Isolation of interstitial fluid from skeletal muscle and subcutis in mice using a wick method

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Markhus, Carl Erik, and Helge Wiig. Isolation of interstitial fluid from skeletal muscle and subcutis in mice using a wick method. Am J Physiol Heart Circ Physiol 287: H2085–H2090, 2004. First published June 24, 2004; doi:10.1152/ajpheart.00379.2004.—Until recent years, mice were sparsely used in physiological experiments, and therefore, data on the basic cardiovascular parameters of mice are lacking. Our aim was to gain access to interstitial fluid and thereby study transcapillary fluid dynamics in this species. Using a modified wick method, we were able to isolate interstitial fluid from subcutis and skeletal muscle in mice. Three-stranded, dry, nylon wicks were inserted post mortem in an attempt to avoid local inflammation and thus eliminate protein extravasation and wick contamination. Colloid osmotic pressure (COP) was measured with a colloid osmometer for submicroliter samples and averaged (means ± SE) 18.7 ± 0.4 in plasma, 9.1 ± 0.4 in subcutis, and 12.3 ± 0.5 mmHg in muscle. HPLC of plasma and wick fluid showed similar patterns except for some minor peaks eluting in the <40-kDa region. Plasma protein extravasation as determined by 125I-labeled human serum albumin showed that contamination of interstitial fluid by plasma proteins was negligible (~2%). Capillary hyperfiltration induced by intravenous infusion of saline (10% of body wt) was reflected in tissue fluid isolated by wicks as shown by the average postinfusion COP values of 14.5 ± 0.6, 6.8 ± 0.3, and 7.7 ± 0.4 mmHg in plasma, subcutis, and muscle, respectively. We conclude that the wick technique can be easily adapted for use in mice and may represent a reliable method to isolate interstitial fluid and study transcapillary fluid flux in this species.

plasma proteins; transcapillary exchange; Starling forces; colloid osmotic pressure

Recent Advances in Genetic manipulations in mice have opened nearly unlimited possibilities for studying the physiological and pathophysiological roles of almost any functional or regulatory protein in the intact animals. These developments have made the mouse an increasingly popular model animal for physiological research. Because mice have been relatively sparsely used in physiological experiments, data regarding their basic parameters are still limited (9, 15, 18). This also applies to the factors that determine transcapillary fluid flux.

One of these factors is the colloid osmotic pressure of the interstitial fluid (COPi). To determine this pressure, it is necessary to isolate fluid that is representative for interstitial fluid. Such fluid is not readily available; therefore, various methods have been developed for fluid sampling. One of these is the wick method, which was first described by Aukland and Fadnes (2). In the original version of the wick method, fluid was isolated after implantation of saline-soaked wicks in rat subcutis in vivo. Owing to problems related to inflammation induced by wick insertion in subcutis (5) and cell damage during implantation in muscle (26), the method was modified. Later studies show that samples representative for interstitial fluid in the skin and muscle of rats can be obtained by implanting dry wicks post mortem (25, 26).

The skin and muscle interstitial fluid amounts to ~60% of the total body interstitial fluid volume (3). Accordingly, transcapillary fluid-balance studies in these organs are of considerable interest. In a recent study, Stohrer et al. (21) measured COPi in subcutis utilizing saline-soaked wicks and reported a mean pressure of 8.3 mmHg in athymic NCr/Sed mice with a corresponding COP in plasma (COPp) of 19.9 mmHg. The former value is significantly lower than that found by us in the same tissue in rats (25). This may be due to a true species difference or to the use of saline-soaked wicks, which are shown to induce dilution of the interstitial fluid (25). The present studies were designed to adapt and validate the wick method for use in mice. Apart from being important for studies of transcapillary fluid balance in mice, such validation is vital for future experiments on signaling molecules at the local level in this species, i.e., in the extracellular fluid bathing the cells. In the present study, our aim was to isolate interstitial fluid from skin and muscle in mice to determine transcapillary COP gradients. The hypothesis was that there are species differences in COP that can be evaluated by use of implanted wicks.

Materials and Methods

All experiments were performed in Naval Medical Research Institute mice (n = 34; body wt, 30–40 g) of both sexes. Mice were anesthetized with 2.5 ml/kg sc of a 1:1 fenntyl-fluanisoin (Hynorm) and midazolam (Dormicum) mixture administered to the dorsal back region. The mice were kept anesthetized at all times during the in vivo experiments and were killed while still anesthetized by stretching of the neck. Body temperature was kept at 36.5–37.5°C using a servo-controlled heating pad. All experiments were performed in accordance with recommendations given by the Norwegian State Commission for Laboratory Animals and were approved by the local ethical committee.

Implantation of wicks in subcutis and muscle. Interstitial fluid was sampled with three-stranded nylon wicks (diameter, 1 mm; length, 3–6 cm) that were prewashed in acetone, ethanol, and distilled water (7). After wicks were dried (at 55°C for 2–4 h), they were stored in a box at 100% relative humidity. To prevent evaporation, all wick handling took place in an incubator kept at 100% relative humidity. All wicks were implanted post mortem to avoid the inflammatory response that develops after in vivo implantation (5, 25).

In subcutis, wicks were implanted using a straight surgical needle as previously described in detail (25).

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Before implantation in the muscle, the wicks were threaded into a 3- to 4-cm-long polyethylene (PE)-160 catheter (26). Wicks were implanted both laterally and medially in each lower limb. For the lateral implantation, an ∼2-mm skin incision was made distally and ventrolaterally on the leg. A 1-mm incision was then made with an iris scissors in the muscle fascia above the tibialis anterior and extensor digitorum longus muscles. The wick-loaded catheter was inserted under the muscle fascia along the space of natural dissection parallel to the muscle fibers. While withdrawing the insertion catheter, we pushed the wick out with a PE-50 catheter and left it behind between the two muscle layers to collect fluid. For medial implantation, a similar incision was made medially on the thigh and in the fascia above the adductor longus and the gracilis muscles. The wick was then implanted as described. After removal of both catheters, the skin was stretched over the wick ends and covered by a drop of oil to reduce evaporation.

In preliminary experiments, an ∼6-cm-long wick was used for implantation in muscle. This gave variable results for COP, probably because of traumatization of the underlying muscles that resulted in exudation of intracellular proteins. Therefore, we reduced the wick length to ∼3 cm. This modification yielded more stable pressures and was used for the data reported here.

After an implantation period of 20–30 min, the wick ends along with any portions that had a tinge of blood were cut off. Blood-stained wicks, which resulted from severing of a blood vessel during implantation, were observed in <5% of implantations. All such wicks were discarded. Accepted wick sections were removed and transferred to mineral oil-filled (density, ∼0.88 g/dl), 2-mL centrifuge tubes containing a funnel (10). Wicks from lateral and medial muscle compartments were pooled. Wick fluid was isolated by centrifugation at ∼1,400 g for 5 min. Fluid was aspirated by a glass capillary and then recentrifuged (at ∼800 g) to separate wick fluid from mineral oil. Plasma was isolated from blood sampled by orbital puncture.

**Analysis of wick fluid.** COP was measured using a colloid osmometer designed for submicrocylinder samples equipped with a PM 30 membrane (Amicon; molecular mass cutoff, 30 kDa), as described previously in detail (24). Usually, samples of 0.5–1 μL were applied to the osmometer membrane. The coefficient of variation for this osmometer is estimated to be 3.2% for rat and 2.8% for human plasma (24).

We also evaluated the graph pattern when the samples were assayed on the colloid osmometer. Whereas recordings from plasma and wick fluid normally became stable within 20–40 s, fluid from contaminated interstitial wicks initially had a higher pressure that gradually decreased to a stable level, which indicates passage of molecules through the osmometer membrane. Such problems were prevalent in the initial experiments when wicks were implanted into muscle but were eliminated by reducing the wick length (see Implantation of wicks in subcutis and muscle).

**Induction of overhydration.** To study whether a net filtration resulting in an increased tissue hydration was reflected in the interstitial fluid, overhydration was induced. After anesthesia, the right external jugular vein was cannulated using a PE-50 catheter. Saline solution (0.9%), which amounted to 10% of the body mass, was given intravenously during a period of 30 min (n = 6 mice). After an equilibration period of 1 h, a blood sample was obtained by orbital puncture, and the mouse was killed. Thereafter, interstitial fluid was sampled and processed as described above.

**Estimation of plasma protein extravasation to wick fluid.** Although wicks were inserted post mortem, the wick implantation procedure may have resulted in plasma exudation from nearby capillaries and thus contamination of wick fluid. To quantify a potential leak, 0.7–1.0 μCi of 125I-labeled human serum albumin (125I-HSA; Institute for Energy Technology, Kjeller, Norway) was injected into the external jugular vein and allowed to circulate for 5 min before the mouse was killed. Wicks were then inserted, and fluid was isolated as described above. Wick fluid volume was measured by means of calibrated microcapillaries (Modulohm; Herlev, Denmark), and the radioactivity values in wick fluid and plasma were determined using an LKB gamma counter (model 1282; Compugamma; Turku, Finland) with a window setting of 120–320 keV. Counts were corrected for background radiation.

The fraction (R) of wick fluid deriving from plasma was expressed as

\[
R = \frac{(\text{cpm}^{125}\text{I-labeled HSA}) \times (\mu\text{L fluid})^{-1}}{(\text{cpm}^{125}\text{I-labeled HSA}) \times (\mu\text{L plasma})^{-1}}
\]

where cpm is counts per minute. Thus R will yield the fraction of plasma albumin that leaks to the interstitium either as capillary sieving during the 5 min of circulation or due to the implantation procedure. During the 5-min distribution period, we can assume that the transcapillary passage of tracer albumin to the interstitium is negligible.

The distribution of macromolecules in plasma and subcutaneous and muscle fluid was determined by HPLC using Superose 12 HR 10/30-size exclusion columns (Pharmacia Biotech) with an optimal separation range of 10–300 kDa. Exact plasma and wick fluid volumes were measured with calibrated microcapillaries and diluted with NaCl buffer (0.15 M, pH 7.4) to a total volume of 110 μL. The diluted solution was injected onto the HPLC system using a Gilson 234 autoinjector (200-μL loop). A constant flow of 0.5 mL/min was obtained by using a Spectr серии P2000 pump (Thermo Separation Products), and the protein concentration in the elution fluid was measured by UV detection at 280 nm (Spectr серии UV 100). The UV signal was digitalized, sampled at 2 Hz, and analyzed on a computer using ChromoQuest 2.51 (Thermo Quest) (22).

**Morphological studies.** The wick position in relation to the surrounding muscle cells during implantation was visualized by histology. Wicks were implanted as described in lateral and medial compartments in two mice. In one of the mice, the wick was left in situ, and in the other, the wick was removed. Then both lower extremities were amputated proximally to the wick-insertion area. Femur, tibia, and fibula were removed carefully without affecting the muscle layers close to the wicks. The amputated limbs were fixed in an acetate-formalin preparation overnight. Fixed preparations were wax embedded, cut horizontally, and stained with hematoxylin and eosin.

**Statistical analysis.** Data are expressed as means ± SE. Data were tested for statistical significance using Student’s t-test (two-tailed) or one-way ANOVA with Tukey’s tests for multiple comparisons. Differences were accepted as statistically significant at the P < 0.05% level.

**RESULTS**

**COP**<sub>i</sub> and **COP**<sub>p</sub>. Typical wick fluid samples were clear and straw colored. The quantity of wick fluid varied according to the collection site. Thus from wicks implanted in muscle, the normal amount of fluid was 2–4 μL, whereas in wicks from subcutaneous areas, 10–15 μL of fluid could usually be isolated.

COP<sub>p</sub> averaged 18.7 ± 0.4 mmHg (n = 26 mice). Subcutaneous and muscular COP<sub>i</sub> values were 9.1 ± 0.4 (n = 26 mice) and 12.3 ± 0.5 mmHg (n = 10 mice), respectively (Fig. 1). The COP<sub>p</sub> and COP<sub>i</sub>, values from subcutaneous and muscular tissues were all significantly different (P < 0.05, ANOVA), which indicates a regional difference in COP, values.

**Responses to changes in transcapillary Starling forces.** Saline solution (10% of total body mass) was injected to induce overhydration. In these mice (n = 6), the amount of interstitial fluid in both muscle and subcutis was increased. As shown in Fig. 1, saline infusion reduced COP<sub>p</sub> as well as COP<sub>i</sub> values. COP<sub>p</sub> averaged 14.5 mmHg with corresponding pressures of 6.8 and 7.7 mmHg in the subcutis and muscle, respectively.
The pressures obtained after overhydration differed significantly from those in the control situations for all tissues studied (*P < 0.05, ANOVA). Furthermore, whereas ANOVA showed that postinfusion subcutis and muscle COP, values did not differ significantly, both of these pressures differed significantly from the corresponding COP; subcutis. Pressures after overhydration were significantly lower than in the corresponding control situations in all compartments; *P < 0.001 for plasma and muscle; ‡P < 0.02 for subcutis.

![Graph](chart.png)

Fig. 1. Colloid osmotic pressures in plasma (COPₚ; n = 26) and in interstitial fluid (COPᵢ) isolated from subcutis (n = 26) and muscle (n = 10) in normally hydrated mice (control) and in mice with overhydration induced by saline infusion of 10% of body wt (n = 6; volume expansion). Values are means ± SE. *P < 0.05, muscle COPᵢ in control situation was significantly higher than the corresponding COPᵢ subcutis. Pressures after overhydration were significantly lower than in the corresponding control situations in all compartments; *P < 0.001 for plasma and muscle; ‡P < 0.02 for subcutis.

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Estimation of extravasation of plasma proteins. The extravasation experiments showed that almost all of the radioactive tracer albumin remained intravascularly. In subcutaneous wick fluid, the ¹²⁵I-HSA concentration averaged 1.2% of that in plasma (Fig. 2). An identical corresponding value was found for wick fluid from muscle (Fig. 2), which shows that the contamination of subcutaneous and muscular wick fluids by plasma proteins was negligible.

Analysis of isolated fluid. The protein pattern of the isolated fluids was determined by HPLC, and the elution patterns of fluids isolated from plasma, subcutis, and muscle are shown in Fig. 3. On the plasma curve, the elution volumes for typical

![Graph](chart2.png)

Fig. 2. Fraction of ¹²⁵I-labeled human serum albumin (¹²⁵I-HSA) tracer recovered in wicks implanted in subcutis and muscle relative to that in plasma. Mean values ± SE are also shown. Difference in the mean values of the two groups is not statistically significant; P = 0.9.

![Graph](chart3.png)

Fig. 3. Representative patterns for plasma and interstitial fluid from subcutis and muscle eluted in a Superose 12 HR column. A: rat plasma indicates elution volumes for typical plasma proteins. B: wick fluid from subcutis. Reduced IgM and IgG fractions and a defined peak at 13.4 ml can be observed. C: wick fluid from skeletal muscle shows an even reduced IgM and IgG fraction and a less-defined peak at 13.4 ml compared with subcutis in B.
plasma proteins are shown. The elution pattern in wick fluid was similar to that of plasma, except for a relatively larger albumin than globulin fraction. Both subcutaneous and muscular wick fluids had some minor peaks eluting in volumes of 13.5–18 ml, but these constituted <6% of the area under the curve. The HPLC curves suggest an insignificant leak of intracellular proteins to the wick fluid.

From the HPLC curves, we calculated wick fluid-to-plasma total protein ratios of 0.54 and 0.58 in subcutaneous and muscular interstitial fluids, respectively, with corresponding ratios for albumin of 0.64 and 0.68.

Morphology

The histological sections of muscle with implanted wicks verified that the wicks were located in the connective space between the muscle fascicles, i.e., in the perimysium. There was no indication of any cell damage in the contact area between the wick and the muscle connective tissue, which is in agreement with previous results in rats (26).

DISCUSSION

The emergence of genetically engineered mice calls for an effort to adapt methods for use in mice and to generate data for mice under control conditions as well as during interventions. Here we have adopted the use of an implanted wick method for sampling interstitial fluid from skin (or subcutis) and muscle in mice thereby enabling studies of, e.g., transcapillary fluid, balance and exact quantification of extracellular fluid signaling substances in the native fluid in these organs. Furthermore, we have verified that this method yields representative samples of such fluid in this species. Muscular and subcutaneous wick fluids sampled post mortem also responded as expected from interstitial fluid to changes in transcapillary fluid pressure. We have found that mice have significantly lower COPi in skin and subcutis than rats, which verifies our hypothesis and shows that species variation in basic parameters must be considered when mice are used as an experimental model. We will discuss methodological aspects and relevant data collected by others before reviewing the physiological implications of our findings.

Methodological aspects. On the basis of experience from previous studies, we chose to implant the wicks post mortem (25, 26) to avoid the circulatory component of inflammation, i.e., that extravasation and hyperemia associated with wick insertion in vivo (5) affects the composition of the sampled fluid. With the use of this approach, the wick fluid may be contaminated by proteins that reside in blood vessels severed during wick insertion. As pointed out by Kramer et al. (12), the addition of plasma that has approximately twice the protein concentration in interstitial fluid does not affect the wick fluid protein concentration due to the low plasma volume in the skin and muscle of 0.6 and 0.4% of tissue wet weight, respectively. That plasma contamination was negligible and did not affect the wick fluid composition was shown by the extravasation of tracer albumin of 1.2%.

Another potential problem associated with using the wick method in skeletal muscle has been contamination of the sampled fluid by nonplasma proteins (25, 26). Modifying the implantation procedure by utilizing a catheter and arresting the circulation (i.e., implanting post mortem) eliminated this problem in rats (26). In preliminary experiments, traces from the colloidal osmometer indicated the existence of molecules smaller than the membrane cutoff size (30 kDa) in sampled fluid; this assumption was verified by HPLC. Refining the implantation technique and reducing the length of the implanted wick eliminated this problem as shown by a negligible amount of nonplasma proteins in wick fluid (see Fig. 3C).

Comparison with other data. The wick method has been used extensively to sample interstitial fluid in several species including humans (for references, see Ref. 3), but data from mice are scarce. To our knowledge there is only one previous study where the topic of interstitial fluid isolation was addressed in mice. Stohrer and coworkers (21) sampled fluid from tumors implanted in athymic NCr/Sed nude mice and used wick fluid from subcutis as a control. They reported an average COPp of 19.9 mmHg with a corresponding COPi in subcutis of 8.3 mmHg. Their absolute mean value for COPi in subcutis was slightly but not significantly lower than our value of 9.1 mmHg for the same tissue. Because COPi varies as a function of COPp (4, 11, 17), a comparison of the COPi/COPp ratio is more relevant. If related to COPp, their COPi/COPp ratio of 0.42 is significantly lower than our corresponding ratio of 0.49. This difference may be due to strain variation but is most likely a result of their use of saline-soaked wicks, which tends to underestimate the true COPi value (12, 25). An implication of this interpretation is that if there is any strain variation in COPi, it is of minor physiological importance. Both of these pressures from subcutis are, however, significantly lower than the COPi of 13.3 mmHg found in the control situation in subcutis of rats when the same method was used. The latter pressure was 62% of the corresponding COPp, which shows that the observed difference was not due to different plasma protein concentrations and thus demonstrates a species difference in COPi, in subcutis.

Physiological implications of the data. The present findings have implications for transcapillary fluid balance and pave the way for additional studies on the extracellular fluid in intact animals. Our method adaptation has enabled us to present novel data on mouse muscle COPi values. Also, we have verified that COPi in mouse subcutis is low and is different from that of rats (cf., our hypothesis) and that there most likely are no strain differences in this parameter. With a closer look at the control data, it is somewhat surprising to note that the average pressures in muscle and subcutis differed by 3.2 mmHg. Theoretically, the cell-rich muscle is more prone to artifacts due to cell swelling, which may remove fluid from the interstitium and thereby increase the concentration of proteins in the interstitial fluid. The effects of wick-implantation time in muscle have been studied in detail in a previous study on rats (26). Protein concentration did not increase as a function of time for a period ≤80 min, which suggests that cell swelling was not the explanation for the high muscle COPi. Another potential explanation for the high COPi value could be contamination from cell proteins, which were shown as able to affect this pressure when the more traumatic needle method was used to implant wicks into muscle (25, 26). Such contamination would have been evident from HPLC curves of muscle fluid and was not present in accepted experiments. Having controlled for potential sources of error, we conclude that the difference in COPi, in subcutis and muscle is physiological and that it emphasizes the importance of determining and compar-
ing basic cardiovascular parameters in mice to species where more extensive data are available.

The question then arising is, What can be the explanation for the observed difference in muscle and subcutaneous COPi values? In rats, the interstitial fluid protein concentrations in subcutis and muscle are similar (26), which is in contrast with the present observations in mice. One possible explanation could be the state of tissue hydration. In CH3 mice, we have observed an interstitial fluid volume in muscle of 10% of wet weight (T. V. Karlsen and H. Wiig, unpublished observations), i.e., corresponding to what has been found in the same tissue with the same tracer in rats (20), whereas corresponding data for skin in mice show a higher volume than in rats. A higher interstitial fluid volume may indicate a greater dilution of interstitial proteins and again result in a lower COPi. In human control subjects, COPi is higher on the thorax than at the more-dependent lower leg (17), which is explained by a higher filtration pressure in the latter site. In agreement with the study in humans, we have found that edema in lymphedematous mice only develops in the paw (T. V. Karlsen and coworkers, unpublished observations), but it is difficult to imagine that a hydrostatic effect on filtration pressure could be the explanation for our observed COPi differences in skin and muscle. What we may conclude is that the interstitial fluid volume is kept stable (“autoregulated”) despite wide variation in filtration forces (3), and the reason for such a potential variation in the “set point” of the skin of mice warrants additional studies of the other determinants of transcapillary filtration in this species.

The importance of COPi as measured here (“global” COPi) as a determinant of transcapillary fluid balance has been questioned: Hu et al. (8) measured filtration across single frog capillaries and found that the effective colloid osmotic gradient was less than predicted from plasma and global tissue fluid albumin concentrations due to significant gradients at the filtration barrier. These observations are in agreement with model predictions (13) and experiments in the rabbit synovium, which has fenestrated capillaries (14). In a recent study, Adamson and coworkers (1) verified that the same phenomenon also applies to nonfenestrated mesenteric capillaries in rats. Such a pressure gradient is most pronounced during states of high filtration. At normal filtration, their model predicts that the effective COP is ~80% of that in the “global” interstitial fluid phase (i.e., COPi as measured here). The tissues studied by us, skin and muscle, have continuous or nonfenestrated capillaries, and even if it has not been established, the same phenomenon may apply to these vessels. Although the importance of the absolute value for COPi is debated in relation to filtration across single capillaries, there is no doubt that this parameter is one of the predictors for the edema-preventive capacity of a tissue (3, 6). In rat skin and muscle, edema does not develop until the COPi is reduced from normal values of ∼10 to ~2 mmHg (4, 19), which suggests that a decrease in COPi could counteract an increase in capillary pressure or a reduction in COPi of 8–10 mmHg. An important implication of the present study is that the interstitium in skeletal muscle is able to compensate for a significantly higher increase in net filtration pressure than in skin or subcutis.

Our study may have consequences for other than transcapillary fluid balance investigations in mice. In a recent report on cytokine response to a systemic and local inflammatory stimulus in rat skin, we have shown that there is a differential response in plasma and tissue and that important extracellular signaling may be missed when only plasma is investigated (16). That study is supported by data from rat bone marrow, which show that extracellular signaling responses to altered hematopoiesis are more clearly reflected locally in the bone marrow interstitium than in plasma (23). Our presentation of a method for isolation of tissue fluid from skin and muscle interstitium clears the path for studies on extracellular signaling in these tissues in normal and engineered mice.

GRANTS

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