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NONINVASIVE PROBING OF THE NEUROVASCULAR SYSTEM IN HUMAN BONE/BONE MARROW USING NEAR-INFRARED LIGHT*

TIZIANO BINZONI[†,‡,¶] and DIMITRI VAN DE VILLE[‡,§,‖]
[†]*Département des Neurosciences Fondamentales*
University of Geneva, Rue Michel-Servet 1
1211 Genève 4, Switzerland

[‡]*Département de l'Imagerie et des Sciences de l'Information Médicale*
University Hospital, Rue Gabrielle-Perret-Gentil 4
1211 Genève 14, Switzerland

[§]*Institute of Bioengineering*
École Polytechnique Fédérale de Lausanne (EPFL)
STI-IBI, BM4.128, Station 17
1015 Lausanne, Switzerland
[¶]*tiziano.binzoni@unige.ch*
[‖]*dimitri.vandeville@epfl.ch*

Understanding the mechanisms of interaction between bone/bone marrow, circulatory system and nervous system is of great interest due to the potential clinical impact. In humans, the amount of knowledge in this domain remains relatively limited due to the extreme difficulty to monitor these tissues continuously, noninvasively and for long or repeated periods of time. A typical difficult task would be, for example, to continuously monitor bone/bone marrow blood perfusion, hemoglobin oxygen saturation or blood volume and study their dependence on the activity of the autonomic nervous system. In this review article, we want to show that near-infrared light might be utilized to solve these problems in part. We hope that the present analysis will stimulate future studies in this domain, for which near-infrared light appears as the best available technology today.

Keywords: Review; laser-Doppler flowmetry; near-infrared spectroscopy; photoplethysmography.

1. Introduction

Contrary to popular belief, bones are very active organs in our body. Indeed, in bone/bone marrow, the circulatory and nervous system play an important role in controlling the physiological process.[1] The vascular tree of these tissues is capable to adapt its geometry to the needs of physiological changes such as during human growth, aging or in pathological conditions.[2] Blood vessels in the bone transport substrates involved in energy metabolism, and osteogenesis and participate in the skeletal homeostasis.[1] Blood flow by itself may be implicated in bone growth and remodeling by

*Dedicated to the memory of Professor Britton Chance and his visionary ideas in biomedical photonics.

stimulating specialized bone cells.[3] Bone contains a rich innervation that participates in the control of bone blood flow and probably in the regulation of bone metabolism.[2,4] A sensitive innervation is also present in bone periosteum.[1] Even if a better comprehension of the above-mentioned mechanisms has always been considered important due to the potential clinical impact, we still need to advance our understanding of the ongoing processes *in vivo*.

Well-known physiological parameters, such as blood perfusion, hemoglobin oxygen saturation or blood volume, remain inaccessible in bone/bone marrow. This is especially true if their evolution or interaction with the nervous system has to be studied in humans. This measurement problem comes from the fact that it is extremely difficult to monitor the bone/bone marrow continuously, noninvasively and for long or repeated periods of time. Measurement techniques that are usually applied in laboratory are difficult or impossible to apply on humans for bone/bone marrow monitoring, and when applicable, they are partially invasive and permit only a few measurements.[2]

For this reason, during the past years, our group and others have tried to use near-infrared light to surmount some of these issues. The developed approaches can be divided in three groups based on three different working principles: near-infrared spectroscopy, photoplethysmography, and laser-Doppler flowmetry at large interoptode spacing. All of these techniques use near-infrared light to probe because it can deeply penetrate into the bone/bone marrow and thus carry desirable information on blood parameters after tissue interaction. One of the major advantages of these techniques is that they are noninvasive. This means that they can also be deployed in special experimental conditions, where the psychological stress of the subject may have a strong influence on the regulatory processes of the vascular system, and thus on the reliability of the measurements.[5,6]

Without complicating this overview with too many technical details, we have chosen to focus on the physiological results with the hope that a larger number of investigators will be stimulated by the findings to develop new interesting studies related to the neurovascular control in bone/bone marrow.

2. Three Complementary Techniques

Each of the techniques presented in the following sections uses a light source (e.g., laser or normal lamp) and a photodetector. The light is perpendicularly "projected" into the tissue and then collected by the photodetector, by means of two optical fibers with the tips positioned on the skin surface where the investigated bone is situated. The distance between the two fibers (interoptode spacing) is typically chosen in the range 10−40 mm. As a rule of thumb, the region of interest detected by these techniques is a "banana shaped" volume with its extremities situated at the position of the two fibers' tips (the term "banana shaped" has been historically coined, thanks to Professor Chance and his studies on photon migration in biological tissues[7]). Now we see how each technique exploits the detected light to obtain physiologically related parameters (e.g., Hb, blood flow, and other physiological parameters). It must be noted that the bones investigated in the experimental works presented in this brief review are always the human tibia diaphysis and the patella. The advantage of these bones is that they are not covered by a skeletal muscle at the point of measurement and thus the signal contamination of the latter tissue can be neglected.

2.1. *Near-infrared spectroscopy*

Near-infrared spectroscopy (NIRS) allows to monitor levels of tissue oxy- (HbO_2) and deoxy-hemoglobin (Hb) concentrations, and tissue blood oxygen saturation (SO_2). It must be noted that nowadays, many different types of NIRS instrumentation exist (for a review see Delpy and Cope[8]), but the most representative among them is certainly the RUNMAN developed by Professor Chance and his team.[9] In fact, this was the first NIRS instrument (with two wavelengths) utilized to perform routine measurements in humans. Thanks to the RUNMAN, human biophotonics has acquired a great momentum and it has never stopped since then. For the sake of precision, note that in the following cited references a slightly different NIRS system has been used; i.e., not two but a continuum of wavelengths (typically in the range 700−800 nm) was used, together with a mathematical treatment not present in the RUNMAN.[8] However, even if this represents a technical improvement, the main philosophy introduced by Professor Chance always remains.

Using NIRS, it has been shown that the speed of resaturation in oxygen of the blood in bone/bone marrow after 3 min ischemia is faster in a skeletal

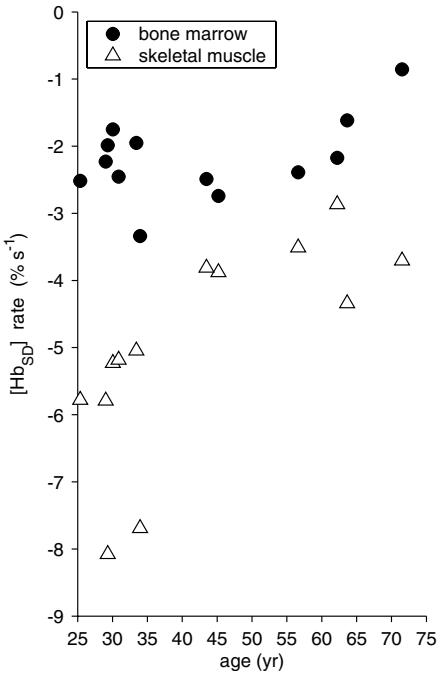

Fig. 1. Reperfusion index of human bone/bone marrow (diaphysis) and *tibialis anterior* muscle expressed in % of Hb_{SD} per sec as a function of age (Figure from Binzoni *et al.*[10] Reprinted with permission). The larger the negative number, the faster the blood reoxygenation speed. The parameter Hb_{SD} is equivalent to Hb in the present manuscript.

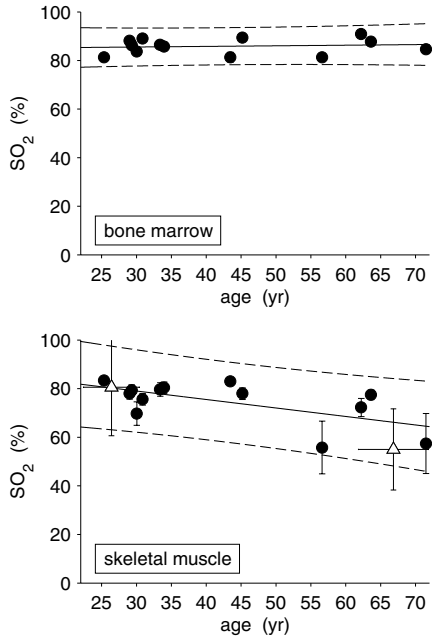

Fig. 2. Mean blood oxygen saturation (SO_2) as a function of age for the human tibia bone/bone marrow (diaphysis) and the *tibialis anterior* (Figure from Binzoni *et al.*[10] Reprinted with permission). The continuous line is the regression line and the hatched lines represent the 95% confidence intervals. Vertical bars are the standard deviations. The triangles represent the same SO_2 measurements but are taken from the literature.[11]

muscle than in bone/bone marrow; see Fig. 1.[10] In this figure one can also observe that the reoxygenation speed decreases with age. It must be noted that, though the experimental interoptode spacing was large enough to observe the marrow, there are probably also contributions from the "bone" (cortex). For this reason we prefer the expression bone/bone marrow. Another interesting observation (see Fig. 2) was that SO_2 is higher in bone/bone marrow than in skeletal muscle (*tibialis anterior*). At the same time, SO_2 in muscle decreased with age, while in bone/bone marrow it remained constant.

The noninvasiveness of NIRS allows to follow the long-term evolution of the blood flow−related parameters in humans exposed to special environments, such as astronauts. Therefore, NIRS has been proposed as a tool for monitoring the effects of microgravity on human bone/bone marrow.[12] Another link between gravity, blood volume distribution and flow is shown in Fig. 3, where $Hb_{tot} = Hb + HbO_2$ in bone/bone marrow (tibia diaphysis) increases when the subject was submitted to orthostatic stress (tilt bed).[13] This means that blood accumulates inside the bone/bone marrow, during transition from supine to vertical position. This phenomenon is probably enabled by the simultaneous displacement of extravascular fluids, however, this hypothesis remains to be investigated. One must highlight the fact that this kind of NIRS-derived measurements are hard to be implemented with any other, even invasive, technique. By studying Hb and HbO_2 independently, it can be inferred that probably an veno-arteriolar reflex might also exist in bone/bone marrow as observed in muscle[a] (see, e.g., Fig. 4, dashed lines and original manuscript[13]).

This topic of monitoring bone/bone marrow noninvasively is interesting and for the moment only near-infrared−related techniques allow its exploration in humans. By computing the rate of change of Hb and HbO_2 during arterial occlusion, it has been possible to estimate the oxygen consumption of the

[a]In response to elevated venular pressures, a local reflex signal from the venule to the arteriole (veno-arteriolar axon reflex) can evoke vasoconstriction.[14]

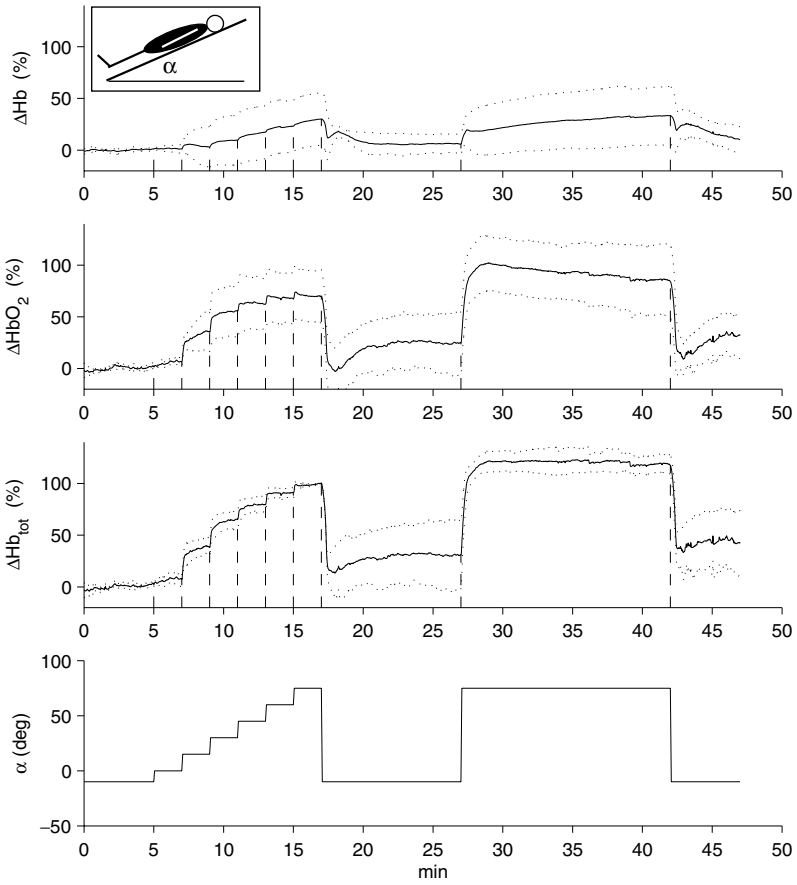

Fig. 3. Oxy- (ΔHbO_2), deoxy- (ΔHb) and total (ΔHb_{tot}) hemoglobin changes as a function of time in human tibia bone/bone marrow (diaphysis) during orthostatic variations for six subjects (Figure from Binzoni *et al.*[13] Reprinted with permission). The orthostatic pressure is changed by varying the tilt bed angle α. The dotted lines represent the standard deviation. The vertical dashed lines represent the changes in bed position (i.e., a change of α). Hemoglobin units are expressed as a percentage of ΔHb_{tot} at 17.0 min for $\alpha = 75°$ (mean over 20 s).

distal trabecular zone of the tibia.[16] As expected, it has been found that bone oxygen consumption does not change from rest to 80% of maximum voluntary contraction (dorsiflection) while in muscle the oxygen consumption does increase.

2.2. *Photoplethysmography*

Photoplethysmography is a simple and low-cost technique to detect tissue blood flow changes.[17] This technique monitors the intensity of the light reaching the photodetector at one wavelength (in the references presented in the following paragraphs, a light emitting diode of 560 nm or 804 nm was used), and it is generally accepted that this intensity may depend on two parameters: (1) the blood volume, Hb_{tot}, in the region of interests and; (2) the "shape" of the red blood cells. In the first case, the detected intensity may change as the light is more or less "absorbed" by the tissue depending on the hemoglobin content. In the second case, the variation of the red blood cells' shape induced by the change in their speed inside the blood vessels induces a variation in the scattering coefficient of the tissue, which results in a change in the light intensity. The second possibility explains why it is possible to observe by photoplethysmography "blood pulsations" in tissues where it is assumed that the vessels cannot expand (typically in cancellous bone).[17] This is also why, according to these authors, the term "blood flow" has been used in the presentation of the data.

The main advantage of this technique is that it can acquire data very fast, making it possible to observe the tissue blood pulsations. In fact, photoplethysmography does not need a complex

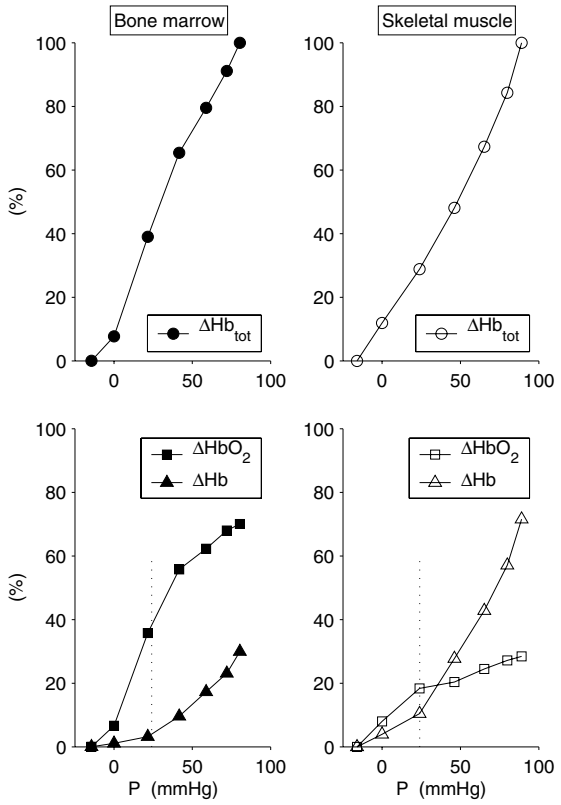

Fig. 4. Oxy- (ΔHbO_2), deoxy- (ΔHb) and total (ΔHb_{tot}) hemoglobin changes as a function of the orthostatic intravascular pressure (P) in human tibia bone marrow (diaphysis; closed symbols) and *Gastrocnemius medialis* (open symbols; data derived from Binzoni *et al.* (2000) for six subjects[15]). Hemoglobin units are expressed as a percentage of ΔHb_{tot} at the maximum P value (corresponding to a tilt bed angle of $\alpha = 75°$) independently for each tissue. The dotted lines define the mean P value for which a veno-arteriolar reflex is induced in the *Gastrocnemius medialis*, producing a decrease in the mean tissue blood speed.[15] Figure from Binzoni *et al.*[13] Reprinted with permission.

data treatment, such as in the case for NIRS or LDF, because it directly relies on the detected light intensity. By exploiting this approach, it has been possible to show that blood flow changes are detectable noninvasively in patellar bone.[18] Subsequently, this technical possibility has allowed the first clinical investigation in bone. So a question arises from this observation: is an ischemic mechanism involved in the pathogenesis of the patello-femoral pain syndrome? It has been found that the decrease in the pulsatile peaks after knee flexion (see Fig. 5) is larger in patients than in normal subjects. According to the authors, this supports the hypothesis that a change in bone blood perfusion may be involved in this pathology.[19]

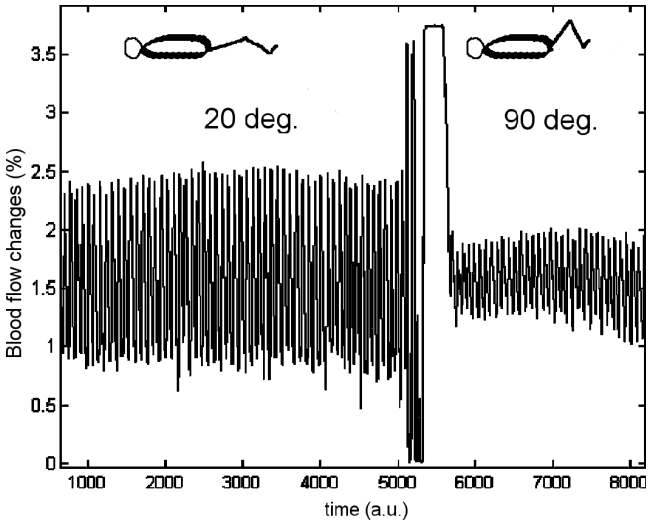

Fig. 5. Photoplethysmographic recordings showing the decrease in pulsatile patellar blood flow changes after passive knee flexion from 20° to 90°, for a typical subject. The abscissa represents the time (units not specified in the original). Figure modified from Näslund *et al.*[19] Reprinted by permission of SAGE Publications.

2.3. *Laser-Doppler flowmetry at large interoptode spacing*

Laser-Doppler flowmetry (LDF) allows measuring tissue blood perfusion (also "flow"), tissue mean blood velocity and the number of moving red blood cells (often wrongly termed "blood volume").[20,21] LDF is based on the laser-Doppler effect resulting in a frequency shift of the light when interacting with a moving red blood cell.

In bone, the LDF technique is typically applied invasively or during surgical operations. Since the LDF setup usually used in these cases have a very short interoptode spacing, it is necessary to insert the optical fibers through the skin to reach the bone (since the measured volume is very small, typically smaller than 1 mm^3). Actually, it is possible to assemble LDF with large interoptode spacing, thus allowing to monitor deep regions of tissue.[22] With this approach, the measurements become completely noninvasive.

In Fig. 6, one can see blood perfusion measurements during a repeated ischemia−reperfusion protocol, obtained from two different types of human tissues: the skeletal muscle (forearm muscle) and the bone (cortex) of the tibia diaphysis. The post-ischemic hyperemia appearing in skeletal muscle can easily be observed in Fig. 6 as large peaks appearing immediately after the ischemic

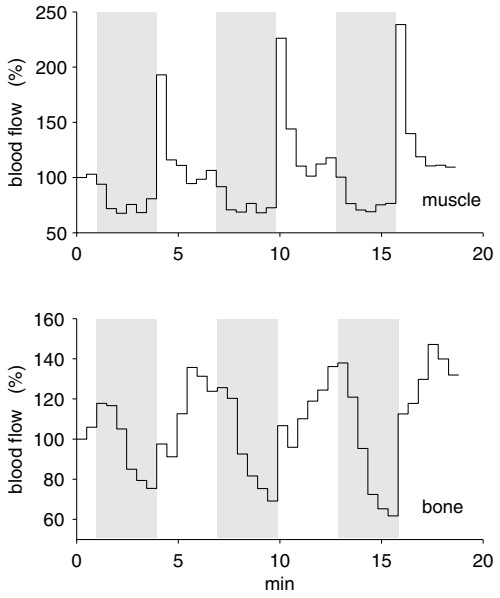

Fig. 6. Laser-Doppler blood flow (perfusion) measurements (wavelength 785 nm) as a function of time, performed during repeated ischemia−reperfusion intervals. The measurements were performed on the forearm muscles and on the tibia diaphysis (cortex) of the same subject. The gray-colored region correspond to the ischemic periods (generated by inflating a pressure cuff).

periods. Note that during ischemia (gray-colored region) the LDF signal does not return to zero, which is the (technical) reason why this baseline level is called the "biological zero". The crucial point is that there is no post-ischemic hyperemia in bone, and that all the flow transients are slower than observed in skeletal muscle. This phenomenon observed is perfectly compatible with the findings presented in Fig. 1. These simple measurements show that LDF can also be used to monitor noninvasively blood flow changes in bone, and adds complementary information to NIRS and photoplethysmography.

3. Conclusions

In the present brief review, we demonstrate that NIRS, photoplethysmography and LDF are complementary techniques that allow monitoring of parameters related to bone/bone marrow blood flow, velocity, volume and oxygen saturation. As mentioned in Sec. 1, it is important to remember that the autonomic nervous system, and as a consequence tissue blood flow perfusion, may be strongly influenced by mental challenges such as stress or hypnosis.[5,6,23] For this reason, any invasive measurement technique can potentially alter the flow-related parameters. Thanks to the noninvasive character of light-derived techniques such as NIRS, photoplethysmography and LDF, it becomes now easy to investigate in real time the role of the autonomic nervous system in the control of the bone/bone marrow blood perfusion, without disturbing the measurement process. Unfortunately, the number of studies (on human subjects) in this field remains very limited for the moment. We hope that the present overview will encourage future research on this interesting and important topic.

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