Muscle blood flow in Duchenne type muscular dystrophy, limb-girdle dystrophy, polymyositis, and in normal controls¹

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SYNOPSIS Muscle blood flow (MBF) was measured using the local ¹³³Xenon injection method in patients with Duchenne type muscular dystrophy (six), limb-girdle dystrophy (four), polymyositis (seven), and in normal controls (11). Measurements were made at rest and during hyperaemia induced by ischaemic exercise and by histamine injection. Capillary diffusion capacity was measured with the ⁵¹Cr-EDTA method in three patients with Duchenne dystrophy and in six normal controls. Patients with Duchenne dystrophy had normal MBF during rest and during hyperaemia after exercise and significantly increased MBF after histamine injection. The capillary diffusion capacity was higher than normal, but not significantly. In limb-girdle dystrophy MBF was normal, while in polymyositis it was significantly decreased in all three circulatory states. The relevance of the findings to the proposed ischaemic aetiology of Duchenne dystrophy is considered.

The muscular dystrophies have been attributed classically to a primary degenerative process involving the muscle cell. However, during recent years several investigators have suggested that a circulatory abnormality might play an important role in the pathogenesis of the Duchenne-type dystrophy. The morphological basis for this hypothesis is as follows. Aortic ligation in the rat followed by treatment with vasoactive amines produces focal muscle fibre necrosis resembling the early stages of Duchenne dystrophy (Mendell *et al.*, 1971; Mendell *et al.*, 1972). Vascular embolization in the rabbit with 20–80 µm dextran particles has a similar effect (Hathaway *et al.*, 1970). If the vascular hypothesis were correct, then the blood flow in clinically affected muscles of patients with Duchenne dystrophy might be reduced in at least some physiological condition. To test this assumption, we measured the

muscle blood flow (MBF) in various neuro-muscular diseases and in normal subjects using the inert gas ¹³³Xenon. In addition, muscle capillary permeability was determined in some patients and controls.

METHODS

Seventeen patients and 11 normal subjects were studied. In each case informed consent was obtained from the patient, or, if a minor, from the parents. Six patients had Duchenne type muscular dystrophy, four had limb-girdle muscular dystrophy, and seven had polymyositis. The diagnosis in each case was based on the history, neurological examination, electromyography, and muscle biopsy. The serum creatine phosphokinase (CPK) level in patients with Duchenne dystrophy ranged from 2,530 to 6,976 units; in patients with limb-girdle dystrophy, from 244 to 1,536 units; in patients with polymyositis from 38 to 700 units (normal values: <55 units for females; <75 units for males). In three of the six patients with Duchenne dystrophy there was evidence of X-linked recessive inheritance (indicated by * in Fig. 2). Asymptomatic mothers of these patients had significantly elevated serum CPK levels and the 5 and 7 year old boys were half-brothers with the same mother but different fathers. Of the seven

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TABLE 1

MUSCLE BLOOD FLOW DURING REST, AFTER ISCHAEMIC EXERCISE, AND AFTER LOCAL HISTAMINE INJECTION*

	Resting blood flow			Blood flow after ischaemic exercise			Histamine induced hyperaemia		
	Mean $\pm$ SE	Range	Patients (no.)	Mean $\pm$ SE	Range	Patients (no.)	Mean $\pm$ SE	Range	Patients (no.)
Normal controls	2.8 $\pm$ 0.07	1.5–3.4	9	59 $\pm$ 0.72	48–75	11	52 $\pm$ 0.88	40–70	11
Duchenne dystrophy	2.6 $\pm$ 0.28	1.7–4.0	4	60 $\pm$ 4.0	56–80	3	76 $\pm$ 4.1†	43–116	6
Limb girdle dystrophy	2.3 $\pm$ 0.46	1.1–3.8	3	60 $\pm$ 3.8	48–80	4	58 $\pm$ 4.5	40–78	4
Polymyositis	1.8 $\pm$ 0.17†	1.3–2.8	4	48 $\pm$ 2.9‡	20–75	7	41 $\pm$ 1.8†	20–56	7

* Results indicate ml./100 g/min. For each type of blood flow average of all measurements in given patient was used in calculating the group mean.

† Significant difference from controls at $P < 0.001$.

‡ Significant difference from controls at $P < 0.01$.

patients with polymyositis three were being treated with prednisone at the time of the study (indicated by † in Fig. 2).

The blood flow in the patients was measured in slightly to moderately weak muscles. Similarly affected muscles were selected for the muscle biopsy and in several instances the blood flow measurements and the biopsies were taken from corresponding but opposite muscles of similar strength.

DETERMINATION OF MUSCLE BLOOD FLOW Muscle blood flow (MBF) was measured with ^{133}Xe dissolved in isotonic saline as described by Lassen and co-workers (1964). Before injection the muscle was carefully palpated to ensure deposition of the isotope into its substance; 0.05 ml of a solution containing 150 μCi ^{133}Xe was injected over 15 seconds with a 25 gauge needle into the muscle studied. In most cases this was the anterior tibial or the brachial biceps muscle. The needle was withdrawn 20 seconds after the injection. The clearance of the isotope was measured with a widely collimated sodium iodide crystal scintillation detector coupled to a ratemeter with a time constant of three seconds and equipped with a logarithmic recorder. The initial counting rate obtained was about 10^5 counts per minute.

Three different types of MBF measurements were performed:

1. *Measurements during rest* After the ^{133}Xe injection the clearance curve was followed for 15 minutes. Because slight hyperaemia can be present during the first 5 to 10 minutes after injection, that part of the curve recorded during the last five minutes was used for the calculations.

2. *Measurements during reactive hyperaemia after ischaemic exercise* Five minutes after the injection of the isotope the circulation to the limb was occluded by a blood pressure cuff positioned around the upper

thigh or arm and inflated to 250–300 mmHg. Next, the injected muscle was exercised as selectively as possible by voluntary flexion and extension until complete exhaustion or intolerable pain appeared (60–100 movements). The cuff was then released and the ^{133}Xe clearance curve was followed for the next five minutes. MBF was calculated from the steepest part of the curve.

3. *Measurements of histamine-induced hyperaemia* 30 μg histamine and 150 μCi ^{133}Xe , dissolved in a volume of 0.05 ml, were injected and the clearance curve was followed for the next three minutes. MBF was calculated from the steepest part of the clearance curve (Lindbjerg, 1965).

In all three types of measurement MBF was calculated from the clearance curves according to the formula:

$$\text{MBF} = 0.70 \times 2.3 \times D \text{ ml/100 g/min}$$

where 0.70 is the muscle to blood partition coefficient for ^{133}Xe ; 2.3 the conversion factor from base 10 to natural logarithm; and D the slope of the logarithmically recorded clearance curve expressed in per cent of decade per minute.

DETERMINATION OF CAPILLARY DIFFUSION CAPACITY Capillary diffusion capacity was studied in three

TABLE 2
CAPILLARY DIFFUSION CAPACITY FOR $^{51}\text{Cr-EDTA}$ *

	Mean $\pm$ SE	Range
Normal controls (6)	3.0 $\pm$ 0.09	2.3–4.0
Duchenne dystrophy (3)	3.9 $\pm$ 0.28	3.2–4.8

* Values represent millilitres of interstitial fluid cleared from 100 g tissue per minute. In each case the diffusion capacity was determined in the right and left anterior tibial muscle and the average value thus obtained was used in calculating the group mean.

FIG. 1. Muscle blood flow after ischaemic exercise (upper panel) and histamine injection (lower panel) in 11 normal subjects. All measurements for each subject are indicated. For a given muscle, measurement on the right side precedes that on the left side. AT: anterior tibial; BB: brachial biceps.

FIG. 2. Muscle blood flow in Duchenne dystrophy, limb-girdle dystrophy, and polymyositis after ischaemic exercise (upper panel) and histamine injection (lower panel). All measurements for each subject are indicated. For a given muscle, measurement on the right side precedes that on the left side. * evidence for X-linked recessive inheritance; † corticosteroid therapy. AT: anterior tibial; BB: brachial biceps; BT: brachial triceps; VL: lateral vastus; LP: long peroneal.

patients with Duchenne dystrophy (those with evidence of X-linked recessive heredity) and in six normal controls. The ^{51}Cr -EDTA- ^{133}Xe method was used in conjunction with histamine injection as described by Lassen and Trap-Jensen (1968) and by Trap-Jensen and co-workers (1970). A solution containing about $70\ \mu\text{Ci}$ ^{133}Xe , $30\ \mu\text{Ci}$ ^{51}Cr -EDTA, and $30\ \mu\text{g}$ histamine was injected intramuscularly. To increase the counting rate, three scintillation detectors were used for recording the clearance curves. The combined pulses were registered by two ratemeters with three-second time constants and logarithmic recorders, and with pulse-height analysers set to count ^{133}Xe or ^{51}Cr , respectively. With maximal counting rates of 10^4 for the ^{51}Cr and 10^5 for the ^{133}Xe , less than 0.5% of the counts by the ^{51}Cr ratemeter were due to ^{133}Xe , and about 1% of the counts by the ^{133}Xe ratemeter were due to the ^{51}Cr . Capillary diffusion capacity was calculated according to the formulas of Lassen and Trap-Jensen (1968) and Trap-Jensen *et al.* (1970).

RESULTS

In patients with Duchenne dystrophy the mean MBF did not differ significantly from the normal control mean during rest or after ischaemic exercise. Histamine injection resulted in a significantly greater mean MBF in Duchenne dystrophy than in the controls (Figs 1 and 2, Table 1).

The mean capillary diffusion capacity determined in three patients with Duchenne dystrophy was greater than the corresponding value in the six normal controls but the two means were not significantly different ($P < 0.1$) (Table 2).

In patients with limb-girdle dystrophy the MBF was within normal limits during rest, after ischaemic exercise, and after histamine injection (Figs 1 and 2, Table 1).

The patients with polymyositis had significantly lower MBF during rest, after ischaemic exercise, and after histamine injection than did the normal controls under similar conditions (Figs 1 and 2, Table 1).

The variation in MBF values between different muscles of a given patient was small and only slightly more marked in patients with myopathies than in the controls (Figs 1 and 2).

For all cases, in the ischaemic exercise study the time interval from the release of the blood pressure cuff to maximal hyperaemia ranged from 10 to 45 seconds. The mean intervals for

each group of cases (17–23 seconds) were not significantly different.

DISCUSSION

The local ^{133}Xe injection method provides an accurate determination of the local MBF. The isotope diffuses freely throughout the tissue and hence its washout curve is determined by the blood flow. Previous studies demonstrated a high degree of correlation between flow values obtained with ^{133}Xe method and values obtained by venous outflow measurements (Tønnesen and Sejersen, 1970).

The calculated blood flow in a given tissue depends on the product of the tissue to blood partition coefficient (λ) and the slope of the ^{133}Xe clearance curve. For muscles λ is relatively low (0.7), while for fatty tissue it is considerably higher. Partial fatty replacement of diseased muscle would increase λ over 0.7 and therefore calculations using 0.7 as λ would yield false low results (Lindbjerg *et al.*, 1966). Thus, if fatty replacement of dystrophic muscle had occurred, the calculated MBF values would be lower than the true value. Therefore our measurements of normal or increased MBF in Duchenne dystrophy might even underestimate the true flow. However, the importance of this error is probably minor because only slightly or moderately affected muscles were studied and excessive fat replacement was not observed in the biopsy specimens.

Our findings may be contrasted with results obtained by other workers in Duchenne dystrophy. Emery and Schelling (1965), using venous occlusion plethysmography, obtained normal MBF values in the resting state. Demos *et al.* (1968), also using plethysmography, found a slightly higher than normal MBF at rest, a still higher MBF at low work loads, and relatively decreased values at high work loads. However, the plethysmographic method measures the total blood flow from the limb, including that from skin and subcutaneous tissues. Therefore, the total work performed by the entire limb of a non-weak person, and hence the total limb blood flow, will be inevitably higher than when a limb with reduced muscle bulk is exercised. This does not necessarily indicate that the blood flow per gram remaining muscle tissue is reduced. In our

dystrophic patients in whom maximal hyperaemia was induced either by the maximum tolerated ischaemic exercise or by histamine, the maximum blood flow per gram muscle was not reduced below the normal level.

The present study demonstrates normal MBF at rest and after ischaemic exercise in Duchenne and limb-girdle muscular dystrophy and a significantly higher MBF after histamine injection in Duchenne dystrophy. The findings indicate that in the region permeated by the injected isotope an appreciable fraction of muscle could not have been ischaemic under the conditions of the study. However, the possibilities remain that (1) focal ischaemic lesions existed in muscle outside the injected regions; or that (2) coexisting focal ischaemia and hyperaemia at the micro-circulatory level could have resulted in a normal or an increased average blood flow. The first assumption appears unlikely because the lesions in Duchenne dystrophy are essentially random in distribution and one might expect that at least some of the 22 injections in six patients after exercise or histamine injection would have coincided with an ischaemic site. If the second assumption were correct, the clearance curves in normal subjects and in Duchenne dystrophy should have had different configurations due to the coexistence of different clearance rates in the latter. This was not observed in the time period during which 80 to 90% of the injected isotope was cleared from muscle.

The significantly increased MBF after histamine injection in patients with Duchenne dystrophy can be related to recent morphometric findings by Jerusalem *et al.* (1974) of an increased capillary size and a normal number of capillaries per unit muscle fibre area in this disease.

Our observations on capillary diffusion capacity suggest that it also may be higher than normal in Duchenne dystrophy. However, with the limited number of patients the difference between the normal and dystrophic values did not reach statistical significance. If the capillary diffusion capacity were, in fact, increased in Duchenne dystrophy then this again would be consistent with the morphometric observations.

In seven patients with polymyositis the mean MBF was significantly decreased under all three conditions of measurements below the corresponding normal control levels. These findings

lend support to the assumption that the micro-vascular bed is compromised in this disease. Morphological evidence for this has been previously reported in polymyositis (Shafiq *et al.*, 1967; Gonzalez-Angulo *et al.*, 1968) as well as in scleroderma and systemic lupus erythematosus (Norton *et al.*, 1968).

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