Non-Thermal Radio Frequency and Static Magnetic Fields Increase Rate of Hemoglobin Deoxygenation in a Cell-Free Preparation

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Abstract

The growing body of clinical and experimental data regarding electromagnetic field (EMF) bioeffects and their therapeutic applications has contributed to a better understanding of the underlying mechanisms of action. This study reports that two EMF modalities currently in clinical use, a pulse-modulated radiofrequency (PRF) signal, and a static magnetic field (SMF), applied independently, increased the rate of deoxygenation of human hemoglobin (Hb) in a cell-free assay. Deoxygenation of Hb was initiated using the reducing agent dithiothreitol (DTT) in an assay that allowed the time for deoxygenation to be controlled (from several min to several hours) by adjusting the relative concentrations of DTT and Hb. The time course of Hb deoxygenation was observed using visible light spectroscopy. Exposure for 10–30 min to either PRF or SMF increased the rate of deoxygenation occurring several min to several hours after the end of EMF exposure. The sensitivity and biochemical simplicity of the assay developed here suggest a new research tool that may help to further the understanding of basic biophysical EMF transduction mechanisms. If the results of this study were to be shown to occur at the cellular and tissue level, EMF-enhanced oxygen availability would be one of the mechanisms by which clinically relevant EMF-mediated enhancement of growth and repair processes could occur.

Introduction

Identification of some of the mechanisms underlying EMF bioeffects and their clinical applications [1] has contributed to the development and more widespread use of more effective therapeutic signals [2]. It is clear that EMF modulation of nitric oxide signalling plays an important role in EMF therapeutics [2–6]. However, there may be other transduction pathways through which EMF signals could modulate tissue repair and growth. For example, several recent studies have reported EMF effects on human Hb in solution and erythrocyte suspensions, including decreases in viscosity [7] and changes in impedance [8], dielectric properties [9], dia- and para-magnetic properties [10], optical absorption [11] and in vivo deoxygenation [12]. In addition, radiofrequency mobile telephone signals have been shown to decrease Hb oxygen affinity in vitro [13] and changes in infrared absorption have been reported for low-frequency EMF [14]. Also, enhanced oxygen delivery by Hb is under investigation as a therapeutic strategy for the treatment of pathologies such as ischemia from stroke, cardiac disease and diabetic ulcers [15]. Thus, the present study aimed to characterize the effects on the rate of deoxygenation of human hemoglobin (Hb) in an in vitro cell-free assay of a pulse-modulated radiofrequency (PRF) signal currently in clinical use for treatment of pain, edema and chronic wounds [1,2], and, applied independently, of a static magnetic field (SMF), from permanent ceramic magnets constructed for therapeutic applications and reported to reduce pain [1]. These non-thermal EMF modalities were chosen for this study due to their demonstrated efficacy in a variety of therapeutically relevant settings [1–6]. The reducing agent dithiothreitol (DTT) was employed for its ability to facilitate control of the time course of Hb deoxygenation by adjusting the ratio of DTT/Hb [16]. This study will show that exposure to PRF and SMF, yielded significant increases in the rate of Hb deoxygenation in the presence of the
EMF Exposure

All hemoglobin samples were exposed to the ambient magnetic field, which was measured using a digital Gauss/Tesla meter (model 7010, F.W. Bell, USA) to be 40.5 ± 0.6 mT. A typical horizontal component value was 34.7 ± 0.2 mT, with a vertical component value of 21.0 ± 0.2 mT. The PRF signal is approved by the US FDA [1–3,18–24]. The PRF signal was delivered with a 20 cm circular horizontal (vertical component = 34.7 mT) sinusoidal carrier (derived from the carrier frequency 27.12 MHz sinusoidal carrier (derived from the carrier frequency)) configured to operate nonthermally through pulse modulation in geometric conditions (control) on the laboratory bench, with a field strength similar to those commonly employed in therapeutic applications [25]. The maximal value of the horizontal and vertical components (both parallel to the magnetic field) was 40.5 ± 0.6 mT. SMF components were measured using a digital magnetometer (Model 450, gaussmeter with MMT-G02-VG transverse Hall effect probe with a 1 mm resolution, Lake Shore Cryotronics, Sunnyvale, CA, USA). The plate remained in the spectrophotometer at 22°C during the reaction, and the time course of deoxygenation was determined using the method of Benesch et al. [17], which employs a weighted linear combination of optical densities at 560, 576 and 630 nm to determine oxyHb, deoxyHb and metHb concentrations. Stock Hb solutions (in Hepes, pH 7.2) and spectra from Hb/DTT data at t = 0 (i.e. after 30 min EMF/Control exposures) were assayed for oxy, deoxy and metHb at 560, 576 and 630 nm [17]. The mean ratio of deoxy/oxy was 3.3% and metHb/DTT was 0.6%. The two datasets do not differ significantly (P = 0.44, n = 5). The mean ratio of metHb/oxyHb was 21.8 ± 3.8% for stock solutions and 25.6 ± 3.7% for Hb/DTT data at t = 0, indicating no significant differences (P = 0.33, n = 5). Optical densities at 540, 560, 576 and 630 nm were measured immediately after EMF exposure and at successive 1 to 30 min intervals, until maximal deoxygenation was observed. Calibration studies confirmed that the kinetics of deoxygenation could be controlled by adjusting the ratio of DTT/Hb concent-
trations [16]. Under the conditions stated above, deoxygenation occurred between 140 and 160 min after EMF exposure. Deoxygenation time was reduced by the addition of 5 M of urea to between 60–85 min after EMF exposure. In the absence of DTT, no significant deoxygenation of Hb occurred up to 3 hours (data not shown). Changes in oxyHb concentration with time are summarized in the figures, and maximal differences between deoxy and metHb for EMF exposures vs controls are reported in the text. All figures and data shown are the results of single experiments that have been repeated 2–10 times.

**Statistical Analysis**

Results were compared using the Student’s t test, or one-way repeated measures ANOVA with Holm-Sidak post hoc analysis, and Fieller’s method for the variance of ratios, as required (Systat 3.0, Systat, Chicago, IL) and are reported as means±SEM. Significance was accepted at P≤0.05.

**Results**

Immediately after 30 min of PRF or SMF exposure, no differences in Hb visible light spectra between PRF, SMF and control (ambient magnetic field only) samples were detected. However, changes in visible light spectra were observed in the 540–630 nm region during the time of most rapid deoxygenation, which occurred in this experiment at 140–160 min after EMF exposure. Figure 1 illustrates typical visible light spectra for PRF, SMF and control exposure conditions, drawn from a single exposure tube for each EMF condition. Deoxygenation occurred at an earlier time following PRF and SMF exposures (trace with one peak), as compared to control samples exposed only to the ambient geomagnetic laboratory environment (trace with two peaks). In trials employing PRF exposure (n = 5 tubes for each EMF exposure condition) using 20 mM DTT, 150 min after a single 30 min exposure, PRF-treated samples exhibited a 30.8±10.3% reduction in oxyHb as compared to controls (14.6±1.3 μM vs. 21.1±2.5 μM, P<0.03) (Figure 2). Concomitant, significant increases in deoxy Hb (86.9±1.1 μM vs. 82.8±1.8 μM, P<0.05) and metHb (42.2±1.4 μM vs. 37.1±1.4 μM, P<0.02) also occurred. For the ratio of DTT/oxoHb concentrations employed in these trials (20 mM DTT; 120 μM oxyHb), 150 min corresponded to the time of most rapid deoxygenation. Addition of 5 M urea to the reagent solution decreased the time to required deoxygenate to approximately 65–75 min after reaction initiation. For these conditions, the maximal difference between PRF-treated and control samples occurred at 75 min, with PRF-treated samples showing a 70.5±9.7% reduction in oxyHb as compared to controls (8.5±2.1 μM vs. 28.8±6.3 μM, P<0.03) (Figure 3). Concomitantly, there were significant increases in deoxy Hb (54.4±2.2 μM vs. 40.9±5.2 μM, P<0.04) and metHb (73.4±1.4 μM vs. 61.0±2.3 μM, P<0.02). Under similar conditions (30 mM DTT, 5 M urea, n = 5) SMF treated samples (10–30 min exposure) underwent nearly complete deoxygenation before the untreated (control) samples began to deoxygenate (P<0.002 for 42 min t<38 min). For these SMF treated samples, the most rapid deoxygenation occurred approximately 10 min earlier than for control samples (Figure 4). Under these conditions, SMF-treated samples substantially completed DTT-induced deoxygenation before untreated samples began to lose O₂, an effect that was visibly observable in the 96-well spectrophotometer plate, as shown in the photo in Figure 5, taken approximately 46 min after the reaction was initiated. Experiments were also carried out for which: 1) Hb was treated with PRF or SMF for 30 min prior to introduction into the deoxygenation solution (20 mM DTT+5M urea in 50 mM Hepes) and 2) the deoxygenation solution was treated with PRF or SMF for 30 min prior to introduction of Hb. In both cases the difference between PRF- or SMF-treated and control samples was not significant. Also, 10 min and 30 min PRF and SMF treatment durations were compared (in 20 mM DTT+5M urea in 50 mM Hepes), with no significant differences observed in the time course of deoxygenation. In the absence of DTT, no PRF or SMF effects were observed on the Hb oxidation state. The SMF sensitivity of this Hb deoxygenation assay was repeated at the University of Bologna, Italy. Initial experiments were performed in New York with Hb from a single donor, and subsequent trials in Bologna used Hb from 3 different donors, with similar results.

**Discussion and Conclusions**

The results from this study indicate that exposure to either PRF or SMF, applied independently, can alter the rate of DTT-induced deoxygenation of human Hb in an in vitro cell-free preparation, resulting in more rapid Hb deoxygenation. To the authors’ knowledge, this is the first report showing that PRF and SMF produced similar effects in the same biological system, in the same study. The rate of Hb deoxygenation is non-linear in time and is dependent upon the ratio of DTT/HbO₂. For this assay, the use of DTT as a reducing agent is preferable to other reducing agents, due to the ease with which the time to deoxygenation can be controlled [16]. It is interesting to note that a similar EMF sensitivity, for Hb exposed to a GSM mobile phone signal, was observed using sodium dithionite [13]. This suggests that EMF acted upon a functional aspect of the Hb deoxygenation process itself, and that this effect was not specific to the reducing agent employed in this study. The observation that EMF pre-treatment of Hb alone, or of the deoxygenation solution itself, failed to yield a significant effect suggests that both PRF and SMF exposures acted upon the interaction of Hb with the deoxygenating solution. The addition of 5 M urea reduced the time to deoxygenate and rendered both the PRF and SMF effects more easily observable. Urea creates an extended cloud of waters that only weakly participates in the hydrogen bonding network of bulk water, facilitating the loosening of the protein structure [26] and destabilizing the water-oxoHb structures that act as key allosteric mediators of the Hb T-R transition [27], thus reducing the energy required to deoxygenate. However, it is unclear whether the addition of urea affected the primary PRF or SMF transduction or merely renders the EMF effects more easily observable. No differences were observed between 10 and 30 min SMF and PRF treatment durations. Effects were observable several min to several hours after PRF or SMF exposure was removed, suggesting that these EMF modalities modified protein/solution structure in a manner that altered the energy required for deoxygenation.

This study does not provide sufficient detail to allow for the elucidation of the mechanisms of action of EMFs on hemoglobin, or to determine if SMF and PRF act upon the same submolecular targets to produce the effects observed here. Also, further studies are required to determine the range of field parameters for which this assay exhibits sensitivity. However, the sensitivity and biochemical simplicity of the assay developed here suggests its usefulness for future studies that may further establish basic biophysical EMF transduction mechanisms. The PRF signal was developed using a theoretical model for the effect of the induced electric field on ion binding kinetics at protein aqueous interfaces [1–3,18]. It should be noted that PRF transduction through the induced electric field is distinct from the transduction pathway involved in SMF effects, for which no electric field is present. This
Figure 1. EMF effect on hemoglobin (Hb) visible light spectra. Spectra representative of the effects of pulsed radiofrequency (PRF) signal and 186 mT static magnetic field (SMF) on Hb visible light spectra during deoxygenation. Data shown are typical of samples drawn from a single tube for each EMF exposure condition, shown here at 150 min after a single 30 min EMF exposure. Deoxygenation of 100 μM Hb was carried out in 50 mM Hepes buffer (pH 7.2) using the reducing agent dithiothreitol (20 mM) at 22°C, and is characterized here by the passage of the spectrum from a two-peaked to one-peaked form. Deoxygenation occurred at an earlier time for EMF exposed samples (traces with one peak), as compared to control samples exposed only to the ambient geomagnetic laboratory environment (trace with two peaks). The EMF effect was observable at the time of most rapid deoxygenation.

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Figure 2. Effect of the pulsed radiofrequency field on time course of hemoglobin deoxygenation. Time course of oxy hemoglobin (HbO₂) concentration, after a single 30 min pulsed radiofrequency electromagnetic field (PRF) exposure of Hb, under deoxygenating conditions. Concentration was determined by visible light spectroscopy at 560, 576, 630 nm. PRF exposure resulted in a significant (30.8±10.3)% reduction in oxyHb concentration, as compared to controls (14.6±1.3 μM vs. 21.1±2.5 μM, P<0.03, n = 5), suggesting an alteration in Hb solution properties that persisted after PRF signal was removed.

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Addition of 5 M urea to the deoxygenation assay reduces the time required for deoxygenation and renders the PRF effect more apparent. The maximal difference between PRF-treated and control samples occurred at 73 min, with PRF-treated samples showing a significant (70.4±9.7)% reduction in oxyHb concentration, as compared to controls (8.5±2.1 μM vs. 28.8±6.3 μM, P<0.03, n=5), in contrast to the 30.8% reduction observed in the absence of urea (cf. Figure 2).

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Effect of the static magnetic field on time course of hemoglobin deoxygenation. Hb deoxygenation after 10 min exposure to 186 mT static magnetic field (SMF) in 5M urea. No significant change in oxy/deoxy ratio was visible until the time of rapid deoxygenation, at approximately 40 min. The time of most rapid deoxygenation occurred approximately 10 min earlier for SMF treated samples (P<0.002 for 42 min < t < 58 min, n=5).

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Electromagnetic Fields on Hemoglobin Deoxygenation

suggestions that more than one mechanism may be responsible for the results reported here. Although direct action on ferrous heme [28] or free radical lifetimes [29,30] may account for the SMF effects observed here, several other models have been proposed for weak, μT-range magnetic field bioeffects [31]. We have previously shown using the Lorentz-Langevin model that μT-range magnetic fields may have an effect on the thermally activated orientation of ionic oscillators and waters bound at the protein surface [32,33] and that the minimum magnetic field required to directly compete with thermal forces to affect dissociation of a bound ion or ligand from a protein binding site is in the 1–10 mT-range [34]. Thus, the effect of the μT-range magnetic field employed in this study could occur by the direct action of the Lorentz force on charges bound at the protein/water interface. Functionally important hemoprotein molecular motions are slaved to the thermal fluctuations of the bulk solvent [35] and protein hydration plays a fundamental role in the stability of dynamics between Hb T-R conformations [36] so that the PRF and SMF interactions described above, acting at the protein/water interface, may suggest a means by which EMF could modulate protein function.

The deoxygenating conditions employed here, using the reducing agent DTT in an in vitro cell-free model, differ substantially from those found in vivo. However, EMF-induced changes in the structure and function of Hb and erythrocyte suspensions shown by others [7–12,14] have been reported in vivo and in aqueous solution (i.e. without chemical deoxygenating agents), thus demonstrating an EMF sensitivity under physiological conditions and in the absence of reducing agents. Enhanced delivery of oxygen has been shown to reduce inflammation [37] and enhance tissue repair [38] and at least one trial has reported an EMF-induced increase in deoxyHb in an in vivo animal model [12]. Allosteric modification of Hb has been suggested as a clinically useful means of enhancing oxygen delivery [39], and is in development for in vivo treatment of ischemia from stroke, cardiac disease and diabetic ulcers [13]. Although much further work is required to ascertain the clinical relevance of the results reported here, enhanced oxygen delivery using PRF or SMF may be important non-invasive, non-pharmacologic therapeutic modalities by which clinically relevant EMF-mediated enhancement of growth and repair processes can occur.

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Author Contributions

Conceived and designed the experiments: DM RL DC PL. Performed the experiments: DM. Analyzed the data: DM RL DC. Contributed reagents/materials/analysis tools: RL DC PL MM. Wrote the paper: DM PL RL PA AB MM GB GA AP DC.

References

1. Pilla AA (2006) Mechanisms and therapeutic applications of time varying and static magnetic fields. In: Barnes F, Greenbaum B, editors. Biological and Medical Aspects of Electromagnetic Fields. Boca Raton, CRC Press. pp. 351–411.
2. Pilla A, Fitzsimmons R, Muchsam D, Wu J, Rohde C, et al. (2011) Electromagnetic fields as first messenger in biological signaling: Application to calmodulin-dependent signaling in tissue repair. Biochim Biophys Acta 1810: 1236–1245.
3. Pilla AA (2012) Electromagnetic fields instantaneously modulate nitric oxide signaling in challenged biological systems. Biochem Biophys Res Commun 426: 330–333.
4. Diniz P, Soejima K, Ito G (2002) Nitric oxide mediates the effects of pulsed electromagnetic field stimulation on the osteoblast proliferation and differentiation. Nitric Oxide. 7: 18–23.
5. Cheng G, Zhai Y, Chen K, Zhou J, Han G, et al. (2011) Sinusoidal electromagnetic field stimulates rat osteoblast differentiation and maturation via activation of NOScGMP-PKG pathway. Nitric Oxide. 25: 316–325.
6. Fitzsimmons R, Jordan SL, Krosberg J, Gaunt T, Pilla AA (2008) A pulsing electric field (PEF) increases human chondrocyte proliferation through a PRF-mediated NO-cGMP signaling pathway. Nitric Oxide. 19: 53–60.
7. Tao R, Huang K (2011) Reducing blood viscosity with magnetic fields. Phys Rev E Stat Nonlin Soft Matter Phys 84(1): 011903.
8. Sosa M, Bernal-Alvarado J, Jiménez-Moreno M, Hernández JC, Guízar-Juárez G, et al. (2005) Magnetic field influence on electrical properties of human blood measured by impedance spectroscopy. Bioelectromagnetics 26: 564–570.
9. Shahaby TE, Shawk MM (2006) Biophysical and biochemical changes in the characteristics of rat blood exposed to combined alternating and static magnetic fields. Rom J Biophys 16: 169–180.
10. Sakhnin L, Khuzaie R (2001) Magnetic behavior of human erythrocytes at different hemoglobin states. Eur Biophys J. 30: 467–470.
11. Ishak LA, Miyakoshi J, Ueno S (2001) Optical absorbance of hemoglobin and red blood cell suspensions under magnetic fields. IEEE Trans Magnetics 37: 2906–2908.
12. Milveckis S, Susczypaikis W (2006) Effects of electromagnetic fields on the meat performance and wool performance of sheep Arch. Tierz., Dummerstorf 49(Special Issue): 219–225.
13. Mousavi SJ, Razi GH, Kamarei M, Aliakbarian H, Sattarzadeh M, et al. (2009) Effects of mobile phone radiodensity on the structure and function of the normal human hemoglobin. Int J Bio Macromolecules 44: 278–285.
14. Magazu S, Calabro E, Campos S (2010) FTIR spectroscopy studies on the bioprotective effectiveness of trehalose on human hemoglobin aqueous solutions under 50 Hz electromagnetic field exposure. J Phys Chem. 114: 12144–12149.
15. Najjar SS, Bottomley PA, Schulman SP, Waldron MM, Steffen RP, et al. (2005) Effects of a pharmacologically-induced shift of hemoglobin-oxygen dissociation on myocardial energetics during ischemia in patients with coronary artery disease. J Cardiovasc Magn Reson 7: 657–666.
16. Herzfeld J, Seidel NE, Taylor MP, Droupad PR, Wang NE (1990) Gentle chemical deoxyribonucleation of hemoglobin solutions. Hemoglobin 14: 399–411.
17. Benschem RE, Bensch RE, Yang S (1973) Equations for the spectrophotometric analysis of hemoglobin mixtures. Anal Biochem 13: 245–248.
18. Pilla AA, Muchsam DJ, Markov MS, Sisken BF (1999) EMF signals and ion/ligand binding kinetics: prediction of bioeffective waveform parameters. Bioelectrochem Bioenerg. 48: 27–34.
19. Strauss B, Patel MK, Navarro A, Berdichevsky M, Pilla AA (2007) Pulsed magnetic fields accelerate wound repair in a cutaneous wound model in the rat. Plast Reconstr Surg 110: 425–430.
20. Strauch B, Patel MK, Rosen DJ, Mahadevia S, Brindzei N, et al. (2006) Pulsed magnetic field therapy increases tensile strength in a rat Achilles’ tendon repair model. J Hand Surg. 31. 1131–1135.

21. Rasouli J, Lekhraj R, White NM, Flamm ES, Pilla AA, et al. (2012) Attenuation of interleukin-1beta by pulsed electromagnetic fields after traumatic brain injury. Neurosci Lett. 519: 4–8.

22. Hedén P, Pilla AA (2008) Effects of pulsed electromagnetic fields on postoperative pain: a double-blind randomized pilot study in breast augmentation patients. Aesthetic Plast Surg 32: 660–666.

23. Rohde C, Chiang A, Adipopou O, Casper D, Pilla A (2010) Effects of Pulsed Electromagnetic Fields on IL-1β and Post Operative Pain: A Double-Blind, Placebo-Controlled Pilot Study in Breast Reduction Patients. Plast Reconstr Surg 123: 1620–1629.

24. Nelson FR, Zvirbulis R, Pilla AA (2012) Non-invasive electromagnetic field therapy produces rapid and substantial pain reduction in early knee osteoarthritis: a randomized double-blind pilot study. Rheumatol Int Mar 27. [Epub ahead of print]

25. Eccles NK (2005) A critical review of randomized controlled trials of static magnets for pain relief, J Altern Complement Med 11(3): 495–509.

26. Roche CJ, Guo F, Friedman JM (2006) Molecular level probing of preferential hydration and its modulation by osmolytes through the use of pyranine complexed to hemoglobin. J Biol Chem 281: 38757–38768.

27. Royer WE Jr, Pardanani A, Gibson QH, Peterson ES, Friedman JM (1996) Ordered water molecules as key allosteric mediators in a cooperative dimeric hemoglobin. Proc Natl Acad Sci U S A. 93(25): 14526–31.

28. Pauling L (1977) Magnetic properties and structure of oxyhemoglobin. Proc Natl Acad Sci USA. 74: 2612–2613.

29. Closs GL (1969) Mechanism explaining nuclear spin polarizations in radical combination reactions. J Am Chem Soc. 91: 4552–4554.

30. Timmel CR, Till U, Brocklehurst B, McLauchlan KA, Hore PJ (1998) Effects of weak magnetic fields on free radical recombination reactions. Molecular Physics 95: 71–89.

31. Binhi VN, Rubin AB (2007) Magnetobiology: the kT paradox and possible solutions. Electromagn Biol Med 26: 45–62.

32. Muehmann DJ, Pilla AA (2009) A Lorentz model for weak magnetic field bioeffects: part I—thermal noise is an essential component of AC/DC effects on bound ion trajectory. Bioelectromagnetics 30: 462–475.

33. Muehmann DJ, Pilla AA (2009) A Lorentz model for weak magnetic field bioeffects: part II—secondary transduction mechanisms and measures of reactivity. Bioelectromagnetics 30: 476–489.

34. Muehmann DJ, Pilla AA (1996) Lorentz approach to static magnetic field effects on bound ion dynamics and binding kinetics: thermal noise considerations. Bioelectromagnetics 17: 89–99.

35. Frauenfelder H, Chen G, Berendsen J, Fenimore PW, Jansson H, et al. (2009) A unified model of protein dynamics. Proc Natl Acad Sci USA 106(13):5129–34.

36. Salvay AG, Grigera JR, Colombo MF (2003) The role of hydration on the mechanism of allosteric regulation: in situ measurements of the oxygen-linked kinetics of water binding to hemoglobin. Biophys J 84(1): 564–76.

37. Bitterman H (2007) Oxygen: an anti-inflammatory drug. Isr Med Assoc J 9: 874–876.

38. Tandara AA, Mustoe TA (2004) Oxygen in wound healing—more than a nutrient. World J Surg. 28: 294–300.

39. Papassotiriou I, Kister J, Griffin N, Stamoulakatou A, Abraham DJ (1998) Modulating the oxygen affinity of human fetal haemoglobin with synthetic allosteric modulators. Br J Haematol. 102: 1163–1171.