all six patients with type III hyperlipoproteinemia in this series who were treated vigorously. When these values were maintained for 3 to 6 months, the PRHBF increased by an average of 55%, a significant improvement (Figs. 6 and 7). Although it has been shown that there is symptomatic improvement in angina pectoris and intermittent claudication with prolonged treatment of patients with type III hyperlipoproteinemia (17), this is the first demonstration of an improvement in blood flow to the extremities, reflecting a reduction in vascular resistance, in these patients.

Two of the six patients with type III hyperlipoproteinemia who were treated had angina pectoris; one improved significantly, and in the other this symptom disappeared entirely during treatment. Of the three patients in this group with intermittent claudication, two improved and one became symptom free. One patient without peripheral pulses below the femoral artery developed a palpable dorsalis pedis pulse after

therapy. When the whole blood viscosity was lowered in two patients by marked caloric restriction over 25 days the PRHBF increased 17%, whereas whole blood viscosity decreased 30%. Over the next 4 months of conventional therapy, during which the serum lipid levels were maintained in the normal range, the blood viscosity decreased only an additional 4.6%. However, the PRHBF increased 57% during this time suggesting that the major determinant of the increased blood flow to the extremities of these subjects was an improvement in their vascular disease (Fig. 8). On the other hand, two patients with type III disease who were not treated showed no improvement in their PRHBF over a 5–6 month period. Therefore, it can be concluded that the increased PRHBF in the patients with type III hyperlipoproteinemia following treatment represents a significant improvement in blood flow to their affected extremities which was only minimally accounted for by alterations in the rheological properties of the blood (26, 27).

It may be presumed either that plaques in the main vessels to these limbs were significantly resorbed or that collateral circulation improved. Since the patients were not on an exercise program, the observed improvement in RHBF is more likely to have occurred secondary to the resorption of cholesterol-rich atheromata. It is not unreasonable to think that the labile lipid portion of atheromatous plaques can be resorbed from the vessel wall to some degree, considering the marked reduction in the size of the xanthoma in patients with type III disease following treatment (17). The recent finding in a type III patient who died of a myocardial infarction that there were endocardial plaques which resembled planar xanthoma, and that this patient's coronary arteries were laden with foam cell lesions (28) further supports the position that the vascular cholesterol in type III patients is very labile. Since, at any given perfusion pressure, the flow in a vessel is proportional to the fourth power of the radius, it is easy to see that a small increase in the size of the vessel can result in a significant improvement in flow.

These observations then, are considered to be best interpreted as evidence to indicate reversal in the progress of atherosclerotic vascular disease by therapy aimed at the sustained reduction of serum lipids. It can be hoped that the improvement in the peripheral circulation demonstrated in these patients also occurred in other vascular beds, notably the coronary and cerebral. That this improvement occurred was suggested by the improvement in angina. It is unknown whether or not prolonged treatment of patients with type II and type IV hyperlipoproteinemia will result in an improvement in their coronary circulations. However, from the results of this study with type III hyperlipoproteinemia, which serves as an ideal experimental model for the short term study of vascular disease, it may be hoped that with prolonged therapy of the other lipid disorders with sustained lowering of blood lipid values, circulatory improvement will also occur.

## ACKNOWLEDGMENTS

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