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Abstract

Improvement in tissue perfusion following surgically induced ischemia in limbs of dogs was experimentally evaluated to clarify the improvement of hemodynamics following walking exercise in chronic, peripheral arterial occlusive diseases. With the use of a computer system in conjunction with medical mass spectrometry, the local tissue perfusion rate was calculated on the basis of the clearance curve of tissue partial pressure of CO2 following electrical stimulation of the ischemic leg to simulate exercise. Ischemia was created in the leg by ligation of the proximal and peripheral arteries. In one month, intermittent claudication improved in accordance with improvement in muscle tissue perfusion. Angiographic evidence of distal runoff became visible six months after surgery, indicating that tissue perfusion played an important role in peripheral hemodynamics. The local tissue perfusion rate improved from 9.51 +/- 2.62 ml/100 g/min to 12.41 +/- 2.42 in one month, to 14.59 +/- 3.19 in three months, to 15.11 +/- 3.24 in six months and to 17.19 +/- 2.63 in twelve months. The improvement of ischemic symptoms following long-term exercise is attributed to improvements in tissue perfusion or collateral circulation.

KEYWORDS: ischemic legs, tissue perfusion, mass spectrometry, walking exercise, intermittent claudication

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Evaluation of Tissue Perfusion in Ischemic Legs of Dogs by CO_2 Clearance Rate

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Improvement in tissue perfusion following surgically induced ischemia in limbs of dogs was experimentally evaluated to clarify the improvement of hemodynamics following walking exercise in chronic, peripheral arterial occlusive diseases. With the use of a computer system in conjunction with medical mass spectrometry, the local tissue perfusion rate was calculated on the basis of the clearance curve of tissue partial pressure of CO_2 following electrical stimulation of the ischemic leg to simulate exercise. Ischemia was created in the leg by ligation of the proximal and peripheral arteries. In one month, intermittent claudication improved in accordance with improvement in muscle tissue perfusion. Angiographic evidence of distal runoff became visible six months after surgery, indicating that tissue perfusion played an important role in peripheral hemodynamics. The local tissue perfusion rate improved from 9.51 ± 2.62 ml/100 g/min to 12.41 ± 2.42 in one month, to 14.59 ± 3.19 in three months, to 15.11 ± 3.24 in six months and to 17.19 ± 2.63 in twelve months. The improvement of ischemic symptoms following long-term exercise is attributed to improvements in tissue perfusion or collateral circulation.

Key words : ischemic legs, tissue perfusion, mass spectrometry, walking exercise, intermittent claudication

As a result of progress in vascular surgery, peripheral arterial reconstructive surgery has been the therapy of choice for chronic occlusive diseases. Peripheral arterial reconstruction, however, even under the most optimum conditions such as when using femoropopliteal bypasses with autovein, has not always produced satisfactory results according to long-term (5-10 years) follow-up studies (1, 2). Furthermore, occlusive disease can sometimes develop significantly faster in an operated limb than in a non-operated one (3).

On the other hand, medical or conservative treatment is justified on the basis of pathophysiological observations that walking exercise is preferred to surgery for ameliorating the peripheral circulation, unless impending or manifest gangrene necessitates immediate surgery (4-9).

This paper presents experimental data

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demonstrating the quantitative increase in tissue perfusion in the ischemic leg following exercise over a period of time, which thus supports the nonsurgical treatment of walking exercise for patients suffering from intermittent claudication.

Materials and Methods

Ischemia was created in the limbs of 15 adult mongrel dogs of both sexes, each weighing approximately 10 kg, by ligation of proximal (internal and external iliac, middle sacral and contralateral internal iliac) and peripheral (femoral) arteries (10).

The dogs were allowed to freely move about in an open area following the preparation of the ischemic limb. The degree of claudication was determined by the length of time the dogs could run at the pace of 700 m per 8 min.

One-shot arteriography was performed using an aortic catheter and 60% meglumine iothalamate under venous anesthesia with sodium pentobarbital. The arteriography was performed one, three, six, and twelve months following the preparation of the ischemic limb.

A medical mass spectrometer, Medspect® (Chemetron, USA), was used to determine the partial pressures of muscle tissue gases. The mass spectrometer/catheter system was calibrated prior to each measurement, and it was operated at a constant sensitivity following calibration. The drift in sensitivity of the mass spectrometer was less than 5% per day. Brief changes in gas tension of ±1 torr could be reliably detected.

Both static (thermal effect) and dynamic (time delay) characteristics of medical mass spectrometric measurement through the catheter were computed for partial pressures of tissue gases. Thereafter, a linear correlation of the thermal effect was confirmed experimentally, and the data read on the machine was converted to the transfer function in order to eliminate the individual time delay of measurements through the catheter (11).

A probe (18 gauge) was inserted into the cranial tibial muscle, being careful to avoid hematoma formation around the sensor area. The puncture was made with a sleeve-clad 14 gauge needle (Medicut®, Sherwood Medical Industries, Inc., USA). The needle was withdrawn, leaving a plastic sleeve through which the catheter was subsequently slipped. After passing the catheter, the plastic sleeve was also withdrawn. A plaster bandage or skin sutures anchored the prove and prevented its movement in the muscle tissue.

Although the Teflon membrane was 0.059 inch in external diameter and 3/4 inches in length, there was never any bleeding along the membrane tract unless a major vessel was inadvertently punctured. The area of the Teflon membrane, through which Medspect measures the gas tensions in the extracellular fluid in which it is immersed, is large enough to ensure an average measurement. An average measurement represents a balance between the cellular production of carbon dioxide and the wash out as well as providing an estimation of tissue perfusion and a quantitative measurement of the available gases (12, 13).

The dog was placed on intravenous anesthesia following intubation and maintained by a constant micro-drip infusion of 3.75 mg/min with sodium pentobarbital. Ten respirations per min on room air were assisted at 10 mmH_2O pressure.

The local tissue perfusion rate of the ischemic limb was calculated on the basis of a clearance curve of the partial pressure of CO_2 in the muscle tissue following electrically stimulated leg exercise. Kety's clearance theory was applied to Fick's principle in determining the regional blood flow volume assuming that CO_2 in the muscle tissue produced by exercise was mainly washed out by the flow of body fluids including blood, lymph and tissue fluid (14, 15). A correlation coefficient of 0.97 was experimentally confirmed between the clearance rate of CO_2 and argon in the muscle tissue (10). The clearance rate of CO_2 in the muscle tissue following exercise was calculated according to the following equation:

$$\text{Clearance rate} = \lambda(\text{Pmax}-\text{Pmin})/\text{A},$$

where Pmax = maximum partial pressure prior to desaturation,
Pmin = minimum partial pressure after desaturation,
A = the area of the desaturation curve lying within the partial pressure and time axes, and
λ = distribution coefficient, presumed to be 1.0 for most tissues except fatty tissue.

The length of time required for a 63.2% decrease in Pmax is the time constant (T). The regional tissue perfusion rate was calculated in units of ml/100 g/min by the computer system (Automatic data acquisition/control system, 9845A, Hewlett-Packard Co., Palo Alato, CA, USA) which acquired data from the mass spectrometer. Each measurement was standardized in 3T time to minimize the deteriorating conditions produced over an extended period (10).

Terminals were inserted into the proximal part of the cranial tibial muscle, and the leg was stimulated for 5 min with 0.1-msec pulses of 100 volts at a frequency of 2 Hz using an EMG Electronic Stimulator (SEM-4201, Nihon Kohden, Tokyo, Japan). Electrical stimulation of the ischemic limb simulated muscle exercise.

The regional tissue perfusion rate of each ischemic limb was directly determined in a series of five measurements: immediately, and one, three, six, and twelve months following the preparation of the limb.

All values were expressed as mean±SD. Intergroup comparisons were made by analyzing the covariance in relation to individual and time variation. Intergroup comparisons of average values with time were made by the *t*-test.

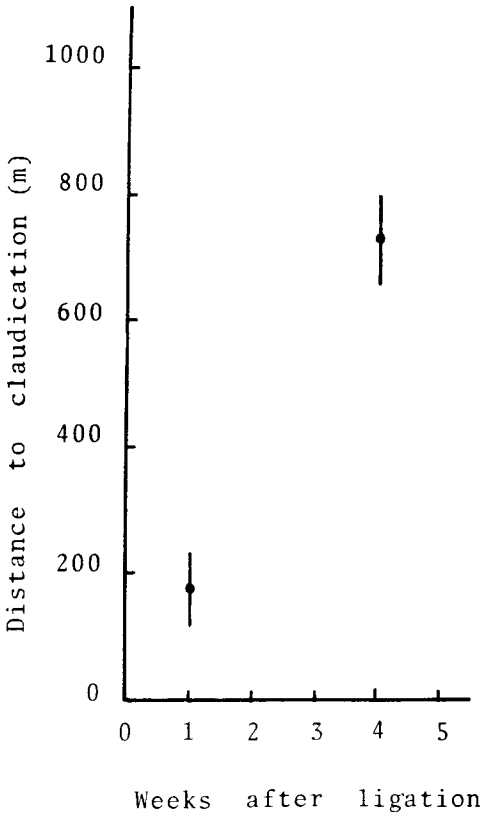

Fig. 1 The distance to claudication was measured with dogs at a pace of 700 m per 8 min in an open area (mean±SD). No claudication was observed after one month.

Results

Claudication. The animal raised the ischemic limb and stopped walking when claudication occurred. Directly following preparation of the ischemic limb, claudication was severe and continued to be so for one week. Claudication developed after running 172±60 (mean±SD) m at about one week following the preparation of the ischemic limb, and 743±75 m at about one month after the operation. No claudication occurred more than one month after the operation (Fig. 1).

Angiographic changes. No main arteries were visible in the ischemic limb immediately following surgery. Radiopaque contrast material could be found in the main arterial branches of the operated limb through the development of collaterals six months after surgery. Twelve months after surgery, well developed collaterals and distal main arterial branches were observable (Fig. 2).

Regional tissue perfusion rate.

Normal limb. The mean partial pressure of CO_2 (pCO_2) in normal muscle was 40.89±11.52 (mean±SD) mmHg at rest, and 52.38±18.00 mmHg following exercise, whereafter it returned to the initial level gradually. The mean partial pressure of O_2 (pO_2) was 21.49±5.44 mmHg at rest, 19.44±5.18 mmHg following exercise, and 23.83±5.25 mmHg 60 min after exercise (n=10). The mean regional tissue perfusion rate following exercise was calculated from 10 nor-

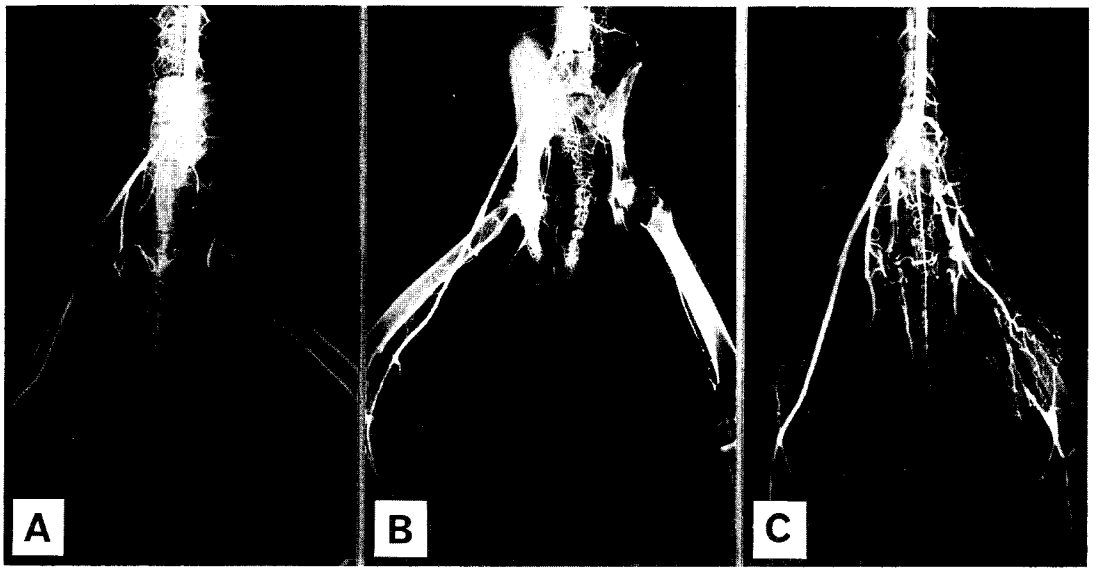

Fig. 2 Arteriography immediately following ligation shows no distal main arterial trees in the ischemic limb (A). Arteriography 6 months after ligation shows distal arterial trees newly developed through collaterals in the ischemic limb (B). Arteriography 12 months after ligation clearly shows distal arterial branches as a result of well-developed collaterals in the ischemic limb (C).

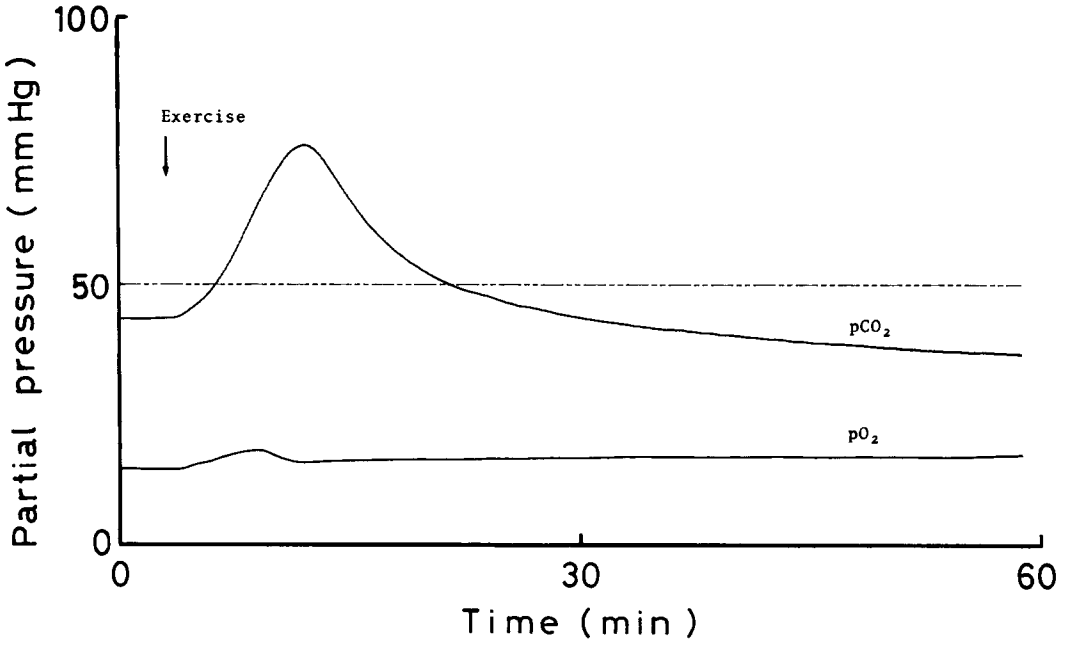

Fig. 3 Typical changes after exercise in the partial pressures of O_2 and CO_2 in normal leg muscles. Muscle pCO_2 increased following exercise and returned gradually to the initial level. The regional tissue perfusion rate of this dog was calculated to be 17.62 ml/100 g/min.

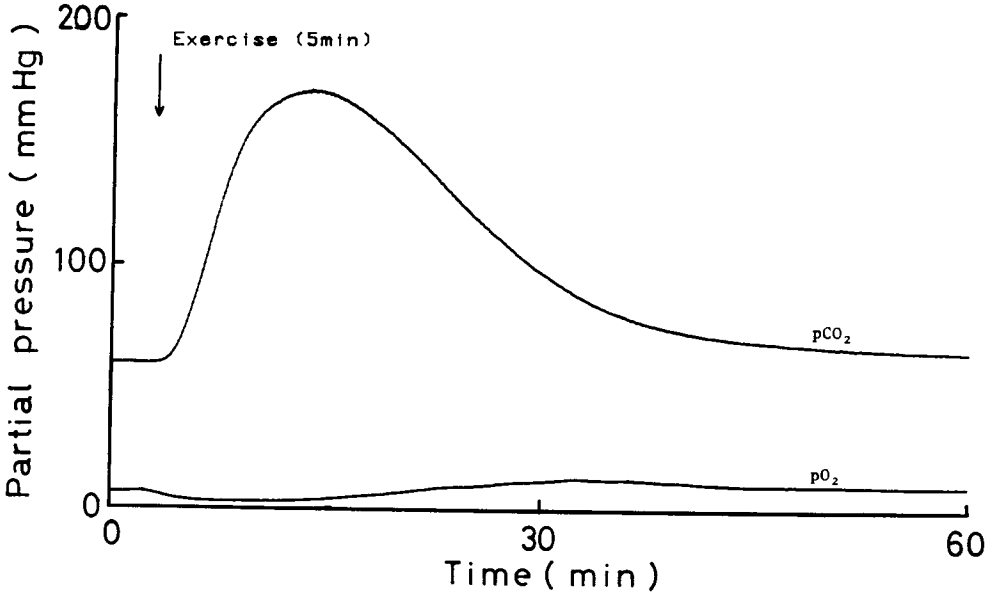

Fig. 4 Typical changes after exercise in the partial pressures of O_2 and CO_2 in ischemic leg muscles immediately following ligation-induced ischemia. Muscle pCO_2 increased sharply and was gradually washed out of the tissue by perfusion. The regional tissue perfusion rate of the dog was 7.51 ml/100 g/min.

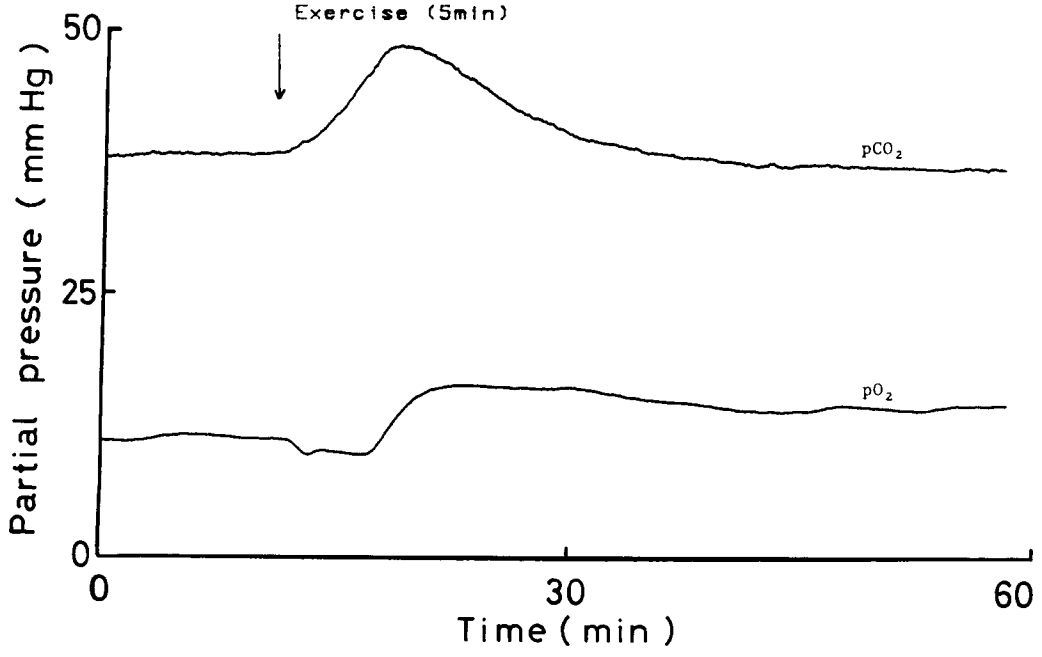

Fig. 5 Typical changes after exercise in the partial pressures of O_2 and CO_2 in ischemic leg muscles one month after ligation. The pCO_2 is similar to that found immediately after ligation; a rather gentle, sloping curve is observed. Tissue perfusion rate was 11.86 ml/100 g/min.

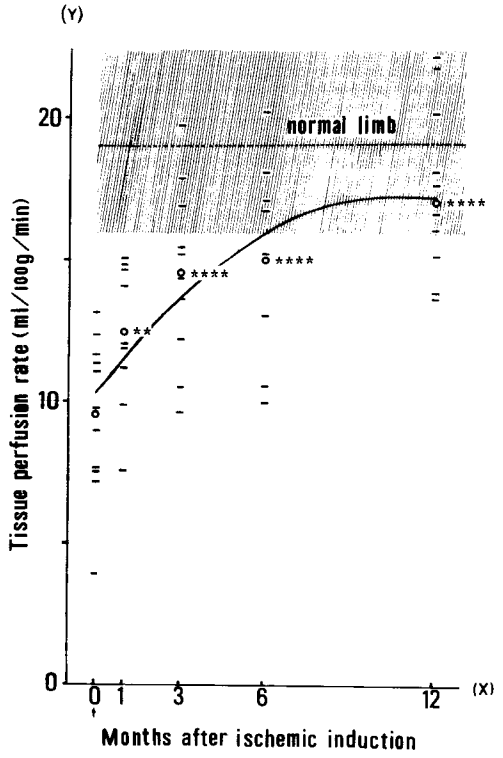

Fig. 6 Tissue perfusion rates following the preparation of ischemic limbs. The regional tissue perfusion rate twelve months after the induction of ischemia is comparable to that of normal limbs. Significant differences were found between the regional tissue perfusion rate immediately after sugery and 1 ($p < 0.02$), 3, 6 and 12 months ($p < 0.001$) after ligation. ------ : average value of normal limbs; / / / /: SD range of normal limbs; ○: average values for each month following ligation; —— : polynomial regression second-order model, $Y = 10.303 + 1.339X - 0.0647X^2$; **, $p < 0.02$; ****, $p < 0.001$.

mal limbs to be 19.09 ± 3.16 ml/100 g/min (Figs. 3,6).

Immediately after the preparation of the ischemic limb. After exercise, pCO_2 in the ischemic muscle sharply elevated and then gradually declined. In contrast, pO_2 in the ischemic muscle declined after exercise, but then it rose above the pre-exercise level (Fig. 4). The mean regional tissue perfusion rate was calculated from 12 ischemic limbs to be 9.51 ± 2.62 ml/100 g/min (Fig. 6).

One month following the preparation of the ischemic limb. Ischemic muscle pCO_2 and pO_2 curves showed similar patterns to those found immediately after surgery with pCO_2 demonstrating a rather gentle, sloping curve (Fig. 5). The regional tissue perfusion rate was calculated to be 12.41 ± 2.42 ml/100 g/min, $n = 10$ (Fig. 6).

Three months following the preparation of the ischemic limb. Ischemic muscle pCO_2 and pO_2 showed gentle curves similar to those seen one month after surgery, but the clearance curve was shifted to the left slightly. The regional tissue perfusion rate was calculated to be 14.59 ± 3.19 ml/100 g/min, $n = 10$ (Fig. 6).

Six months after preparation of the ischemic limb. Ischemic muscle pCO_2 and pO_2 curves were similar to those occurring 3 months after surgery. The regional tissue perfusion rate was calculated to be 15.11 ± 3.24 ml/100 g/min, $n = 10$ (Fig. 6).

Twelve months after preparation of the ischemic limb. The ischemic muscle pCO_2 curve was similar to that occurring 6 months after surgery, but the pO_2 after 12 months showed a gradually rising slope followed by a drop after exercise. The regional tissue perfusion rate was 17.19 ± 2.63 ml/100 g/min, $n = 12$ (Fig. 6).

Test for determining the significant differences between regional tissue perfusion rates for each postsurgical period. One month following surgery, there was a significant ($p < 0.02$) increase in regional tissue perfusion values. These differences were observed immediately after surgery. There were significant ($p < 0.001$) improvements in the regional tissue perfusion values at any time period (3, 6 and 12 months) following surgery when compared with the value immediately after surgery. There also were sig-

nificant ($p < 0.05$) improvements found when comparing the levels of one month and 6 months after surgery, and the levels of 3 months and 12 months after surgery.

Discussion

In this experiment, dogs were allowed to freely move about in an open animal shelter, although in pain from postoperative ischemia. This freedom of movement was to simulate the exercise of daily walking in humans. The study demonstrated an amelioration of intermittent claudication in accordance with improved muscle tissue perfusion ($p < 0.02$) one month following the induction of ischemia. Distal runoff became angiographically visible 6 months after ischemia was induced; this is quite suggestive of the importance of tissue perfusion in peripheral hemodynamics.

The expected tissue perfusion rate following the preparation of the ischemic limb is shown by the polynomial regression second-order model,

$$Y = 10.303 + 1.339X - 0.0647X^2.$$

This model approximates the actual average values by statistically compensating for individual variations over time (Fig. 6). Tissue perfusion is a dynamic peripheral system of circulating body fluids including blood, lymph and tissue fluid. Arteriography provides clear and useful information on ischemia, but is limited to measuring only vessels greater than 0.1 mm. Therefore, hemodynamics or functional consequences of pathology should be evaluated with tissue circulation or perfusion methods that depend principally on blood flow.

Medspect is a mass spectrometer designed for *in vivo* measurement of tissue gases. It allows for overall measurement of changes in the partial pressures of tissue gases in body fluids. Increased muscle pCO_2 caused by exercise returns to pre-exercise levels following the wash out of CO_2 by tissue perfusion. Our previous data indicate that the less ischemic pathology is recognized in the limb, the faster pCO_2 returns to pre-exercise value.

A good correlation ratio of 0.97 was observed in our previous study between the perfusion rates calculated from pCO_2 and pAR (partial pressure of locally applied argon) clearance curves following muscle exercise. Therefore, the tissue perfusion rate can be estimated from the clearance curve of pCO_2 (10, 15). Thus, it seems appropriate to regard the local blood flow as the physiologically local tissue perfusion rate.

The average values of the local tissue perfusion rate for the periods 3, 6 and 12 months following the induction of ischemia were significantly ($p < 0.001$) higher than the value for the acute stage. The tissue perfusion rate observed 12 months following the preparation of ischemic limbs was comparable to the tissue perfusion rate of normal limbs. These data, therefore, support earlier comments by others that a one year period from the onset of intermittent claudication is advisable before making any decision about performing peripheral arterial reconstructive surgery, unless impending or manifest gangrene necessitates immediate surgery (4-8).

Direct reconstructive surgery is obviously suitable for occlusive arterial lesions. However, restoration of the blood supply through small-diameter vessels is not always successfully maintained for a long period of time because of various inevitable reactions that take place in the living organism such as scar contraction and intimal thickening, leading to narrowing the lumen. In addition, about half of these patients are lost postoperatively due to other cardiovascular com-

plications, though a direct operative mortality of less than 1% is expected in peripheral arterial reconstructive surgery (1, 2). A survey performed on 104 patients by Imparato *et al.* suggested that intermittent claudication was relatively benign, although intermittent claudication came to amputation in 5.8% of the patients as was shown in a follow-up study which lasted a mean of 2.5 years. It is reasonable to assume that the majority of patients remained stable or improved due to improvements made in collateral circulation (6-9).

Increased tissue perfusion in the ischemic leg occurring after some time may be explained by the development of latent collaterals (16, 17). As increased tissue perfusion is enhanced by walking exercise, nonsurgical treatment based on the natural history of chronic peripheral arterial occlusive diseases, particularly intermittent claudication, appears feasible.

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