

Human *tibia* Bone Marrow: Defining a Model for the Study of Haemodynamics as a Function of Age by Near Infrared Spectroscopy

Tiziano Binzoni^{1,2)}, Terence Leung⁵⁾, Veronica Hollis⁵⁾, Stefano Bianchi¹⁾, Jean H. D. Fasel³⁾, Henri Bounameaux⁴⁾, Emile Hiltbrand¹⁾ and Delpy Delpy⁵⁾

1) Department of Radiology, Geneva University Hospital, Switzerland

2) Department of Physiology, University of Geneva, Switzerland

3) Department of Morphology, University of Geneva, Switzerland

4) Division of Angiology and Haemostasis, Geneva University Hospital, Switzerland

5) Department of Medical Physics & Bioengineering, University College London, UK

Abstract A human model allowing the non-invasive study of bone marrow haemodynamics has been developed. A decrease in postischaemic tissue reperfusion capability (postischaemic hyperaemia) as a function of age (range 25–72 years) was observed both in the human *tibia* and *tibialis anterior* muscle. In the *tibia* bone marrow the reperfusion capability started to decrease after 50 years and was lower than for muscle for all the age range. Mean basal muscle O₂ saturation (80.8% at 25 years) decreases as a function of age ($-0.35\% \pm 0.13\%$ per year) whereas it remains constant for bone marrow ($84.8 \pm 2.8\%$). A Monte Carlo simulation has been performed to evaluate the accuracy of the derived O₂ saturation measurements and has shown that this parameter is robust even in the presence of substantial noise. It has also been demonstrated that it is necessary to use a multi wavelength NIR spectrometer and a second derivative based fitting algorithm to obtain reliable measurements from the bone marrow, and that the tissue scattering changes occurring during the protocol do not allow the use of the standard near infrared spectroscopy algorithms. The human *tibia* bone marrow model presented here and the related measurement technique should enable access to new areas of physiological research. *J Physiol Anthropol Appl Human Sci* 22 (5): 211–218, 2003 <http://www.jstage.jst.go.jp/en/>

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Introduction

Impaired bone haemodynamics appear to be related to many physio-pathological conditions (Laroche, 2002). Bone lesions seen in blood diseases such as sickle cell anaemia are caused by blood vessel occlusion (Kim and Miller, 2002).

Vascularization is increased in pagetic bone (Arlet and Mazieres, 1975). Increased bone flow was observed in patients with acute leukaemia (Petraakis et al. 1953; Lahtinen et al., 1982) or multiple myeloma and with hyperplastic marrow (Petraakis et al. 1953). Bone angiogenesis is indispensable to growth (Gerber and Ferrara, 2000) and fracture repair (Rhineland, 1974). Reflex sympathetic dystrophy syndrome is also associated with vascular changes (Driessens et al., 1999). Demmler et al. (1983) have demonstrated reduced numbers of arterial capillaries and sinuses per unit area for osteoporotic bone, with a reduction of haematopoietic marrow and an attendant increase in fat cells (Dunnill et al., 1967).

These are a few among many examples highlighting the interrelation existing between bone haemodynamics and specific physio-pathological conditions. For this reason, some authors have started to consider the possible role of impaired bone haemodynamics in triggering pathological states. For example, Laroche (1996) proposed vascular aging and arteriosclerosis within the bone as possible causes of osteoporotic change through an ischaemic mechanism. This hypothesis has been corroborated by the fact that the rate of vertebral bone marrow perfusion revealed a significant decrease in subject older than 50 years (Chen et al., 2001). Thus, it seems reasonable to assume that bone marrow blood perfusion decreases with age, possibly resulting in ischaemia and poor tissue oxygenation (Demmler et al., 1983; Chen et al., 2001). However, to our knowledge, no measurements have been performed in humans demonstrating that reduced perfusion and/or increasing age results in a real reduced bone marrow blood O₂-saturation (SO_2).

In the light of above, if one wants to study bone marrow haemodynamics and SO_2 in humans, it is important to take into account the following points. First, as is well known, it appears very difficult to follow fast haemodynamic changes in human bone marrow with the classical clinical techniques such as

positron emission tomography or nuclear magnetic resonance. Second, it is practically impossible to use these techniques for repeated measurements due to the utilization of radiotracers or contrast agents. Third, the measure of the blood flow, or of a blood flow index, does not give enough information to assess the real tissue SO_2 since these parameters are not trivially related. For the above reasons, the aim of the present work was to: 1) define a human model allowing one to study the bone marrow haemodynamics; 2) development of a non invasive technique permitting one to assess some parameters related the haemodynamics, such as a perfusion index or bone marrow SO_2 ; and, in particular, 3) to investigate the relationship existing between age, blood perfusion and bone marrow SO_2 in healthy human subjects.

One hopes that the human *tibia* bone marrow model presented here and the related measurement technique will enable access to new areas of physiological research, such as the possible role of the bone marrow sympathetic and sensitive nervous systems in the control of blood flow (Chenu, 2001). The SO_2 measurements should also potentially allow in the future to detect non invasively metabolic or blood perfusion related pathologies of the bone marrow.

Methods

Subjects

The present investigation was conducted on 13 healthy and non-smoking subjects, uniformly distributed in the age range 25–72 years. The subjects gave informed consent to the procedures which conformed with the Declaration of Helsinki and were approved by the human ethics committee of the Department of Medecine, University Hospitals of Geneva. All volunteers had an ankle-brachial index (ABI) (Weitz et al. 1996) between 0.9 and 1.3, thereby ruling out haemodynamically significant arterial disease at rest (see below).

Ankle-brachial index

To obtain the ABI the subject was laid prone on the examining table with the back of the bed positioned at 30°. A resting period of 10 min was adopted before the measurements. The ABI was obtained by dividing the systolic pressure measured at the ankle level by the one measured at the arm level. More precisely, a pneumatic cuff was placed around the ankle or arm. The systolic wave was detected by means of an ultrasound Doppler probe (PARKS Medical Electronics Inc., Beaverton, Oregon, USA). At the ankle level the probe was placed first over the *arteria dorsalis pedis* and then over the *arteria tibialis posterior* (two measurements). The higher value between the two measurements was chosen for the ABI assessment. At the arm level the probe was placed over the *arteria radialis* (one measurement). The range of normality was defined in conformity with the guidelines of the “Science Advisory and Coordinating Committee of the American Heart Association” (February 1996). The ABI

measurement was performed on a separate day before the experimental protocol.

Near Infrared Spectroscopy

A CCD-based near infrared spectrometer was used for the experiment (Cope et al., 1989). Light coming from a stabilized tungsten halogen light source (Oriel 77501, Stratford CT, USA) was conveyed to the medial surface of the *tibia* (or the *tibialis anterior* muscle; see protocol) using one fibre optic bundle. Transmitted light was collected from a second fibre optic bundle and focused onto the entry slit of a spectrograph (Spex 270M, Instrument SA Group, Edison, NJ, USA). Spectra with a resolution of 0.32 nm/pixel were acquired between 677 and 998 nm on the cooled CCD-based (Wright Instruments, Enfield, London, UK) near infrared (NIR) spectrophotometer (NIRS) and were stored on a PC. The sampling time for 1 spectrum was 1 s.

Changes in concentration of oxy- ($[\Delta HbO_2]$) and deoxyhaemoglobin ($[\Delta Hb]$) were obtained as usual by fitting changes in the attenuation spectra, which had been corrected for the wavelength dependence of pathlength (Essenpreis et al., 1993). Summarizing, the attenuation spectra (A_n) were obtained by using the first spectrum (I_1) of the time series (I_n) as reference spectrum, i.e.:

$$A_n(\lambda) = -\text{Log}_{10}(I_n(\lambda)/I_1(\lambda)) \quad (1)$$

where n corresponds to the n^{th} spectrum and λ is the wavelength. Specific absorption spectra of Hb, HbO_2 , cytochrome oxydase (CtOx) and water (H_2O) were utilized as a model to fit each A_n . The wavelength fitting range was 780–900 nm. Total haemoglobin changes were expressed as: $[\Delta Hb_{tot}] = [\Delta HbO_2] + [\Delta Hb]$. All the concentrations changes are given in arbitrary units ($\mu\text{M cm}$).

To obtain mean tissue SO_2 it has been necessary to compute values proportional to real oxy- and deoxyhaemoglobin concentration values. Since:

$$SO_2 = \frac{[HbO_{2SD}]}{[HbO_{2SD}] + [Hb_{SD}]} \quad (2)$$

where $[HbO_{2SD}]$ and $[Hb_{SD}]$ corresponds to *absolute* values in $\mu\text{M cm}$ (SD, means that they were obtained by second derivative method; see below). Any proportionality factor is obviously cancelled by the ratio. In accordance with the method proposed by Matcher et al. (1993) and Matcher and Cooper (1994), the values for $[HbO_{2SD}]$ and $[Hb_{SD}]$ were computed by fitting the second derivative, $(d^2/d\lambda^2)A_n(\lambda)$, of the absorption spectra with the second derivative of the specific absorption spectra of Hb, HbO_2 , cytochrome oxydase (CtOx) water (H_2O) and a non calibrated absorption spectrum of human fat (epiploic adipose tissue). The wavelength fitting range was 700–800 nm. In this case, the absorption spectra were obtained as:

$$A_n(\lambda) = -\text{Log}_{10}(I_n(\lambda)/I_{air}(\lambda)) \quad (3)$$

where $I_{air}(\lambda)$ is an air reference spectrum. $I_{air}(\lambda)$ was acquired as usual with a home made integrating sphere coated with paint (White reflecting coating, Munsell Color, New Windsor, NY).

From this fitting procedure, water values ($[H_2O_{SD}]$) have also been obtained but over the range 810–880 nm (Matcher et al., 1993; Matcher and Cooper, 1994). Over this wavelength range water manifests a strong absorption feature and this improves considerably the precision of its estimation.

The effect of noisy $[HbO_{2SD}]$ on SO_2 : Monte Carlo simulation

It is well known, that $[HbO_{2SD}]$ data computed using the second derivative method (see above) display a high noise level. A Monte Carlo simulation was performed to show the effect of this noise on SO_2 estimation (eqn. 2). Values for $[Hb_{SD}]$ were chosen from 80 to 280 in steps of 20 μM cm. Values for $[HbO_{2SD}]$ were 500, 600, 700 and 800 μM cm. For each possible $[Hb_{SD}]$ – $[HbO_{2SD}]$ couple, 100 different random realization of a normal noise with mean zero and standard deviation of 50 μM cm were added to the $[HbO_{2SD}]$, i.e.:

$$SO_2 = \frac{([HbO_{2SD}] + noise)}{([HbO_{2SD}] + noise) + [Hb_{SD}]} \quad (4)$$

Then, the mean and standard deviation of the 100 SO_2 values were compared with the SO_2 values without noise on the identity line. The $[Hb_{SD}]$ and $[HbO_{2SD}]$ magnitudes were chosen in accordance with typical experimental data (see results). Of course, any scaling factor simultaneously applied to $[Hb_{SD}]$ and $[HbO_{2SD}]$ will have no effect on the SO_2 .

Reperfusion index

To estimate the tissue blood perfusion, a perfusion index (PI) was defined as in Binzoni et al. (2002). In summary, the PI was obtained as the maximum speed of $[Hb_{SD}]$ recovery after 3 min of ischaemia (see Protocol). In practice the PI was computed from the first derivative of the $[Hb_{SD}]$ -time curve by means of a Savitzky-Golay algorithm (Savitzky and Golay, 1964). The minimum value of the derivative corresponds to the “maximum speed of recovery”. The data from the different subjects was normalized by defining the baseline level as $[Hb_{SD}] = 0$ and the maximum $[Hb_{SD}]$ value reached after 3 min ischaemia as $[Hb_{SD}] = 100\%$ (Binzoni et al., 2002). For each subject the PI was obtained by taking the mean over 3 different measurements.

Protocol

The subject was asked to lie prone on the examining table, with the back of the bed positioned at 30°, during a resting period of 10 min. The optodes coming from the NIR spectrophotometer were positioned on the medial surface of the tibia at the half distance between the malleolus medialis and the condylus medialis tibiae. To allow the sampling of the bone marrow by NIRS, the interoptode spacing was set to 3 cm (Binzoni et al., 2002). The optodes were covered with a black

cloth to eliminate any possible influence of the natural laboratory illumination. A pneumatic cuff of suitable size was placed around the thigh. Arterial occlusion was obtained by inflating the cuff rapidly to 270 mmHg. NIR spectra were continuously acquired during the final 1 min of rest followed by three periods of 3 min arterial occlusion and a subsequent 5.2 min recovery.

After at least one day the same protocol was repeated but with the optodes positioned on the *tibialis anterior* muscle at the same level as for corresponding bone marrow measurements.

Results

The ABI values were in the normal range for all subjects. There was no statistical correlation between ABI values and age and the mean value obtained was 1.121 (± 0.047).

Fig. 1 shows the calculated bone marrow $[\Delta Hb]$, $[\Delta HbO_2]$, $[Hb_{SD}]$ and $[HbO_{2SD}]$ signals as a function of time for one subject. It must be noticed that there is a larger time dependent baseline increase for $[\Delta Hb]$, $[\Delta HbO_2]$ than for $[Hb_{SD}]$ and $[HbO_{2SD}]$. The noise appearing in the $[HbO_{2SD}]$ values is definitively larger than for $[Hb_{SD}]$ while the $[\Delta Hb]$ and $[\Delta HbO_2]$ data has the same noise level. This observation was true for all the subjects. While, $[\Delta Hb]$, or equivalently $[Hb_{SD}]$, always increased monotonically during ischaemic periods (horizontal black bars) for all the subjects, the values for $[\Delta HbO_2]$ (or $[HbO_{2SD}]$) showed no consistent pattern, some decreasing, others increasing or unchanged and change did not correlate with the age of the subject. For example the $[\Delta HbO_2]$ behaviour in Fig. 1. cannot be clearly determined; however, $[\Delta Hb]$ is clearly increasing during ischaemia. Table 1 summarizes these observations for each subject. The same mixed response was also observed in the muscle studies.

In Fig. 2 the total changes in water concentration from the beginning ($[H_2O_{SD}]_{start}$) to the end ($[H_2O_{SD}]_{end}$) of the protocol are reported for all subjects as a function of the corresponding change in $[\Delta Hb]$ i.e., $[\Delta Hb]_{end} - [\Delta Hb]_{start}$. The changes in $[H_2O_{SD}]$ and $[\Delta Hb]$ are linearly related ($p < 0.05$). Data for both bone marrow and muscle are shown on the same figure because this highlights that this is a “physical” phenomenon independent of the tissue type (see Discussion).

In Fig. 3 the $[\Delta Hb_{SD}]$ concentration as a function of time is shown for one subject (top) together with its first derivative (bottom). This clearly shows the three minima corresponding to the maximum reperfusion velocity that were utilized to compute the reperfusion index (see Methods). Similar tracings were found for the *tibialis anterior* muscle.

The PI values for the bone marrow and muscle as a function of age are reported in Fig. 4. Large negative values correspond to a high blood perfusion rate (negative $[Hb_{SD}]$ -time slope). The *tibialis anterior* muscle PI values are clearly lower than for the tibia bone marrow, resulting in general in a higher reperfusion capacity for the muscle. It can be seen that the reperfusion capacity decreases as a function of age in the

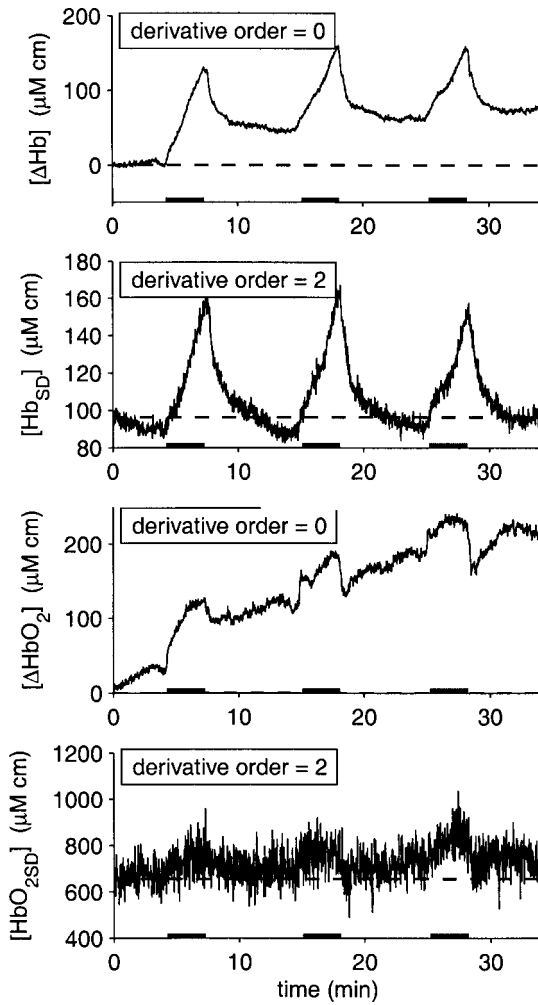


Fig. 1 Time course of deoxyhaemoglobin ($[Hb_{SD}]$), oxyhaemoglobin ($[HbO_{2SD}]$), changes in deoxyhaemoglobin ($[\Delta Hb]$) and changes in oxyhaemoglobin ($[\Delta HbO_2]$) ($\mu M cm$) in the human *tibia* bone marrow for one subject. The parameters $[\Delta Hb]$ and $[\Delta HbO_2]$ were computed from the measured OD using a standard NIRS algorithm (i.e. no derivative) whereas $[Hb_{SD}]$ and $[HbO_{2SD}]$ were obtained from the same experimental data set but by using the second derivative method (see Methods). The black bars correspond to the ischaemic periods. The hatched line represents the starting baseline (set to zero value for $[\Delta Hb]$ and $[\Delta HbO_2]$).

muscle. For the bone marrow the PI is more stable but it starts to increase after 50 years of age.

Fig. 5. reported the results of the Monte Carlo simulation. It can be seen that although the SO_2 data is noisy they lie well on the identity line. This means that the effect of noise on the $[HbO_{2SD}]$ data is not large and that the SO_2 is mainly determined by the $[Hb_{SD}]$ level. Each vertical bar corresponds to the standard deviation over 100 noise realizations.

Fig. 6 reported the SO_2 values for the *tibia* bone marrow and the *tibialis anterior* muscle as a function of age. While bone marrow SO_2 is constant at $84.8 \pm 2.8\%$, the SO_2 value for muscle decreases as a function of age: $-0.35\% \pm 0.13\%$ per year ($p < 0.05$).

Table 1 Behaviour of $[\Delta Hb]$ and $[\Delta HbO_2]$ during the ischaemic periods. The arrows mean that: \uparrow) the curve is monotonically increasing; \downarrow) the curve is monotonically decreasing; \leftrightarrow) there is no clear behaviour.

Age	Bone marrow		Muscle	
	$[\Delta Hb]$	$[\Delta HbO_2]$	$[\Delta Hb]$	$[\Delta HbO_2]$
25	\uparrow	\uparrow	\uparrow	\downarrow
29	\uparrow	\leftrightarrow	\uparrow	\downarrow
29	\uparrow	\uparrow	\uparrow	\downarrow
30	\uparrow	\uparrow	\uparrow	\uparrow
30	\uparrow	\leftrightarrow	\uparrow	\leftrightarrow
33	\uparrow	\uparrow	\uparrow	\downarrow
33	\uparrow	\leftrightarrow	\uparrow	\uparrow
43	\uparrow	\uparrow	\uparrow	\uparrow
45	\uparrow	\downarrow	\uparrow	\uparrow
56	\uparrow	\uparrow	\uparrow	\downarrow
62	\uparrow	\downarrow	\uparrow	\leftrightarrow
63	\uparrow	\uparrow	\uparrow	\leftrightarrow
71	\uparrow	\downarrow	\uparrow	\uparrow

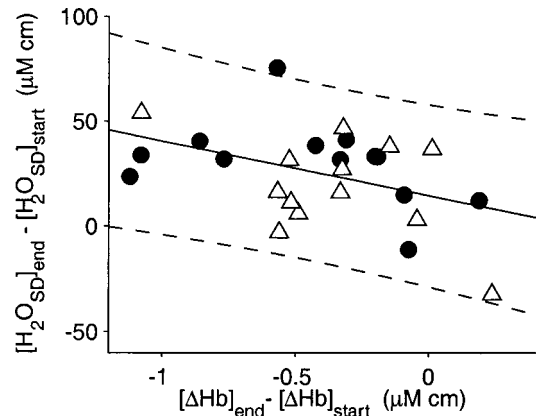


Fig. 2 Changes in the water concentration in arbitrary units ($\mu M cm$) from the beginning of the protocol ($[H_2O_{SD}]_{start}$) compared to the end ($[H_2O_{SD}]_{end}$) as a function of the changes in deoxyhaemoglobin ($[\Delta Hb]_{end} - [\Delta Hb]_{start}$). The parameter $[H_2O_{SD}]$ was obtained by the second derivative method and $[\Delta Hb]$ using the standard method. Data for the human the *tibia* bone marrow (\bullet) and the *tibialis anterior* muscle (Δ) are on shown the same plot.

Discussion

In the present work we have shown that the oxidative metabolic activity of the *tibia* bone marrow is large enough to

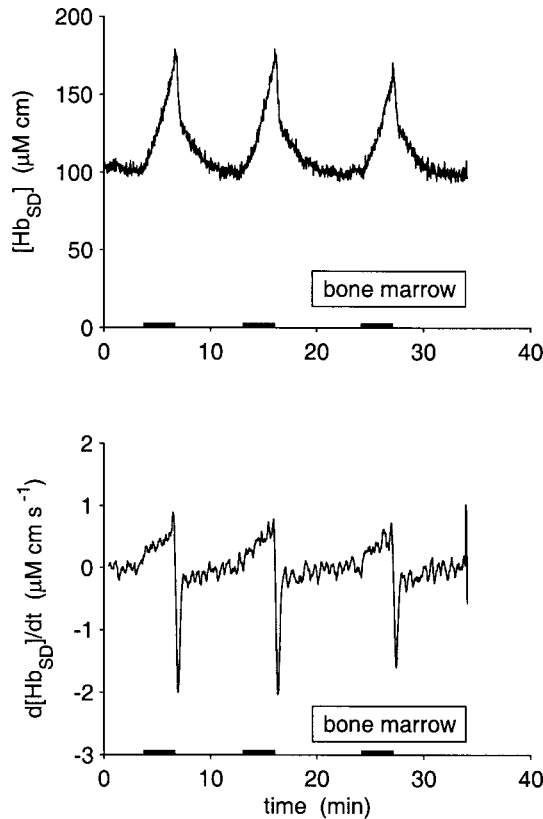


Fig. 3 top) Time course of deoxyhaemoglobin ($[Hb_{SD}]$) ($\mu M \text{ cm}$) as a function of time in the human *tibia* bone marrow for one subject; bottom) Second time derivative of the same $[Hb_{SD}]$ data; The black bars represents to the ischaemic periods.

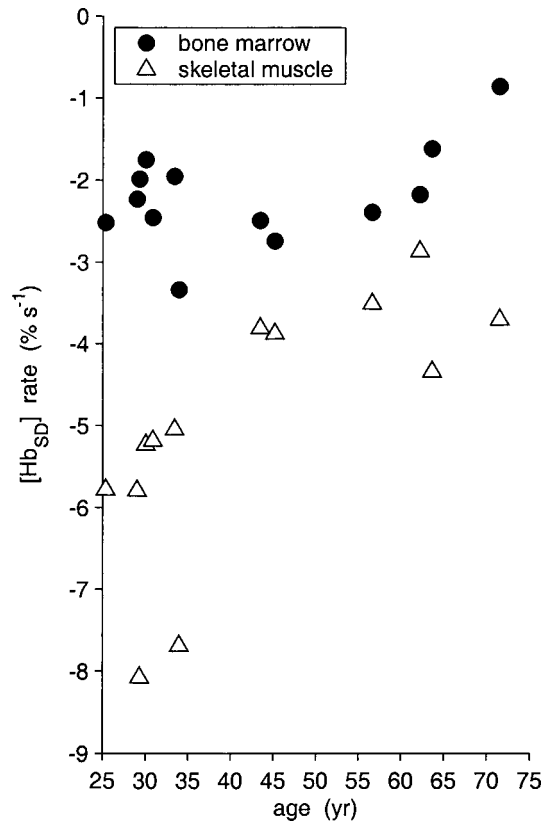


Fig. 4 Reperfusion index expressed in % of $[Hb_{SD}]$ per second (see Methods) for human the *tibia* bone marrow (●) and the *tibialis anterior* muscle (Δ) as a function of age. The reperfusion index describes the reperfusion capacity of a tissue after a 3 minute ischaemic period. Large negative values mean a good (fast) blood reperfusion capacity.

allow us to detect $[Hb_{SD}]$ and $[HbO_{2SD}]$ changes during 3 min ischaemia (Fig. 1). The global behaviour of the bone marrow $[Hb_{SD}]$ and $[HbO_{2SD}]$ time course was similar to that found for the *tibialis anterior* muscle of the same subjects. However, the calculated perfusion was methodically higher in skeletal muscle (Fig. 4) as previously observed (Binzoni et al., 2002). This means that, after an acute ischaemic event, the relative reperfusion capacity of the muscle is higher compared to the bone marrow (the larger is the negative value of the PI the larger the reperfusion capacity). It has been also shown that muscle PI becomes less negative as a function of age. This observation is in agreement with invasive measurements performed in humans, showing that the basal limb blood flow (femoral artery) was 25–30% lower in the 55–75 year age range compared to 20–35 year olds (Dinenno et al., 2001). Bone marrow mass in the human constitute approximately 1–2.5% of the total body weight (Donohue, 1958) and thus has a small influence on whole limb measurements of blood flow or energy metabolism.

For the above reasons, if one wants to assess any parameter related to *tibia* bone marrow perfusion capacity it is necessary to “isolate” the tissue and make specific measurements. To our knowledge, NIRS appears to be the unique technique allowing one to obtain this result non-invasively. In the present work,

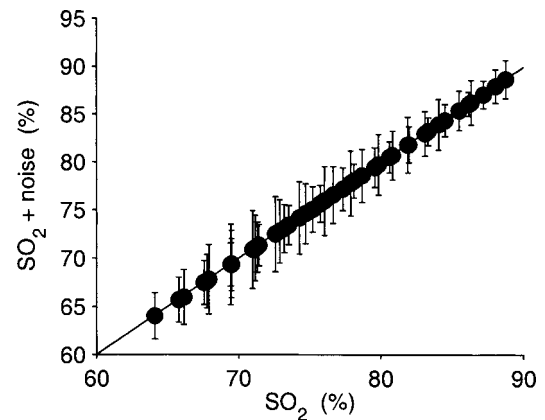


Fig. 5 Monte Carlo simulation showing mean tissue blood O_2 saturation (SO_2) computed using noisy $[HbO_{2SD}]$ data ($SO_2 + \text{noise}$) compared with the exact SO_2 values (i.e. $[HbO_{2SD}]$ without noise). The vertical bars represents the standard deviations computed over 100 random realizations of the noise.

tibia bone marrow PI appears to reduce after 50 years old (Fig. 4). Interestingly, MRI has shown, by using a contrast agent, that the rate of vertebral bone marrow perfusion also shows a

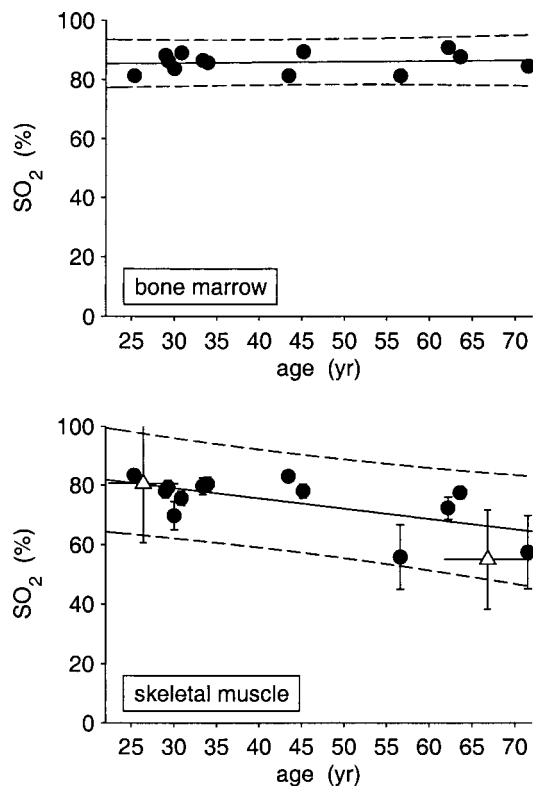


Fig. 6 Mean tissue blood O_2 saturation (SO_2 , ●) as a function of age for the human *tibia* bone marrow and the *tibialis anterior* muscle. The continuous line is the regression line and the hatched lines represents the 95% confidence interval. The vertical bars are the standard deviations. The triangles are data taken from the literature (Costes et al., 1999) for comparison.

significant decrease in subjects older than 50 years (Chen, 2001). It should be noticed, that the standard ABI of our subjects was not sensitive to the normal age related physiological changes detected by NIRS.

As highlighted in the Introduction, a low perfusion (or a less negative PI) does not necessarily mean that the tissue is hypoxic. In fact, mean muscle SO_2 never reaches critical levels (Fig. 6). There is however a linear decrease as a function of age, and the resting SO_2 values found in the present work correspond well to those measured by Costes et al. (1999) in resting human *vastus lateralis* (Fig. 6, triangles). Our approach and that of Costes give the same consistent results; however, in our case it was not necessary to use arterial occlusion for up to 10 min to calibrate the NIRS data. This should allow one to apply the technique to estimate SO_2 in other human tissues. The present muscle SO_2 values may be explained by the fact that PI changes with age. In other words, a decrease in blood flow may by definition increase the muscle oxygen extraction capacity, resulting in a lower SO_2 .

Surprisingly, the *tibia* bone marrow SO_2 remains constant and the saturation level appears to be higher than in the muscle (Fig. 6). Higher SO_2 might be explained by a different metabolic activity between muscle and bone marrow but also

by a smaller bone marrow venous reservoir, compared to muscle. No data confirming this hypothesis was found in the literature. SO_2 constancy, may be related to the observed decrease in the number of sinusoids (“venous” capillaries) with increasing age (Burkhardt et al., 1987). On the other side, the observed SO_2 constancy combined with a reduction in PI above 50 years, might be induced by a decrease in oxidative metabolism, compensating for the age related flow change. In this case, the augmented tissue oxygen extraction (low $[HbO_{2SD}]$) would be compensated by a lower oxygen consumption. Unfortunately, to our knowledge, there are no measurements on humans demonstrating this hypothesis. However, it has been shown in the dog that the bone marrow blood flow is linear related to the oxygen consumption (Sim and Kelly, 1970) which is in agreement with the above hypothesis. Looking at the bone marrow $[Hb_{SD}]$ data appearing in Fig. 1, one can see that it would be possible to directly calculate O_2 consumption by the well tested method proposed by De Blasi et al. (1993). Unfortunately, this method will probably depend on the bone cortex thickness in a non linear way and our current study did not obtain this information. The problems of the measurement of O_2 consumption in bone marrow have to be further investigated. The PI and SO_2 parameters presented here are by definition “normalized” and so they should not, theoretically, be too sensitive to the bone cortex thickness. The ability to observe specifically the *tibia* bone marrow and the adjacent *tibialis anterior* muscle is also dictated by the fact that the adipose tissue thickness is not too large in this region of the leg and that the thickness of the fat layer over the bone marrow and the muscle is nearly the same. This will in this case influence in the same manner the NIRS based measurements of the subjacent tissues (Binzoni et al., 1998; Yamamoto et al., 1998; Van Beekvelt et al., 2001) and make data comparison easier. Of course, the choice of the *tibia* was also dictated by the fact that the bone marrow is easily accessible by NIRS.

In the present work the quantity $[Hb_{SD}]$ has been utilized instead of $[\Delta Hb]$, even when it was not strictly necessary. Theoretically, to compute the PI one might use $[\Delta Hb]$, since as a result of its mathematical derivation is less subject to noise (see Methods and Fig. 1). However, the problem with $[\Delta Hb]$ clearly appears in Fig. 2. In fact, during the protocols, measurable water concentration changes were detected by the NIRS instrument. As shown by Matcher et al. (1993) and Matcher and Cooper (1994) these water concentration variations introduce some artifacts in the NIR spectra that may manifest themselves as an arbitrary DC baseline shift (scattering losses plus unknown absolute photometry) and a quasi-linear background due to the wavelength-dependent scattering losses. These artefacts lead to a large shift of the estimated $[\Delta Hb]$ concentration (compare $[\Delta Hb]$ and $[Hb_{SD}]$ in Fig. 1) during the time. This problem may be bypassed by using the second derivative fitting method (see Methods) and thus $[Hb_{SD}]$. The practical consequence of this is that if one wants to study human bone marrow it is necessary to utilize a

NIR spectrometer able to acquire a range of wavelengths. Only in this way it is possible to compute the second derivative and to correct the large concentration errors. Standard NIR instruments using only 2 or 4 wavelengths seem not to be suitable for making studies of bone marrow haemodynamics.

When measuring muscle or bone marrow oxygenation, it would be more “natural” for a physiologist to use $[\text{HbO}_{2SD}]$ instead of $[\text{Hb}_{SD}]$. However, when looking at Table 1 one can clearly see that $[\text{HbO}_{2SD}]$ is more sensitive to blood volume changes and the behaviour is less correlated to the oxidative metabolic activity. From the metabolic point of view, $[\text{HbO}_{2SD}]$ should be the “mirror image” of $[\text{Hb}_{SD}]$, but possible blood volume changes may break this symmetry (e.g. the $[\Delta\text{Hb}]$ and $[\Delta\text{HbO}_2]$ during the ischaemic periods in Fig. 1). It has already been noted that in some case, e.g. during strenuous exercise on a cycloergometer (*vastus lateralis* muscle; Cerretelli and Binzoni, 1997), the $[\text{Hb}_{SD}]$ signal seems to be less influenced by volume changes and thus more representative of muscle metabolism. Concerning the present protocol, Table 1 summarizes well this observation; $[\Delta\text{Hb}]$ should increase and $[\Delta\text{HbO}_2]$ decrease linearly during the ischaemic periods (neglecting of course the baseline shift due to the scattering changes). Actually, the only variable changing monotonically and always in the expected “physiological” direction related to the metabolic activity is the $[\Delta\text{Hb}]$ (up-vertical arrows in Table 1).

The large amount of noise on the $[\text{HbO}_{2SD}]$ data (e.g. Fig. 1) would lead one to think that the computed basal SO_2 values (eqn. 2) are also strongly influenced. However, the Monte Carlo simulations clearly show that for the range of the present $[\text{Hb}_{SD}]$ and $[\text{HbO}_{2SD}]$ experimental values, SO_2 is mainly determined by $[\text{Hb}_{SD}]$ (Fig. 5) and that the mean over many experimental points gives reliable SO_2 values.

In conclusion, it appears that even if *tibia* bone marrow blood flow changes as a function of age, the SO_2 level remains constant in normal subjects. It has also been shown that bone marrow SO_2 is higher than muscle SO_2 for any age. Apart from the application reported here, the present human *tibia* bone marrow model might open other exciting areas of physiological research.

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Correspondence to: Tiziano Binzoni, PhD, PD, Centre Médical Universitaire, Département de Physiologie, 1211 Genève 4, Switzerland

Phone: ++41 22 70 25 358

Fax: ++41 22 70 25 402

e-mail: Tiziano.Binzoni@medecine.unige.ch