A New Measurement of Tissue Capillarity: The Capillary-to-Fibre Perimeter Exchange Index

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Catalogue Data

Key words: capillary supply, muscle fibres, oxygen flux, training, diabetes
Mots-clés: capillarisation, fibres musculaires, diffusion d’oxygène, entraînement, diabète

Abstract/Résumé

The surface area of contact between capillaries and muscle fibres has been suggested to be the site of greatest oxygen flux density in the movement of oxygen from the capillaries to the muscle fibres. A new measurement of tissue capillarity, designed specifically for use on non-perfusion fixed muscle tissue (i.e., that obtained via needle biopsy), is presented that describes the capillary supply from this perspective. The Capillary-to-Fibre Perimeter Exchange Index (the CFPE Index) is derived as the quotient of the individual capillary-to-fibre ratio (i.e., the capillary-to-fibre ratio calculated for each fibre individually) and the fibre perimeter. This method is suggested to provide a means of quantitating potential alterations in the capacity for oxygen flux (e.g., as may occur in response to a training intervention) and any carrier- or receptor-mediated aspect of blood-tissue exchange between the capillaries and muscle fibres (e.g., insulin or glucose delivery).

La surface de contact entre les capillaires et les fibres musculaires serait, semble-t-il, la zone qui présente la plus grande diffusion d’oxygène des capillaires vers les fibres musculaires. L’article décrit une nouvelle technique d’évaluation de la capillarisation tissulaire conçue spécifiquement pour l’analyse des spécimens sans perfusion obtenus par biopsie. L’indice de diffusion capillaire-périmètre de la fibre (CFPE index) correspond au quotient du ratio capillaire-fibre par le périmètre de la fibre. Cette mesure pourrait servir à quantifier les modifications potentielles de la capacité de diffusion d’oxygène (e.g., qui peut se manifester au cours de l’entraînement) et de toute autre forme d’échange entre le

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sang et la fibre via un complexe transporteur-récepteur (e.g., diffusion d’insuline ou de glucose).

Theoretical Basis for the Measurement

Krogh (1919) originally proposed the concept that the distance from the centre of a capillary to the centre of a muscle fibre (i.e., the maximal diffusion distance) was of primary importance in determining the maximal rate of oxygen delivery to muscle fibres. Considerable advancements in our understanding of the mechanisms underlying oxygen transport have followed, with mounting evidence suggesting that the greatest resistance to the diffusion of oxygen occurs between the red blood cell and the first few microns subjacent to the capillary wall-muscle fibre sarcolemma interface (Gayeski and Honig, 1986; Groebe and Thews, 1990; Honig et al., 1991). Although the possible contribution of myoglobin-facilitated diffusion in explaining the steep PO₂ gradient for oxygen between capillary and muscle fibre is still debated (Honig et al., 1991; Papadopoulos et al., 1995) (Figure 1), the aggregate

![Figure 1. Schematic depiction of the capillary supply to muscle fibres, demonstrating the rapid drop in PO₂ as one moves from the red cell towards the center of the muscle fibre (i.e., radial diffusion of oxygen). As can be seen from the graph, the drop in PO₂ is greatest in the first few microns from the red cell to just inside the sarcolemmal membrane. This figure is based upon theoretical and experimental evidence describing oxygen gradients between capillaries and muscle fibres (Groebe and Thews, 1990; Honig et al., 1991). Mb represents myoglobin. The contribution of myoglobin-facilitated oxygen diffusion remains controversial (Honig et al., 1990; Papadopoulos et al., 1995).](image-url)
surface area of the mitochondria, which is some 500 times greater than the aggregate surface area of red-cell containing capillaries, dictates that the oxygen flux as a function of the available surface area (i.e., the oxygen flux density) is highest at the points of contact between the muscle fibres and the capillaries (Honig et al., 1992; Mathieu-Costello, 1993).

As a result of these findings, several variables have evolved to describe the capillary supply to the muscle fibres from the perspective of the determinants of maximal oxygen flux at the muscle-capillary interface, for example, the capillary-to-fibre perimeter ratio (Mathieu-Costello et al., 1991) and the capillary-to-fibre contact length (Sullivan and Pittman, 1987). However, it is noteworthy that these previous measurements required an accurate quantitation of the capillary perimeter and, thus, required that the muscle tissue be fixed via vascular perfusion, since this approach prevents collapsing of the capillaries (Mathieu-Costello, 1993). In contrast, perfusion fixation is not possible in living human subjects, resulting in the capillaries in muscle tissue collected via the percutaneous needle biopsy approach being collapsed to varying degrees.

Therefore, a new measurement describing the muscle fibre capillary supply is proposed that also takes into account current theory regarding the locus of the greatest resistance to the diffusion of oxygen at the muscle–capillary interface and that can be derived from common measurements made on transverse-sections of nonperfusion fixed muscle, that is, as obtained via the Bergstrom (1962) needle biopsy technique. Since this new measurement is assessed on each fibre individually, rather than being a global descriptor of the tissue as a whole (unlike measurements of capillary density), this variable would allow expressions of various aspects of the metabolic profile of individual fibres to be made relative to their capillary supply (e.g., fibre phenotype, mitochondrial content, TCA cycle enzyme activities, could be related to the capillary supply, allowing differentiation between the different fibre types), thus providing a means of examining structure and function relationships on a fibre-by-fibre basis (see below). Given the heterogeneity of fibre type distribution in many muscles, and the resulting sharing of capillaries by fibres of potentially quite different metabolic demands, the capability for evaluation of the capillary supply relative to fibre type using the new method is an important benefit provided by this approach.

**The Capillary-to-Fibre Perimeter Exchange Index**

Plyley and Groom (1975) proposed the concept of the sharing factor (SF) to account for aspects of the capillary supply from the reference point of the capillary. Thus, the sharing factor (related to capillary contacts [CC] and capillary-to-fibre ratio [C:F] by the equation: \( SF = \frac{CC}{C:F} \)) acknowledges that capillaries are shared by two or more muscle fibres, except under those circumstances where a capillary occurs at the juncture between the edge of a fascicle and a single muscle fibre. The sharing of capillaries by more than a single fibre has implications for the size of the domain that is supplied by a given capillary (Kreuzer et al., 1991). Therefore, since the sharing factor may alter as a result of angiogenesis (Aquin et al., 1980;
Poole and Mathieu-Costello, 1996) or capillary degeneration (Pyley and Groom, 1975), any new expression describing the capillary supply should incorporate this concept of the capillary sharing factor in order to account for potential alterations in the two-dimensional capillary-fibre geometric arrangement.

The proposed measurement describes the capillary supply to the perimeter of the muscle fibre in muscle transverse sections, taking into account the two-dimensional geometric arrangement of the capillaries and muscle fibres (i.e., the capillary sharing factor). Thus, the new variable, which we term the Capillary-to-Fibre Perimeter Exchange Index (CFPE), is derived by the equation: CFPE Index = C:F/P, where C:F is the capillary-to-fibre ratio assessed on individual fibres (see Figure 2 for description of this method), and P is the fibre perimeter. Briefly, calculation of the C:F is performed by first determining the number of capillary contacts for the fibre in question. The sharing factor for each of the capillaries around the fibre is then determined. By taking the sum of the fractional contribution of each capillary contact, the capillary-to-fibre ratio for that individual fibre is obtained. The CFPE index is then derived as the quotient of C:F, and the perimeter of the fibre in question.

![Microscope viewing area](image)

**Figure 2.** This figure depicts the means by which the individual capillary-to-fibre ratio (C:Fi) is calculated on a transverse section of muscle. Briefly, for the fibre indicated, of the six capillaries surrounding the fibre (CC = 6), five are in contact with three fibres (i.e., the sharing factor for each of these capillaries is 3), and one is in contact with two fibres (i.e., the sharing factor for this capillary is 2). By taking the sum of these two proportions, one obtains the C:F for that fibre: C:F = (5 × 1/3) + (1 × 1/2) = 2.17.
While determination of C:F is more laborious than the population-based C:F (derived as the quotient of the capillary density and fibre density), an advantage afforded by this method is that measurements of the CFPE Index (and many of the other capillary measurements) can be performed relative to the fibre type. Furthermore, by utilization of a histochemical method that simultaneously stains for capillaries and fibre types (e.g., the Rosenblatt lead-ATPase stain; Rosenblatt et al., 1987), the assessment of the capillary supply on both Type I and Type II fibres can be performed on a single section.

**Previous Measurements for Nonperfusion Fixed Tissue**

Andersen and Henriksson (1977) proposed the expression, fibre area supplied per capillary contact (FA/CC), to allow quantitation of the capillary supply relative to the cross-sectional area of muscle fibres. This measurement is useful in that it incorporates both the capillary supply and the fibre area of individual fibres, both of which can be altered through growth (Aquín et al., 1980; Hudlicka, 1982), disuse (Coggan et al., 1992), hypoxia (Hoppeler et al., 1990), physical training (Hoppeler et al., 1985; Ingjer and Brodal, 1978), and other interventions. However, as has been described above, the recent findings regarding the site of greatest resistance to oxygen flux suggest that expressions based upon fibre area may not reveal the magnitude of the changes in the capacity for oxygen flux, particularly if changes in fibre size accompany changes in the capillary supply (see below).

These findings do not render the area-based measurements (such as FA/CC and capillary density) insignificant; rather, these findings suggest the value of these measurements in interpreting potential changes in the capillary supply may be better applied to other processes of blood-tissue exchange. For example, there is considerable evidence to suggest that, because of the localization of lipoprotein lipase in the capillary endothelial wall (Scow et al., 1976), the capillary density, which helps determine the capillary surface area per fibre volume, is expected to, and does, demonstrate a high correlation with both lipoprotein lipase activity and the uptake of fatty acids by skeletal muscle fibres (Lithell et al., 1981). Thus, the area-based measurements of the capillary supply seem to relate important information regarding the capacity for metabolic substrate delivery (Simoneau, 1995). Moreover, Tesch et al. (1981; Tesch and Wright, 1983) have found that the clearance of lactic acid from muscle fibres is also related to the capillary density, indicating the utility of the area-based measurements in describing the capacity for removal processes as well. Given these considerations, it is suggested that the CFPE Index be utilized as part of an array of morphometric measurements (e.g., measures of fibre size, fibre type distribution, CC, C:F, SF, capillary density [CD], and CFPE Index) in order to gain a more complete understanding of the capacity for the different processes that capillaries serve in blood-tissue exchange.

The utility of the CFPE Index in providing information different from that obtained using more common measurements of the capillary supply has been recently demonstrated in an investigation of the effect of resistance training on the
capillary supply in older men (Hepple et al., in press). Specifically, we observed a significant increase in fibre size that was accompanied by a significant increase in the number of capillaries around the muscle fibres. While these changes in the morphometric profile resulted in a maintenance of the capillary supply as a function of the fibre area supplied per capillary (i.e., FA/CC:F₁) and the diffusion distance (R₉₅, which is strongly related to capillary density), the CFPE Index was observed to increase, consistent with an increase in the capillary supply to the perimeter of the muscle fibres. The CFPE Index was found to explain a greater proportion of the variance in \( \dot{V}O_2 \)peak (\( \dot{V}O_2 \)peak also increased with resistance training in this study) than any of the other measures of the capillary supply (i.e., CC, C:F₁, FA/CC:F₁, or R₉₅). These results demonstrate the importance of using morphometric measures from the perspective of both the area (e.g., CD, R₉₅) and the perimeter capillary supply (e.g., CFPE Index) in order to more fully characterize the significance of the alterations in the morphometric profile.

**Implications and Limitations of the Measurement**

The CPFE Index describes the capillary supply of a given fibre relative to the outer membrane perimeter of that fibre, and since it accounts for the capillary sharing factor, it is sensitive to alterations in the two-dimensional capillary-fibre geometric arrangement. This feature, in addition to being calculated on an individual fibre basis, is what sets the CPFE Index apart from previous measurements of this nature (Egginton and Johnston, 1983; Flood, 1979). Thus, the CPFE Index is a two-dimensional measurement describing the capillary supply to the surface area of the muscle fibre (note that the perimeter of a muscle fibre in transverse section is related to the three-dimensional surface area of that muscle fibre).

It is important to realize, however, that this new measurement does not take into account the degree of capillary tortuosity. This will result in the potential for error when making comparisons between different muscle groups or different species, due to the variability in sarcomere length and the degree of capillary tortuosity that can occur between such samples (Mathieu-Costello, 1994; Mathieu-Costello et al., 1991). Since the degree of capillary tortuosity does not appear to alter with training (Poole et al., 1989), the "noise" or error introduced into the CPFE Index is only a function of potential differences in sarcomere length between muscle samples (providing the samples were from the same muscle and the same species). In this respect, the new measurement is subject to the same limitations as many of the other two-dimensional morphometric variables (e.g., the capillary density, FA/CC). In particular, because the cross-sectional area of a muscle fibre in transverse section increases linearly with a decreasing sarcomere length (Mathieu-Costello, 1987), whereas the fibre perimeter increases in proportion to the square root of sarcomere length with a decreasing sarcomere length (Mathieu-Costello et al., 1991), differences in sarcomere length between muscle samples could influence the fibre size, and thereby affect the precision of the CPFE Index. In this regard, it has been suggested that the sarcomere length for human muscle fibres obtained via the
Bergstrom needle method displays a fairly small deviation, with mean values between 1.85 ± 0.01 microns (range = 1.75–1.91 µm; O. Mathieu-Costello, personal communication, December 1995) and 1.92 ± 0.02 microns (H. Hoppeler, personal communication, December 1995).

The small degree of variability between samples has been hypothesized to be due to a uniform degree of contraction induced by the cutting of the muscle, and the subsequent release of sarcoplasmic Ca²⁺ stores (H. Hoppeler, personal communication, December 1995). Utilizing data describing the variability in fibre area as a function of sarcomere length (Mathieu-Costello, 1987), we have estimated that the perimeter of a muscle fibre appearing in transverse section would alter by approximately 4% (i.e., the square root of the reported variability of fibre area) between a sarcomere length of 1.71 and 1.98 µm (i.e., a slightly greater variation in sarcomere length than is generally indicated). Thus, the noise introduced into the CFPE Index by the variability in sarcomere length between biopsy samples would appear to be rather small with the needle biopsy technique.

Suggested Applications of the CFPE Index

The CFPE Index is proposed as a means of quantitating the degree of capillary-muscle fibre contact in transverse sections of nonperfusion fixed muscle tissue. Since, as was mentioned above, this region has been suggested to offer the greatest resistance to oxygen flux, alterations in the CFPE Index will provide a means of determining the implications of the alterations in the capillary supply on the capacity for oxygen flux to the skeletal muscle fibres following numerous interventions. However, it is important that comparisons using this measurement be limited to the same muscle group, and to the same species. With regard to studies of physical training, since many training interventions are often accompanied by alterations in muscle fibre size and since simple geometry dictates that alterations in muscle fibre perimeter will not change at the same rate as the fibre cross-sectional area on muscle transverse-sections (i.e., assuming muscle fibres are roughly circular in cross-section, increases in the fibre area are proportional to the radius² and increases in the fibre perimeter are proportional to the radius¹), the CFPE Index provides another measure of the capillary supply that, along with the area-based measurements, may facilitate a more complete and accurate interpretation of the ramifications of changes in capillary supply when fibre size is altered (i.e., in models associated with muscle fibre hypertrophy or atrophy), either in conjunction with, or independent of, alterations in the numbers of capillaries.

Other potential uses of the CFPE Index are under conditions where one is interested in obtaining an index of the significance of alterations in the capillary supply from the perspective of any carrier- or receptor-mediated event at the muscle-capillary interface. For example, adult-onset diabetes has been suggested to be accompanied by alterations in both muscle fibre size and the capillary supply (Eriksson et al., 1994; Lithell et al., 1982). Since insulin and glucose are dependent upon receptor- and transporter-mediated events (both of which are then a
function of the surface area of contact between capillaries and muscle fibres), respectively, the CFPE Index could be utilized to provide a measure of the effects of the alterations in muscle fibre-capillary morphometry (i.e., altered fibre size, capillary number, or both in muscle transverse sections) on insulin and glucose delivery to the skeletal muscle fibres that is more relevant to the capacity for these processes than the capillary density measurements that have been utilized previously (Eriksson et al., 1994; Krotkiewski, 1994; Lillioja et al., 1987). In addition, as was mentioned above, calculating the CFPE Index on an individual fibre basis provides the investigator with the opportunity to determine potential differences in the capillary supply between fibre type populations.

Modelling the CFPE Index

In order to depict how increases in the capillary supply might affect the CFPE index, we modelled the relationship between the CFPE Index and the capillary-to-fibre ratio (Figure 3), based upon the data provided in Table 1. Certain assumptions were made in order to facilitate the computation of the derived values presented in Table 1 (i.e., SF and C:F) and, thus, to provide a logical platform upon which to examine the resulting changes in the CFPE Index with an increasing capillary supply. In the calculations, a constant fibre perimeter of 300 microns (value chosen based upon direct measurements of the fibre perimeter in older men; Hepple, 1996), and a hexagonal arrangement of fibres (Figure 4) was assumed.

![Figure 3](image_url)

**Figure 3.** The capillary-to-fibre perimeter exchange index (C:F/P) as a function of an increasing capillary supply. C:F = the capillary-to-fibre ratio calculated on an individual fibre basis (i.e., the individual capillary-to-fibre ratio).
Table 1  Raw Data From Which the Alteration of the Capillary-to-Fibre Perimeter Exchange Index (C:F/P) Is Calculated

<table>
<thead>
<tr>
<th>CC</th>
<th>SF</th>
<th>C:F&lt;sub&gt;i&lt;/sub&gt;</th>
<th>C:F/P (caps·1000µm&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
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<tr>
<td>2</td>
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</tr>
<tr>
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<td>3.000</td>
<td>2.000</td>
<td>6.667</td>
</tr>
<tr>
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<td>2.450</td>
<td>8.167</td>
</tr>
<tr>
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<td>2.750</td>
<td>2.909</td>
<td>9.697</td>
</tr>
<tr>
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<td>2.667</td>
<td>3.375</td>
<td>11.250</td>
</tr>
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<td>2.600</td>
<td>3.846</td>
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</tr>
<tr>
<td>11</td>
<td>2.545</td>
<td>4.322</td>
<td>14.407</td>
</tr>
</tbody>
</table>

*Note. CC = number of capillary contacts; SF = sharing factor; C:F<sub>i</sub> = capillary-to-fibre ratio calculated on an individual fibre basis; P = fibre perimeter, as determined on transverse sections of muscle tissue.*

Figure 4. Hexagonal arrangement of capillaries and muscle fibres. In this arrangement, there are six potential positions in which capillaries could be shared by three fibres (the crosses represent capillaries in these positions).
We assumed that new capillaries will first fill spots where capillaries are shared by three fibres up to, and including, six capillaries around a fibre (i.e., CC = 6). Further increases in the number of capillary contacts were assumed to occur at positions between two adjacent fibres; that is, it was assumed that these capillaries were being shared by only two fibres. These assumptions have a direct bearing on the relationship between CC and C:F; since the order in which the "open" positions between fibres are filled by new capillaries will directly influence the capillary-sharing factor (SF). In addition, while both hexagonal and square arrays (Kayar et al., 1982; Pyley and Groom, 1975; Snyder, 1987) have been proposed to describe the two-dimensional geometric organization of the capillaries and muscle fibres, the reality is more likely a random pattern somewhere in between these two extremes (Snyder, 1990). This consideration, as well as the variability in fibre size within a sample, will influence the nature of the relationship between CC and C:F, since there may not be exactly six positions around a fibre where capillaries could be shared by three fibres (deviation on either side is indicated from direct observations). While these assumptions should not be applied to direct measurements, use of this model does allow one to characterize the relationship between the capillary-to-fibre perimeter exchange index and an increasing capillary supply without incurring serious error.

Summary

In summary, we have introduced a new measurement of the capillary supply that will provide information regarding the potential for carrier- or receptor-mediated processes of blood-tissue exchange, including the capacity for oxygen flux from the capillaries to muscle fibres. This measurement can be easily determined from standard morphometric measurements obtained from transverse sections of muscle tissue, and is valid for use on nonperfusate fixed muscle, such as that obtained via the needle biopsy technique, since, unlike previous measurements of this nature, it does not require quantification of the dimensions of the capillary profiles.

References


Krogh, A. (1919). The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. J. Physiol. 52: 409-415.


Acknowledgments

This work was partially supported by grants from the Canadian Fitness and Lifestyle Research Institute and the Ontario Ministry of Tourism, Transport and Recreation. I would like to thank Dr. Mike Plyley for his thoughtful insights and helpful discussion of the new measurement.

Received May 28, 1996; accepted in final form July 27, 1996.