Acta vet. scand. 1989, *30*, 113–119.

Improved Method to Estimate Oxygen Consumption, Diffusing Capacity and Blood Flow of Synovial Membrane

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Svalastoga, E., T. Kiær and J. Grønlund: Improved method to estimate oxygen consumption, diffusing capacity and blood flow of synovial membrane. Acta vet. scand. 1989, 30, 113–119. – The joint cartilage is depending on the oxygen diffusing from the capillaries of the synovial membrane through the synovial tissue and synovial fluid.

In the present study we describe a new method to calculate the diffusing capacity (DO_2), oxygen consumption (VO_2) and blood flow of the synovial membrane. The principle of the method is to perfuse the joint cavity with two saline solutions, one with high and one with low oxygen and nitrogen partial pressures and to measure the oxygen and nitrogen partial pressures in the perfusate flowing out of the joint. Using a model of gas exchange between the joint and the membrane a set of equations was derived expressing the relationship between the blood flow, diffusing capacity and oxygen consumption and the oxygen and nitrogen flowing to and from the joint in the two different perfusion situations. In 12 rabbit knee joints we found a blood flow of 0.388 ml/min (SEM 0.027), VO_2 of 0.495 µl/min (SEM 0.196) and DO_2 of 0.024 µl/min/Torr SEM 0.003 (mean).

joint perfusion; joint model; mass spectrometry.

Introduction

The joint cartilage is oxygenated by diffusion of O_2 from the capillaries of the synovial membrane through the synovial tissue and joint fluid. The oxygen transport depends on the diffusing capacity and the rate of oxygen consumption of the tissue barriere between the capillaries and the cartilage. These parameters may be affected in osteoarthritis due to inflammation of the synovial membrane which is likely to increase the diffusion distance and the rate of oxygen consumption.

The present study describes a new method to estimate oxygen consumption, diffusing capacity and blood flow of the synovial membrane. The principle of the method is to perfuse the joint cavity with saline at a high and a low oxygen nitrogen partial pressures. At the low PO_2 and PN_2, oxygen nitrogen diffuse from the synovial capillaries to the perfusate and at the high partial pressure the flows go in the opposite direction. Using a simple model of gas exchange between the joint cavity and the synovial membrane a set of equations was derived for the relationship between on the one side the O_2 and N_2 flows at the high and the low PO_2 and on the other side the oxygen consumption (VO_2), the diffusing capacity

(DO_2) and the blood flow of the synovial membrane. VO_2, DO_2 and Q were determined by insertion of the measured O_2 flows into these equations which were then solved using an iterative algorithm.

Materials and methods

Theory

The joint model used in the data analysis has 3 components: i) the joint cavity, ii) the synovial membrane and iii) the capillaries of this membrane. The joint cavity was perfused with saline equilibrated with 2 different gas mixtures. Mixture 1 consisted of atmospheric air with 0.96 % He. Mixture 2 was pure He. When the joint cavity was perfused with either of these mixtures oxygen and nitrogen were exchanged between the perfusate and the capillary blood. The change in the O_2 content of the blood multiplied by the blood flow is equal to the amount of oxygen removed from or added to the perfusate less the amount consumed by the synovial membrane:

$$(CtaO_2 - CtvO_2\,(1)) \cdot Q = JO_2\,(1) - VO_2 \quad (1)$$

$$(CtaO_2 - CtvO_2\,(2)) \cdot Q = JO_2\,(2) - VO_2 \quad (2)$$

where $CtaO_2$ and $CtvO_2$ are the oxygen contents in the arterial and venous blood and Q is the blood flow through the capillaries. JO_2 is the oxygen flow from the perfusate and VO_2 is the rate of oxygen consumption in the synovial membrane. The indices 1 and 2 refer to the 2 different gas mixtures. The oxygen content in blood is a function of PO_2 (the oxygen binding curve):

$$CtO_2 = f(PO_2) \quad (3)$$

JO_2 is equal to the difference between the oxygen contents in the inflowing and outflowing perfusate multiplied by the perfusion rate:

$$JO_2\,(1) = F \cdot \alpha O_2 \cdot (PiO_2\,(1) - PoO_2\,(1)) \quad (4)$$

$$JO_2\,(2) = F \cdot \alpha O_2 \cdot (PiO_2\,(2) - PoO_2\,(2)) \quad (5)$$

where F is the joint perfusion rate and αO_2 is the solubility of oxygen in the perfusate. PiO_2 and PoO_2 are the oxygen partial pressures in the perfusate flowing in and out of the joint cavity.

Insertion of (3), (4) into (1) and (2) gives:

$$(f(PaO_2) - f(PvO_2\,(1)) \cdot Q = F \cdot \alpha O_2 \cdot (PiO_2\,(1) - PoO_2\,(1)) - VO_2 \quad (6)$$

$$(f(PaO_2) - f(PvO_2\,(2)) \cdot Q = F \cdot \alpha O_2 \cdot (PiO_2\,(2) - PoO_2\,(2)) - VO_2 \quad (7)$$

where PaO_2 and PvO_2 are the arterial and venous oxygen partial pressures.

A previous analysis of the described joint model (*Svalastoga & Grønlund* 1985) has shown that the oxygen flow to or from the perfusate can be approximated by:

$$F \cdot \alpha O_2 \cdot (PiO_2\,(1) - PoO_2\,(1)) = DO_2 \cdot (PpO_2\,(1) - PcO_2\,(1)) + VO_2/2 \quad (8)$$

$$F \cdot \alpha O_2 \cdot (PiO_2\,(2) - PoO_2\,(2)) = DO_2 \cdot (PpO_2\,(2) - PcO_2\,(2)) + VO_2/2 \quad (9)$$

where $PpO_2 = (PiO_2 + PoO_2)/2$ and $PcO_2 = (P(PaO_2 + PvO_2)/2$.

When the joint is perfused with saline equilibrated with gas mixture 2 (without nitrogen and oxygen, nitrogen diffuses from the perfusate to the capillaries of the synovial membrane. Mass conservation for nitrogen gives:

$$F \cdot \alpha N_2\,(P) \cdot (PiN_2\,(2) - PoN_2\,(2)) = Q \cdot \alpha N_2\,(B) \cdot (PaN_2 - PvN_2\,(2)) \quad (10)$$

where αN_2 (P) and αN_2 (B) are the solubilities of nitrogen in the perfusate and blood. assuming that PN_2 in the outflowing perfusate equilibrates with the nitrogen partial pressure in venous blood ($PoN_2 = PvN_2$) gives:

$$Q = F \cdot \alpha N_2\,(P) \cdot (PiN_2\,(2) - PoN_2\,(2))/(\alpha N_2\,(B) \cdot (PaN_2 - PoN_2\,(2)) \quad (11)$$

Eqs. (6), (7), (8), (9) and (11) form a system of 5 equations with 5 unknowns: Q, PvO_2 (1), PvO_2 (2), DO_2 and VO_2. The partial pressure of nitrogen and oxygen in the inflowing perfusate are identical to those of the gas mixtures and the partial pressures at the outlet can be measured. the arterial PO_2 and PN_2 can be respectively measured and

calculated from the composition of the inspiration gas. The oxygen binding curve of blood was derived from *Bartels & Harms* (1959). This system of equations were solved using an iterative technique to find the zero point of multidimensional non-linear functions. The program was written in FORTRAN 77[1] and processed by an Olivetti M24 SP Personal Computer equipped with an 8087 coprocessor.

Experiments

The experimental procedure and setup are very similar to that of a previous study (*Svalastoga & Grønlund* 1985). 12 rabbits were anaesthetized by an initial dose of pentobarbitone (20 mg/kg) which was supplemented when necessary. The rabbits were kept on spontaneous ventilation and breathed atmospheric air. Hence, the arterial nitrogen partial pressure is given by:

$PaN_2 = (P_B - 47 \text{ Torr}) \times 0.79$

where P_B is equal to the barometric pressure. Arterial blood samples were obtained from a catheter in the carotid artery. The skin over the infrapatellar and the medial collateral ligaments was anaesthetized (0.5 ml lidocaine, 20 mg/ml) and a 20 gauge cannula was inserted into the joint cavity via the infrapatellar ligament. A second cannula was inserted into the medial femoro-tibial joint compartment through the medial horisontal approach and the joint cavity was flushed to remove synovia and debris. The rabbit was then moved into a box with a temperature of 37°C and the joint was perfused by an infusion pump (Harvard Apparatus, Millis, U.S.) at a rate of approximately 2.0 ml/min with 0.9 % saline equilibrated with either of the 2 gas mixtures. The perfusate was led through a T-tube (Fig. 1) with a blood gas catheter (*Lundsgaard et al.* 1980) connected to a mass spectrometer (VG MCROMASS, Middlewich, U. K.). The mass spectrometer in combination with the catheter enables continuous and simultaneous measurement of PO_2 and PN_2 in the perfusate. After attainment of steady O_2 and N_2 readings (usually within 5–10 min) an arterial blood sample was taken to measure PaO_2.

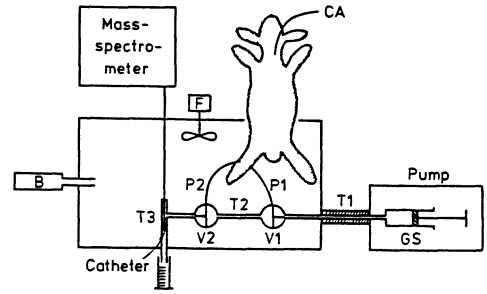

Figure 1. Experimental setup. For details see text and *Svalastoga & Grønlund* (1985).

Results

In the calculations αO_2 was set to 0.0326 μ/ml/Torr. αN_2 (B) and αN_2 (P) were assumed equal and thus cancelled in eq. (11). We used the oxygen binding curve for rabbit blood reported by *Bartels & Harms* (1959). The curve was digitized and incorporated in the computer program. Table 1 summarizes the results of the study. The first 5 columns show the measured values of PaO_2, PoO_2 and PoN_2 in each of the experiments. Columns 6–8 show the calculated oxygen consumptions (VO_2), diffusing capacity (DO_2) and blood flow (Q).

Discussion

The validity of the calculated oxygen consumptions, diffusing capacities and blood flows depend on the magnitude of errors in the measured partial pressures of oxygen and nitrogen in the outflowing perfusate.

[1] Program is available on request.

Table 1. Estimated oxygen consumption, diffusing capacity and blood flow in synovial membrane of rabbits.

Experiment	PaO_2 (Torr)	PvO_2 (Torr)	PoO_2 I (Torr)	PoO_2 II (Torr)	PoN_2 (Torr)	VO_2 (µl/min/Torr)	DO_2 (µl/min/Torr)	Blood flow (ml/min)
1.000	68.300	87.13	115.990	28.340	96.500	0.103	0.036	0.393
2.000	55.300	59.41	101.510	24.040	132.100	0.381	0.040	0.582
3.000	86.600	109.36	127.160	30.420	96.800	0.014	0.034	0.436
4.000	87.250	108.04	135.160	18.560	68.700	0.093	0.019	0.292
5.000	69.200	79.65	128.870	16.310	87.100	0.093	0.020	0.384
6.000	81.300	101.75	132.460	14.500	59.700	0.401	0.018	0.249
7.000	58.500	59.78	113.900	0.720	78.600	1.991	0.019	0.325
8.000	71.500	79.69	112.850	18.330	102.400	1.189	0.031	0.445
9.000	46.900	48.20	142.090	8.800	95.700	-0.606	0.007	0.430
10.000	57.700	61.27	109.260	14.070	106.300	1.076	0.031	0.489
11.000	57.700	61.38	121.730	8.900	73.900	0.744	0.019	0.317
12.000	72.100	79.26	131.400	9.890	72.900	0.452	0.014	0.312
Mean N = 12.000	67.696	77.877	122.698	16.080	89.225	0.495	0.024	0.388
STD	12.355	20.584	11.584	8.240	19.077	0.649	0.010	0.091
SEM	3.725	6.20	3.493	2.484	5.752	0.196	0.003	0.027

Furthermore, the validity depends on to what extend such errors are amplified in the algorithms used to calculate VO_2, DO_2 and Q. To evaluate the importance of this error source we have altered the measured values of PoO_2 (1), PoO_2 (2) and PoN_2 (2) by ± 20 and ± 10 % and recalculated VO_2, DO_2 and Q. Figs. 2–4 show the effects of such errors.

The sensitivities to errors in PoO_2 (2) and PoN_2 are quite small whereas errors in PoO_2 (1) cause large errors in VO_2 and DO_2 and can in fact cause the calculated oxygen consumption to become negative. Only one of the experiments showed a negative VO_2. PoO_2 (1) is accurately measured by the mass spectrometer because the instrument is cali-

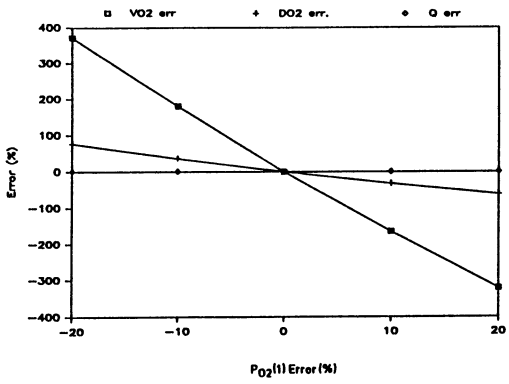

Figure 2. Effect on estimated VO_2, DO_2 and Q values of altering PoO_2 (1) ± 20 %.

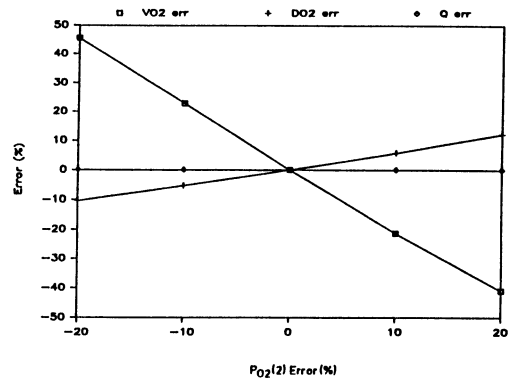

Figure 3. Effect on estimated VO_2, DO_2 and Q values of altering PoO_2 (2) ± 20 %.

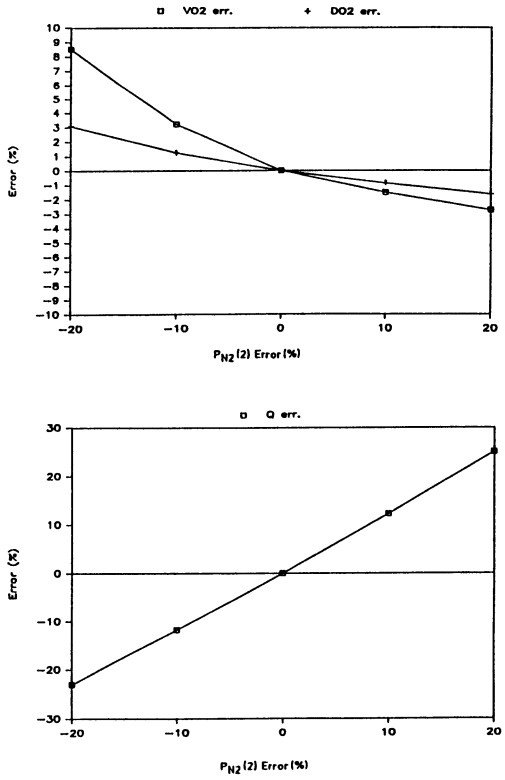

Figure 4. Effect on estimated VO_2, DO_2 and Q values of altering PoN_2 (2) ± 20 %.

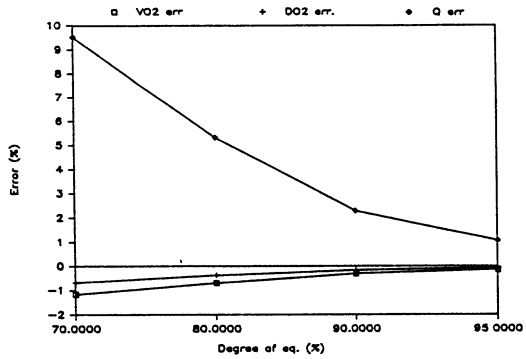

Figure 5. Influence on the estimated VO_2, DO_2 and Q values of assuming that respectively 30 %, 20 %, 10 % and 5 % of the intial PN_2 difference between the inflowing blood and the perfusate remains between the outflowing blood and perfusate.

brated in salt water saturated with atmospheric air which has an oxygen partial pressure (≈ 150 Torr) close the typical value of PoO_2 (1) (Table 1). The accurate measurement of PoO_2 (1) probably explains that only one of the experiments showed a negative VO_2.

The other main source of error is the lack of complete equilibration between nitrogen in the outflowing perfusate and the venous blood. Fig. 5 shows the influence on the results of assuming that respectively 30 %, 20 %, 10 % and 5 % of the intial PN_2 difference between the inflowing blood and the perfusate remains between the outflowing blood and perfusate. It is interesting to note that the results are not critically dependent on the fulfillment of the assumption of the equilibration of nitrogen between outflowing perfusate and venous blood.

In a previous study (*Svalastoga & Grønlund* 1985), we have determined the diffusing capacity and the rate of oxygen consumption in the synovial membrane using a method based on measuring flow of oxygen from the perfusate across the synovial membrane to the capsular capillaries. By altering the joint perfusion rate we obtained 63 sets of data on the PO_2 in the outlet versus the perfusion rate in 14 rabbit knee joints. Using a least squares technique these data gave a single estimate on the VO_2 and DO_2. To determine the standard deviations of these estimates it was necessary to use a computer simulation based on a simplified statistical model. In the present study we obtained 12 individual values of VO_2 and DO_2 which allowed calculation of the standard deviations. In addition to providing an experimental estimate of the random variation in the calculated

VO_2 and DO_2 values the new method has the advantage of giving an estimate of the blood flow. Finally, the assumption of identical oxygen partial pressures in the capsular arteries and capillaries is avoided. The results shown in Table 1 indicate that avoidance of this assumption is an important improvement because in most experiments the venous PO_2 is far from the oxygen partial pressure in the effluent perfusate.

We have not been able to find data in the literature which are comparable to ours. The mean VO_2 was found to be 47 % and the mean DO_2 38 % less than the estimates of the previous study. A possible explanation for these differences is the lack of equilibration of oxygen across the synovial membrane.

The partial pressure difference across the synovial membrane required to overcome the oxygen consumption can be estimated by insertion of $JO_2 = 0$ into eq. (4) and (8):

$PO_2\,(C) - (O_2\,(JC) = VO_2/2 \times DO_2$

where PO_2 (JC) is the oxygen partial pressure of the joint cavity in the case where the net oxygen flow across the synovial membrane is zero. Insertion of the mean values for VO_2 and DO_2 gives an oxygen partial pressure difference across the membrane of about 10 Torr. This value compared to the arterial value of approx. 68 Torr is small and leaves ample reserve for the oxygenation of the joint cartilage.

Our data on synovial blood flow of 0.388 ml/min are difficult to assess because most other publications report flows in ml/min/g. To compare our results with recent investigations of blood flow in the synovial membrane using radio labelled microsphere technique, we dissected the synovial membrane from 2 rabbit knee joints. In contrast to *Phelps et al.* (1972), we found it impossible to differentiate between the layers of the membrane. We were able to recover approx. 1 g of synovial tissue and it was estimated that this amount corresponds to about half of the total amount of synovial tissue. This gives a synovial blood flow of approx. 20 ml/min/100 g for the rabbit knee joint. Using labeled microsphere technique in rabbit synovial membrane *Hıerton* (1981) found a flow of 10 ml/min * 100 g. *Bünger* (1987) reported a flow of 1 ml/min * 100 g in juvenile canine knee joint capsule, and *Lucht et al.* (1983) calculated a flow of 10 ml/min * 100 g in the juvenile canine hip joint capsule.

The clinical applicability of the new method has not been tested. However, it would be easy to perform the measurement under arthroscopic examinations because the arthroscope already includes facilities for infusion of salt water into the joint.

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Sammendrag

Forbedret metode til at måle iltforbrug, diffusionskapacitet og blodgennemstrømning i synovialmembranen

Ledbruskens iltforsyning er afhængig af diffusion fra synovialmembranens kapillærer samt transport gennem synovi. I nærværende arbejde redegøres for en metode til at bestemme synovialmembranens blod flow (Q), diffusions kapacitet (DO_2) og metabolisme (VO_2) Princippet i metoden er at perfundere leddet med to saltvandsopløsninger, én med lavt og én med højt indhold af ilt og kvælstof. Ved anvendelse af en model for gasudveksling genereres ligninger, der udtrykker forholdet mellem Q, VO_2 of DO_2 og ilt- og kvælstofflow til og fra perfusionsvæsken.

Ved undersøgelse af 12 kaninknæled fandt vi Q = 0.388 ml/min (SEM 0.027), VO_2 = 0.495 µl/min (SEM 0.196) og DO_2 = 0.024 µl/min/Torr (SEM 0.003) (mean).

(Received March 22, 1988, accepted June 29, 1988)

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