Lymph flow dynamics in exercising human skeletal muscle as detected by scintography

Eino Havas*†‡, Tapani Parviainen§, Juha Vuorela§, Jarmo Toivanen§, Tuomo Nikula‖ and Veikko Vihko*

*LIKES-Research Center, University Campus, FIN-40100 Jyväskylä, §Isotope Laboratory, Central Hospital of Central Finland, Keskussairaalantie 19, FIN-40640 Jyväskylä, ‖MAP Medical Technologies OY, Elementtitie 27, FIN-41160 Tikkakoski and †The Department of Biology of Physical Activity, University of Jyväskylä, PO Box 35, Fin-40351 Jyväskylä, Finland

1. The effects of dynamic and isometric muscle contractions on the lymph flow dynamics in human skeletal muscle were studied with a scintographic method.

2. Radioactively labelled human serum albumin (^{99m}Tc-HSA) was injected bilaterally into the vastus lateralis muscles of eight men ($n = 16$), four of whom had had an endurance training background. The subjects performed 100 submaximal contractions in 10 min as (i) dynamic knee extensions (CONS), (ii) isometric contractions with the knees at full extension (IMExt), or (iii) isometric contractions with knees fixed at 90 deg angle flexion (IMFlex). The exercises were separated by 65 min periods in supine rest. The level of radioactivity at the injection site was monitored by a gamma-camera, and the clearance rate of radioactivity (CR) was calculated as the fractional decrease during the periods of interest (CR unit = % min^{-1}).

3. The clearance rate was low during the rest periods (0·04 ± 0·05% min^{-1}), though higher in the trained than in the sedentary subjects (0·06 ± 0·05 *vs.* 0·03 ± 0·03% min^{-1}; $P = 0·008$). Exercise increased the clearance rate three- to sixfold, to 0·16 ± 0·16% min^{-1} during CONS, 0·20 ± 0·15% min^{-1} during IMExt and 0·09 ± 0·11% min^{-1} during IMFlex. There were no differences between the subject subgroups.

4. The higher clearance rate during IMExt than during IMFlex ($P = 0·02$) demonstrates the importance of muscle deformations on lymph propulsion and experimentally confirms the current concepts of lymph formation and propulsion in voluntarily active skeletal muscle. It is suggested that lymph propulsion by working muscle is most efficient when the muscle is able to shorten close to its minimum length.

Numerous studies have shown that physical activity increases lymph flow, both peripherally in the collecting ducts (Olszewski & Engeset, 1985; McGeown, McHale & Thornbury, 1987; Coates, O'Brodovich & Goeree, 1993) and centrally in the thoracic duct (Lindena, Küpper & Trautschold, 1984). Most of the exercise-related lymph flow data are derived from measurements in skin and subcutaneous lymphatics (Olszewski, Engeset & Sokolowski, 1977; McGeown *et al.* 1987), and data on skeletal muscles are scarce. Direct cannulation studies in animals have shown that active contractions evoked by electrical stimulation of the muscle increase the lymph flow to twice the resting values (Jacobsson & Kjellmer, 1964*a*,*b*; Bach & Lewis, 1973), but even in that case the flow is only 1/10 of the flow from the skin and the subcutaneous tissue (Bach & Lewis, 1973).

The lymphatics in skeletal muscle consist entirely of lymphatic capillaries, which have no smooth muscle (Skalak, Schmid-Schönbein & Zweifach, 1984), and are thus unable to contract spontaneously. The lymphatics lie adjacent to arcading and transverse arterioles, and are surrounded by muscle fibres, to both of which the lymphatic endothelial cells are firmly connected by collagen fibres (Skalak *et al.* 1984; Schmid-Schönbein, 1990). Consequently, the arterial pulsations, and especially muscle fibre deformations, can cause opening and closing of the lymphatic capillaries (Skalak *et al.* 1984; Mazzoni, Skalak & Schmid-Schönbein,

‡To whom correspondence should be addressed at the LIKES-Research Center, University Campus, FIN-40100 Jyväskylä, Finland.

1990). When a muscle is stretched, the lymphatics open (Mazzoni *et al.* 1990), and the interstitial fluid enters the lymphatics. Accordingly, when the muscle contracts, the lymphatics collapse and propel fluid forward, with the valvular structure of the lymphatic capillaries allowing only unidirectional flow of lymph (Skalak *et al.* 1984).

Physical exercise is brought about by muscle contractions, and the muscles can contract in several ways. Although the general effects of exercise on lymph flow have been documented, the specific roles of different types of muscle contractions on the muscle lymph flow are not known. When such effects are to be studied, humans are appropriate subjects. The cannulation of deep lymphatics draining the muscles, which has been successfully performed in cats (Jacobsson & Kjellmer, 1964*a*, *b*) and rabbits (Bach & Lewis, 1973), is not suitable for use in human studies. Therefore, we applied the scintographic tracer method (Szabó, Magyar & Molnár, 1973; Reed, Johansen & Noddeland, 1985) to study the lymph flow in contracting human skeletal muscle. The transport routes for intramuscularly injected tracers were established by Szabó *et al.* (1973), whose finding that the great majority of intramuscularly injected albumin is cleared via lymphatics was confirmed by the studies of Reed (1985) and Reed *et al.* (1985).

This study had two main objectives. First, it aimed to establish the applicability of the scintographic tracer method in human studies. Second, it was designed to clarify the role of muscle deformations during contractions on lymph flow (as deduced from the rate of albumin clearance). A preliminary report of this study has been presented as an abstract (Havas, Parviainen, Nikula & Vihko, 1996).

METHODS

Subjects

Eight healthy men (25–55 years) volunteered for the study after having been thoroughly informed of the nature of the measurements, and after giving their written consent to the study. The experimental protocol was approved by both the Jyväskylä Central Hospital, and the University of Jyväskylä Ethical Committees. Four of the subjects had an endurance training background, whereas the others had not participated in regular athletic training.

General set-up

The subjects reported to the laboratory at 11 a.m. After measurements of the maximal contraction intensity of the vastus lateralis muscles (see Electromyography), the subject was placed in a hospital bed, where he remained in a supine position throughout the experiment. The radioactively labelled tracer was injected bilaterally after a 15 min period of supine rest, and the first scintographic image was taken 1 min after the injection. Thereafter, images were taken at time points shown in Fig. 1. After 20 min rest the subjects were moved so that their lower legs were hanging over the edge of the bed for 10 min (vertical dashed line in Fig. 1). After a control image with legs in full extension, the first exercise session was commenced and it lasted for 10 min. The exercise was followed by a 65 min rest period. There were three such sessions in a row. When required, the laboratory staff moved the subject to the edge of the bed so that he was able to perform the exercises, which required having the knees flexed to a 90 deg angle.

The radioactive tracer and injection

We decided to use non-colloidal albumin for two reasons. First, it is a protein molecule and convectively transported, unlike colloids, which are usually digested by macrophages. Second, we found in preliminary experiments, with somewhat heavier exercise (bicycling), that the overall clearance of albumin was linear during a 4 h period, whereas the clearance of ^{99m}Tc-labelled Dextran 70 was faster and non-linear in a similar experiment.

Human serum albumin (HSA; Blood Transfusion Service, Finnish Red Cross, Helsinki, Finland) was labelled with ^{99m}Tc eluted from a ^{99}Mo/^{99}Tc generator (MAP Medical Technologies, Tikkakoski, Finland) with 9 mg ml^{-1} NaCl solution. All other reagents were prepared from commercial analytical grade chemicals.

HSA was radiolabelled by direct attachment of ^{99m}Tc via partial reduction of the thiol groups of the protein by ascorbic acid. The method described by Thakur & DeFulvio (1991) was applied with minor modifications (Leppälä *et al.* 1995).

The average labelling efficiency was 92%, the radionuclide purity was 99·99%, and the average radiochemical purity of the injected preparation was 96% (range, 94·5–97·5%).

A 100 μl injection, containing 2 mg ^{99m}Tc-HSA in saline, was injected bilaterally into the vastus lateralis muscle 15 cm above the patella with a 26G needle to a depth of 25 mm. The radioactivity in the injected volume varied between 70–120 MBq, according to the specific activity of the preparation on different days. Szabó and colleagues (1973) have shown that an intramuscular injection of 100 μl causes a transient pressure peak which lasts about 30 s (Szabó *et al.* 1973). Normal tissue pressure will be attained within a few minutes (Szabó *et al.* 1973). The microinjections may also

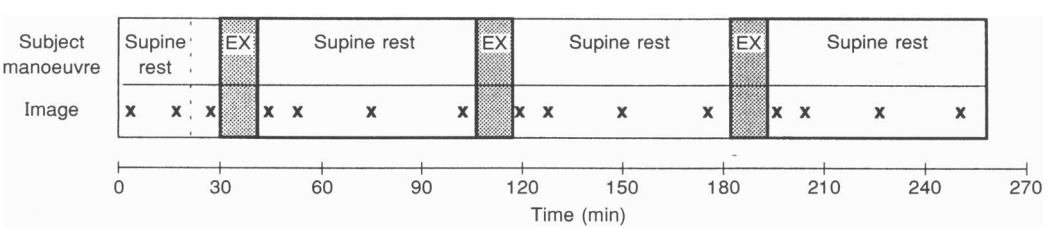

Figure 1. The experimental design of the study

Subjects rested in a supine position during the entire experiment. Ten minute exercise bouts (EX; shaded areas) were performed in a random order, and the radioactivity at the injection site was measured frequently (at times marked ×) with a gamma-camera.

induce a small local increase in blood flow (Hickner, Bone, Ungerstedt, Jorfeldt & Henriksson, 1994), as well as swelling of the interstitium for up to 20 min (Tönnesen & Sejersen, 1970); however, the injection *per se* has been shown not to affect the removal rate of injected tracer (Szabó *et al.* 1973; Reed *et al.* 1985).

Scintographic imaging

The subjects were imaged using a Siemens Digitrack 370-gamma-camera equipped with LEAP large field of view parallel-hole collimator (view diameter, 39 cm) connected to a computer with Gamma-11 software (Nuclear Diagnostics, Stockholm, Sweden). Planar images were collected using a fixed 60 s acquisition time and a 140 keV ± 10% energy window. The images were stored on a computer disk as 128 × 128 pixel (16 × 16 bit) matrices for later analysis. The horizontal levelling of the collimator was achieved by spirit levels attached to the collimator. The distance of the collimator from the legs was set to a minimum, and care was taken always to keep the distance of the collimator from the subjects legs the same for a given subject.

Exercises

Three different exercises, which were performed in a random order, were used. (i) The first exercise consisted of a dynamic knee extension starting with a knee angle of 90 deg and ending with a knee angle of 0 deg (full extension) with a short period of isometric contraction at the end of extension (CONS). This exercise provided the largest range of muscle deformation during the contraction. During the rest periods between contractions the knee angle was 90 deg. (ii) The second exercise consisted of isometric contractions of the knee extensor muscles with the knees fixed at a 90 deg angle (lower legs in dependent position) (IMFlex). In this exercise the muscles were in the elongated (stretched) state during both rest and contractions, and the deformations were highly restricted. (iii) The third exercise consisted of isometric contractions of the knee extensors with a knee angle of 0 deg (knees at full extension, IMExt). In this case the muscles were free to shorten from their relaxed length, and during the rest periods the muscles were also in a relaxed unstretched state. The rhythm of the exercise (10 contractions per minute) was provided by a metronome giving a 1·5 s beep every 6 s. Each exercise session lasted for 10 min (i.e. it included 100 contractions).

Electromyography

Electromyography (EMG) was used to control the true rhythm of the contractions and to help to adjust the intensity of contractions to approximately similar levels in all exercises. Electromyograms of both the vastus lateralis muscles were recorded with ME3000P-equipment (Mega Electronics, Kuopio, Finland) using bipolar surface electrodes. The raw signal was recorded at a bandwidth of 20–500 Hz with a 1000 Hz collection frequency, and was full-wave rectified and averaged for 10 ms periods (IEMG) later. The maximal voluntary IEMG was recorded 60 min before injecting the tracer separately for each of the three exercises (see above). During the exercise periods the IEMG was displayed on-screen to the subject who attempted to adjust the intensity of each contraction to 30% of the maximal IEMG level which was also displayed on the screen. The true mean rhythm of the contractions, as well as the average IEMG during the contractions, was calculated from the stored IEMG signal using the ME3000P software.

Image analysis

The original image containing both legs was first enlarged to contain one leg only (128 × 128 pixel matrix zoomed into a 64 × 64 pixel matrix). The total number of counts and maximum pixel count were recorded, and a region of interest was automatically drawn around the injection site so that it included all pixels having at least a given percentage (15, 5 or 1%) of the counts in the pixel having the maximum pixel count. The number of pixels in the region of interest, as well as the total number of counts, were recorded. In this paper, we considered the injection site to be the area of pixels having at least 1% of the counts of the maximum pixel count in the particular image.

Calculations

The radioactivity in the images was first corrected for the half-life time of ^{99m}Tc (362 min), with the moment of injection taken as being zero. The clearance rate of radioactivity (CR) from the injection site is expressed as a percentage per minute (% min^{-1}) for each period of interest, calculated using the following formula:

$$\mathrm{CR} = (((A_1 - A_0)A_0^{-1})100)(t_1 - t_0)^{-1},$$

where A_0 is the radioactivity (counts min^{-1}) at the beginning of the period of interest, A_1 is the radioactivity at the end of the period of interest, and t_0 and t_1 are the times (min) at A_0 and A_1, respectively.

Statistics

The results are given as means ± S.D. The clearance rates during different periods of the experiment were first tested by analysis of variance (ANOVA), and by Student–Neuman–Keuls *post hoc* comparisons where appropriate. Pearson's coefficient of correlation was calculated in specific cases. The coefficient of variation between measurements from the left and right legs of the same subject was calculated using the formula:

$$\sqrt{(\Sigma d^2/2n)}(100/\bar{x}),$$

where d is the difference between the right and left leg measurements, n is the number of paired determinations ($n = 8$) and $\bar{x}$ is the mean of all measurements.

RESULTS

Anatomical appearance of the lymphatics

Within the first 20 min of supine rest the bladder was seen in each subject. One major lymphatic vessel was visualized in every subject. The vessel became detectable in each of the subjects after the first exercise bout (Fig. 2*A* and *B*). There were three forms of the vessel: one leaving the injection site as medially bound (seen in 6 of 16 legs), one as clearly laterally bound (7 of 16 legs) and one in which the vessel went straight upwards (3 of 16 legs). In one subject, two vessels left the injection site (Fig. 2*C*). The tracer did not markedly accumulate in the lymph nodes, but clearly detectable lymph nodes were seen in three different locations: very close (at most 5 cm apart) to the injection site (seen in 6 of 16 legs), along the lymphatic vein (seen in 6 of 16 legs) and in the inguinal region (seen in 8 of 16 legs). The images of the left and right leg were similar in most subjects, but in some cases there was a clear difference between the legs of the same subject (Fig. 2*C*).

The exercises

The mean durations of contractions were 2·2 s for CONS, 2·0 s for IMExt and 1·9 s for IMFlex. The rest periods between the contractions lasted 3·8, 4·0 and 4·1 s, respectively, with no significant differences between exercises. The mean IEMG of contractions was 22 ± 9% for CONS (of the respective maximum), 33 ± 11% for IMExt, and

$12 \pm 3\%$ for IMFlex. All subjects reported difficulties in achieving the instructed IEMG in IMFlex contractions. There was a good correlation between IEMGs for the left and right vastus lateralis muscles during all exercises ($r^2 = 0{\cdot}66$, $P < 0{\cdot}01$, $n = 24$), but there was no significant correlation between IEMG and clearance rates in any of the exercises.

Inter- and intrasubject variation in the clearance of tracer

The coefficient of variation between measures from the left and right leg of the same subject was 8·6% for IMExt, 29·8% for IMFlex and 24·1% for CONS; the values for the subsequent rest periods were 27·3, 11·8 and 18·7%, respectively. The coefficient of variation between measures of two randomly chosen subjects yielded similar values: 6·3, 22·3 and 27·9%, for the exercises, and 27·0, 20·0 and 29·2%, for the respective rest periods. There was only a weak and non-significant correlation between the clearance rates from the left and right leg during the exercise periods ($r^2 = 0{\cdot}12$, $n = 24$, $P > 0{\cdot}1$). The lack of significant correlation between the subject's right and left legs, differences in the anatomical appearance, and similar intra- and intersubject variations suggested that both legs should be considered as independent observations.

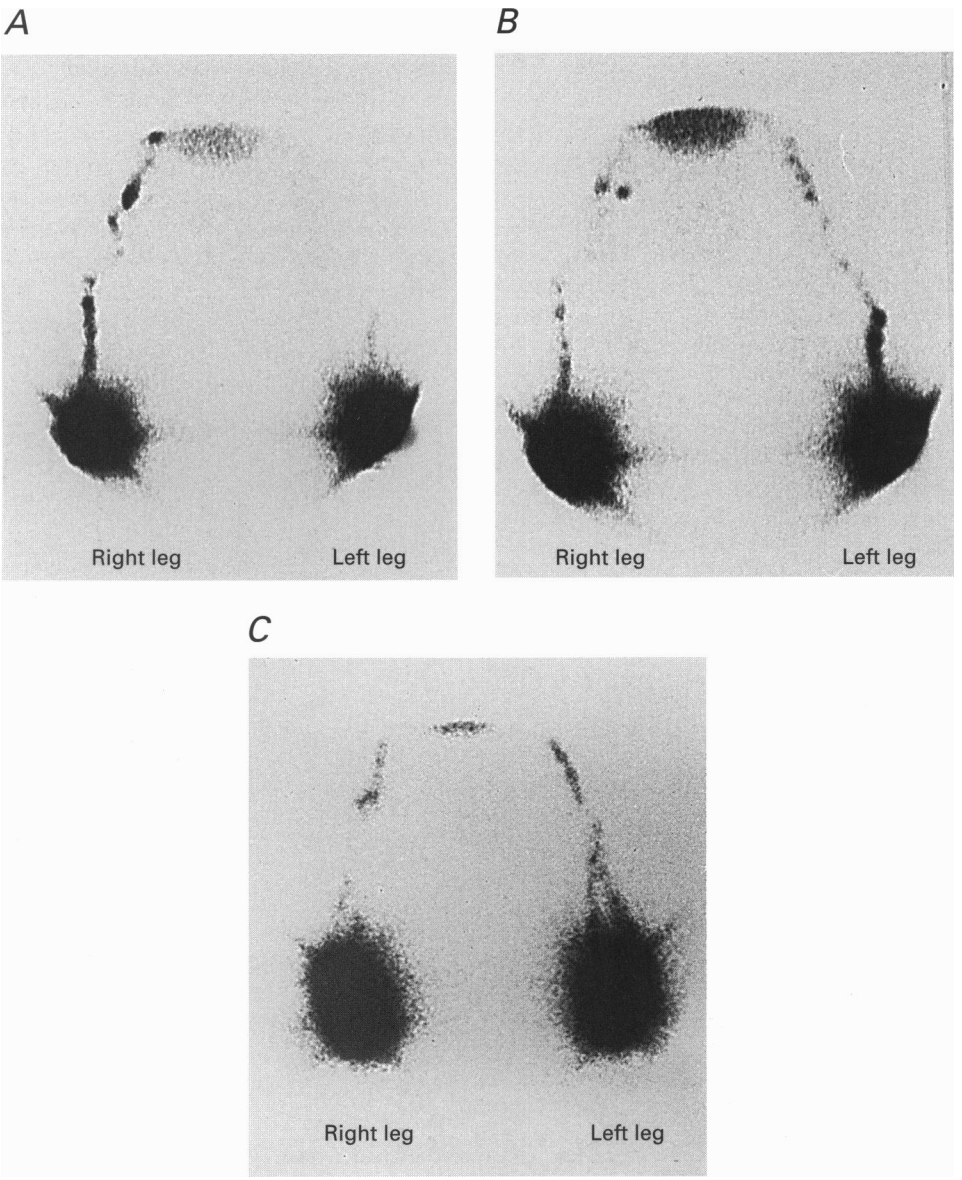

Figure 2. Visualization of the lymphatic vessels draining m. vastus lateralis

^{99m}Tc-HSA was injected bilaterally into m. vastus lateralis. Before image *A* was taken, the subject had performed dynamic knee extensions of the right leg only. Before image *B* was taken (15 min later), the same subject had performed the same 10 min exercise with the left leg only. Images are from a pilot experiment. The image *C* is from another subject after bilateral isometric contractions of the quadriceps muscles.

Clearance rates during rest and exercise

The mean clearance rates during the rest periods between exercises were very low, varying between 0·02 and 0·06% min^{-1}. The only exception was the first 20 min rest period after the injection of the tracer, during which the clearance rate was 0·17 ± 0·16% min^{-1}. When the lower legs were hanging over the edge of the bed (knee angle, 90 deg), the clearance rate decreased to the same level as during the rest periods between exercises (0·06 ± 0·32% min^{-1}).

The clearance rate was always increased during exercises. The mean clearance rate was 5·9 times higher than during the preceding rest period during CONS, and 3·6 times higher during both IMExt and IMFlex (Fig. 3). The clearance rates were similar during CONS and IMExt (0·16 ± 0·16 and 0·20 ± 0·15% min^{-1}, respectively), but during IMExt the clearance rate was twice as high as that during IMFlex (0·09 ± 0·11% min^{-1}; $P = 0·02$). The intersubject ranges of clearance rates were 0·03–0·48, 0·00–0·52 and 0·00–0·29% min^{-1} for IMExt, CONS and IMFlex, respectively. The largest intrasubject ranges (between the legs of one subject) were 0·16–0·36, 0·00–0·35 and 0·00–0·24% min^{-1} for IMExt, CONS and IMFlex, respectively. The mean clearance rates of tracer at the injection site before, during and after each exercise are shown in Fig. 3.

The clearance rate decreased to pre-exercise levels within 10 min after both CONS and IMExt, and remained low during the entire 65 min rest period. However, after IMFlex, the clearance rate did not decrease significantly from the exercise level during the subsequent 65 min rest period (Fig. 3).

The clearance rates were similar in the two subject subgroups (endurance trained and untrained) during all the exercise and rest periods. However, when the clearance rates of all separate rest periods were summated, the clearance rate in the group of trained subjects was higher than in the untrained group (0·06 ± 0·05 *vs.* 0·03 ± 0·03% min^{-1}; $P = 0·008$). Such a difference could not be found for clearance rates during the exercise periods.

DISCUSSION

This is the first study to report the effects of controlled voluntary muscle contractions on the removal of interstitially injected labelled albumin in human vastus lateralis muscle. The effects of muscle contractions on albumin clearance were, in general, similar to those described in previous animal tracer studies. In freely moving rats, the clearance rate of interstitially injected albumin from the gastrocnemius and tibialis anterior muscles was found to be about 8% h^{-1} (Reed *et al.* 1985), and in electrically stimulated dog biceps femoris muscle about 9·6% h^{-1} (Szabó *et al.* 1973). In the present study, clearance rates in contracting human vastus lateralis muscle were 5·4, 9·6 and 12% h^{-1} during IMFlex, CONS and IMExt, respectively. In rat gastrocnemius muscle, the clearance rate during anaesthesia is about one-fourth of that during spontaneous activity (Reed *et al.* 1985). A similar difference was found in dog biceps muscle between rest and electrical stimulation at 1 Hz (Szabó *et al.* 1973). In the present study, we found a consistent three- to sixfold increase in the clearance rates due to muscle contractions.

The scintographic method can measure total clearance of the radioactive tracer, but it cannot distinguish between convective removal via the lymphatics and dissipative transport via blood capillaries. However, in the clearance of interstitially injected albumin, convective transport is of major importance because the lymphatics remove at least 75% of the interstitial albumin (Reed *et al.* 1985). Szabó *et al.* (1973) found that, at rest, more than 20% of the cleared radioactivity entered the circulation directly (dissipative transport), but during electrical stimulation of the muscle,

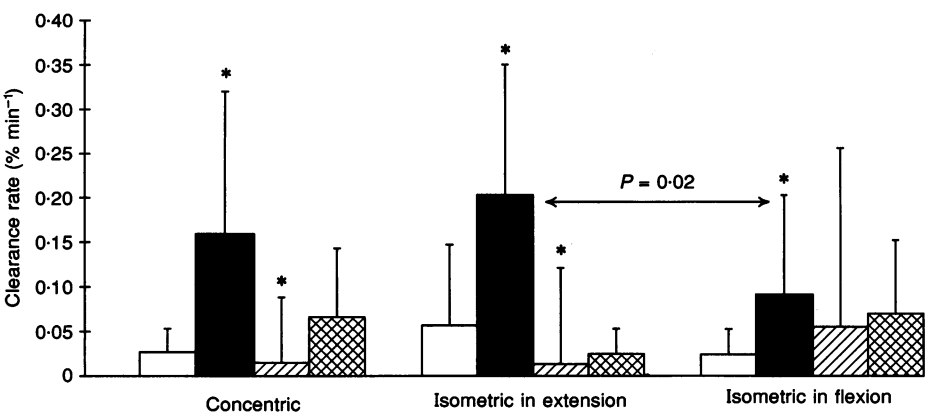

Figure 3. The effects of muscular exercise on the clearance of intramuscular albumin

The clearance rates (% min^{-1}) of ^{99m}Tc-HSA from human vastus lateralis muscle before (□), during (■) and 10 min (▨) and 65 min (▩) after exercises consisting of 100 submaximal voluntary contractions performed over 10 min. Bars show means ± S.D., $n = 16$. Asterisks denote a significant difference ($P < 0·05$) in clearance rates compared with preceding value. P value shows the difference between two exercises.

when total clearance was increased fourfold, the amount of direct dissipative transport decreased to 9% of the total clearance. As the total clearance also covers dissipative transport, the lymph flow calculations based on the albumin removal rates may overestimate the true lymph flow at low flow rates, and the percentage increase in the removal rate will correspondingly underestimate the true increase in the lymph flow (Auckland & Reed, 1993).

The high mean clearance rate and large variation during the first 20 min of the experiment was most probably due to the radiochemical properties of the ^{99m}Tc-HSA tracer used. The radiochemical purity of the injected preparation was within 94·5–97·5%. It is common for these kinds of impurity levels to appear in Tc preparations, and these impurities, especially free unbound [^{99m}Tc]pertechnetate, are likely to gain rapid access into the circulation (Chilton, Callahan & Thrall, 1990). This assumption is supported by the rapid visualization of the bladder in the present experiments.

Because the lymphatics in skeletal muscle are unable to contract (Skalak *et al.* 1984), the propulsion of the lymph in skeletal muscle is dependent upon the external compression of the lymphatics (Skalak *et al.* 1984; Schmid-Schönbein 1990). This compression can be produced by arterial vasomotion (Skalak *et al.* 1984), or by muscle fibre deformations (Mazzoni *et al.* 1990). If the observed low clearance rate at rest was not exclusively due to the dissipative transport of albumin, vasomotion is the only force which could maintain lymph flow. This concept agrees well with the observation that endurance trained subjects had slightly higher overall resting clearance rates than untrained subjects ($0·06 \pm 0·05$ *vs.* $0·03 \pm 0·03$% min^{-1}; $P = 0·008$). Endurance training is known to increase vascularity in skeletal muscles, and the enhanced resting clearance rate suggests adaptations of the lymphatic system as well.

As expected, the clearance rates were increased during the exercises. During exercise, the muscle fibre deformations during and between contractions play a major role in lymph propulsion. The volume of the lymphatics depends upon the diameter of the muscle fibres, so that when muscle fibres are lengthened (stretched), the volume of the lymphatics increases, and, conversely, when fibres are shortened, the lymphatic volume decreases (Mazzoni *et al.* 1990). The different clearance rates for IMFlex and IMExt agree well with the concepts of lymph formation (Skalak *et al.* 1984) and propulsion (Mazzoni *et al.* 1990). When IMExt was performed, the muscle was at its normal relaxed length. During contraction, no external work was done, but the muscle was able to shorten to its anatomical minimum length. Therefore, the cross-sectional area of the vastus lateralis muscle fibres was increased, which in turn collapsed the lymphatics and propelled fluid along the lymphatic system.

During IMFlex, the vastus lateralis muscle was in the same stretched state, during both contractions and rest periods between contractions. Stretching of the muscle enlarges the lymphatic volume, and thus enhances lymph formation (Mazzoni *et al.* 1990). However, as the legs were fixed into the same position, the degree of muscle shortening during contraction was limited. As a consequence of limited deformation, the clearance rate during IMFlex was relatively low (although it was 3·6 times higher than during the preceding rest period). The low clearance rate demonstrates the significance of muscle fibre deformation for lymphatic pumping.

The greatest change in muscle fibre dimensions undoubtedly occurred during CONS, when the vastus lateralis muscle contracted from the stretched state and reached its minimum length (in full knee extension) at the end of the contraction. Similar clearance rates for CONS and IMExt suggest that the phase when the muscle shortens to its anatomical minimal length is the most efficient in lymph propulsion.

During the rest periods between contractions, lymphatic volume recovers as the muscle fibres lengthen, and the lymphatics are refilled. The duration of the refilling period is important for lymph flow. McGeown and colleagues (1988) showed that as the length of the refilling period was increased the amount of lymph propelled during external compression was also increased. In the present experiment, the rhythm of the contractions during exercises was similar in each exercise.

The possibility that the contraction intensities, which, as judged by the IEMG data, were different during IMFlex and IMExt, contributed to different clearance rates cannot be entirely excluded. The blood flow into working muscles increases as the contraction intensity increases (Hickner *et al.* 1994), and, for example, in sheep lung the lymph flow follows changes in cardiac output (Coates *et al.* 1993). However, Jacobsson & Kjellmer (1964*b*) were not able to show such a clear relationship in skeletal muscle. Theoretically, the intensity of contraction affects the strength and degree of fibre deformation and could thus affect also lymph flow. However, in the present experiments the degree of muscle deformation was different in different exercises. Thus evaluation of the significance of the contraction intensities in IMFlex and IMExt is not appropriate.

The time course of the exercise-induced increase in the clearance rate suggests that the difference really lies in lymph propulsion during exercise. The subjects were kept in a supine position throughout the experiment. Thus, the resting values after each exercise were comparable and can also be considered to reflect the long-lasting effects of the exercises. Clearance rates during individual rest periods did not differ significantly from one another, but when they were compared with those during the preceding exercise period, it was found that they decreased rapidly (within 10 min) after CONS and IMExt, but not after IMFlex, when no significant decrease was observed during the entire 65 min supine rest period (Fig. 3). This phenomenon is similar to that observed after electrical stimulation of

muscle (Jacobsson & Kjellmer, 1964*a*, *b*; Bach & Lewis, 1973), namely that '... during and after severe exercise there is a leakage of fluid into the extravascular space of the muscle which takes some time to clear after cessation of exercise' (Bach & Lewis, 1973). The present results suggest that improper lymphatic pumping during IMFlex was compensated for during the subsequent rest period.

In conclusion, the present results demonstrate experimentally the significance of muscle deformations on the interstitial albumin clearance from skeletal muscle. These results support earlier observations on the clearance rates of intramuscularly injected radioactively labelled tracers in animals and extend these observations to human subjects. The exact contribution of variation of the muscle length to lymph flow from exercising skeletal muscle, as well as the role of the intensity of the muscle contraction, warrant further studies.

AUKLAND, K. & REED, R. (1993). Interstitial-lymphatic mechanisms in the control of extracellular fluid volume. *Physiological Reviews* **73**, 1–78.

BACH, C. & LEWIS, P. G. (1973). Lymph flow and lymph protein concentration in the skin and muscle of the rabbit hind limb. *Journal of Physiology* **235**, 477–492.

CHILTON, H. M., CALLAHAN, R. J. & THRALL, J. H. (1990). Radiopharmaceuticals for cardiac imaging. In *Pharmaceuticals in Medical Imaging: Radiopaque Contrast Media, Radiopharmaceuticals, Enhancement Agents for Magnetic Resonance Imaging and Ultrasound*, ed. SWANSON, D. P., CHILTON, H. M. & THRALL, J. H., pp. 419–461. Macmillan Publishing Co., Inc., New York.

COATES, G., O'BRODOVICH, H. & GOEREE, G. (1993). Hindlimb and lung lymph flows during prolonged exercise. *Journal of Applied Physiology* **75**, 633–638.

HAVAS, E., PARVIAINEN, T., NIKULA, T. & VIHKO, V. (1996). Lymphoscintography of exercising human vastus lateralis muscle. *The Physiologist* **39**, A44.

HICKNER, R. C., BONE, D., UNGERSTEDT, U., JORFELDT, L. & HENRIKSSON, J. (1994). Muscle blood flow during intermittent exercise: comparison of the microdialysis ethanol technique and ^{133}Xe clearance. *Clinical Science* **86**, 15–25.

JACOBSSON, S. & KJELLMER, I. (1964*a*). Flow and protein content of lymph in resting and exercising skeletal muscle. *Acta Physiologica Scandinavica* **60**, 278–285.

JACOBSSON, S. & KJELLMER, I. (1964*b*). Accumulation of fluid in exercising skeletal muscle. *Acta Physiologica Scandinavica* **60**, 286–292.

LEPPÄLÄ, J., KALLIO, M., NIKULA, T., NIKKINEN, P., LIEWENDAHL, K., JÄÄSKELÄINEN, J., SAVOLAINEN, S., GYLLING, H., HILTUNEN, J., CALLAWAY, J., KAHL, S. & FÄRKKILÄ, M. (1995). Accumulation of ^{99m}Tc-low-density lipoprotein in human malignant glioma. *British Journal of Cancer* **71**, 383–387.

LINDENA, J., KÜPPER, W. & TRAUTSCHOLD, I. (1984). Enzyme activities in thoracic duct lymph and plasma of anaesthetised, conscious resting and exercising dogs. *European Journal of Applied Physiology and Occupational Physiology* **52**, 188–195.

MCGEOWN, J. G., MCHALE, N. G. & THORNBURY, K. D. (1987). The role of external compression and movement in lymph propulsion in the sheep hind limb. *Journal of Physiology* **387**, 83–93.

MCGEOWN, J. G., MCHALE, N. G. & THORNBURY, K. D. (1988). Effects of varying patterns of external compression on lymph flow in the hindlimb of the anaesthetized sheep. *Journal of Physiology* **397**, 449–457.

MAZZONI, M. C., SKALAK, T. C. & SCHMID-SCHÖNBEIN, G. W. (1990). Effects of fiber deformation on lymphatic volumes. *American Journal of Physiology* **259**, H1860–1868.

OLSZEWSKI, W. L. & ENGESET, A. (1985). Studies on the lymphatic circulation of humans. In *Experimental Biology of the Lymphatic Circulation*, ed. JOHNSTON, M. G., pp. 395–422. Elsevier Science Publishers, Amsterdam.

OLSZEWSKI, W. L., ENGESET, A. & SOKOLOWSKI, J. (1977). Lymph flow and protein in the normal male leg during lying, getting up and walking. *Lymphology* **10**, 178–183.

REED, R. K. (1985). Transcapillary extravasation rate of albumin in rat skeletal muscle. Effect of motor activity. *Acta Physiologica Scandinavica* **125**, 719–725.

REED, R. K., JOHANSEN, S. & NODDELAND, H. (1985). Turnover rate of interstitial albumin in rat skin and skeletal muscle. Effects of limb movements and motor activity. *Acta Physiologica Scandinavica* **125**, 711–718.

SCHMID-SCHÖNBEIN, G. W. (1990). Microlymphatics and lymph flow. *Physiological Reviews* **70**, 987–1028.

SKALAK, T. C., SCHMID-SCHÖNBEIN, G. W. & ZWEIFACH, B. W. (1984). New morphological evidence for a mechanism of lymph formation in skeletal muscle. *Microvascular Research* **28**, 95–112.

SZABÓ, G., MAGYAR, Z. & MOLNÁR, G. (1973). Lymphatic and venous transport of colloids from the tissues. *Lymphology* **6**, 69–79.

THAKUR, M. L. & DEFULVIO, J. D. (1991). Technetium-99m-labelled monoclonal antibodies for immunoscintigraphy. Simplified preparation and evaluation. *Journal of Immunological Methods* **137**, 217–224.

TÖNNESEN, K. H. & SEJERSEN, P. (1970). Washout of ^{133}Xenon after intramuscular injection and direct measurement of blood flow in skeletal muscle. *Scandinavian Journal of Clinical Laboratory Investigations* **25**, 71–81.

Acknowledgements

The entire laboratory staff of Isotope Laboratory, Central Hospital of Central Finland is acknowledged for skilful technical assistance. We also thank Mr Jyri Manninen for revising the English language of the manuscript. This project has been financially supported by the LIKES-Foundation for the Promotion of Sport and Health Sciences, University of Jyväskylä, and the Artturi and Ellen Nyyssönen Foundation, Jyväskylä, Finland.

Author's email address

E. Havas: havas@maila.jyu.fi

Received 21 January 1997; accepted 19 June 1997.